Association of APOE polymorphisms with serological lipid and inflammatory markers

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Abstract

Background

The study aims to assess the association of apolipoprotein E (APOE) gene polymorphisms with serological lipid and inflammatory markers to determine their potential role in predicting the risk of cardiovascular diseases (CVD) and Alzheimer's disease (AD).

Methods

A total of 915 individuals underwent testing for lipid and inflammatory biomarkers at Vibrant America Clinical Laboratory. Clinical data, blood lipid and inflammatory profiles, and APOE genotyping were analyzed using PCR-RFLP.

Result

Compared to the E3/E3 genotype, individuals with E2/E3 genotypes showed higher levels of HDL, triglycerides, APOA, HSCRP, and MPO. E2/E4 genotype carriers had higher levels of HDL, triglycerides, Lp(a), and BNPNT. E3/E4 genotypes were associated with elevated levels of total cholesterol, LDL, Lp(a), HSCRP, SDLDL, OXLDL, MPO, LDL-CAL, PLAC, and APOB. The E4/E4 group displayed higher concentrations of total cholesterol, LDL cholesterol, APOB, Lp(a), HSCRP, SDLDL, OXLDL, MPO, LDL-CAL, and PLAC compared to E3/E3 carriers. These findings highlight the atherogenic effect of the ε4 allele and the potential protective effect of the ε2 allele on lipid and inflammatory markers.

Conclusion

This study provides strong evidence linking APOE gene polymorphism to abnormal serum lipid and inflammatory profiles. Individuals carrying the ε4 alleles exhibited dysregulated lipid metabolism and abnormal inflammatory markers, increasing their risk of CVD and AD. Early detection and prompt diagnosis are crucial for implementing therapeutic, dietary, and lifestyle interventions to mitigate risks and prevent or delay lipid and inflammation-related disorders.

1. Introduction

Apolipoprotein E (APOE) is a lipoprotein composed of 299 amino acids and weighs 34 kDa. It is encoded by the APOE gene located on chromosome 19q13.32. (Chen, Y et al. 2021). The APOE gene has three common alleles at the APOE locus, namely ε2, ε3, and ε4, which give rise to six major genotypes: three homozygous (APOE2/2, APOE3/3, and APOE4/4) and three heterozygous (APOE2/3, APOE2/4, and APOE3/4). These genotypes differ in the amino acids at positions 112 and 158, resulting in the production of APOE2 (Cys112; Cys158), APOE3 (Cys112; Arg158), and APOE4 (Arg112; Arg158). The APOE3/3 genotype is the most common occurring in approximately 60–75% of individuals, followed by APOE2/3 and APOE3/4, which account for about 15–25% and 10–20% of the population, respectively (Singh, C. S 2019). The APOE4/4 genotype, associated with a higher risk of disease is less prevalent,
occurring in approximately 2–15% of individuals, depending on the population studied (Ryan, J et al. 2018). The variations in APOE genotypes lead to differences in APOE isoforms, which in turn influence various cellular functions in the central nervous system (CNS) and peripheral tissues (Miao, G et al. 2023; Parhizkar, S and Holtzman, D. M 2022).

In the CNS, APOE isoforms have distinct effects on different cell types. Astrocytes are the primary source of APOE synthesis, although microglia and neurons can also produce it in certain circumstances (Lanfranco, M. F et al. 2020). The APOE produced by different cell types in the CNS is subsequently lipidated by the ATP-binding cassette transporter A1 (ABCA1) to form lipoprotein particles. The role of ABCA1 is to regulate the efflux of cholesterol and phospholipids from the cell onto high-density lipoprotein (HDL) in plasma (Koldamova, R et al. 2010). It has been proposed that ABCA1 catalyzes the initial transfer of cholesterol onto the lipid-devoid APOE protein and that the ATP-binding cassette transporter G1 (ABCG1) completes the full lipidation of the apolipoprotein. Following lipidation, the APOE-HDL-like lipoparticles are endocytosed by specific members of the low-density lipoprotein receptor (LDLR) family (including LDLR, LDLR-related protein (LRP), APOER2, and VLDLR) present on both neuronal and nonneuronal cells. APOE endocytosis provides cholesterol to the neuron that can subsequently be used for synthesis of plasma membranes, synaptogenesis, and dendritic proliferation (Koldamova, R et al. 2010; Vance, J. E and Hayashi, H 2010). APOE isoforms exhibit differential abilities of binding/transporting cholesterol and phospholipids with APOE2 having the highest ability, followed by APOE3, and APOE4 having the poorest ability to bind to lipid molecules. (APOE2 > APOE3 > APOE4). Consequently, APOE4 is poorly lipidated compared with APOE2 and APOE3 (Fernández-Calle, R et al. 2022). APOE4 specifically disrupts lipid metabolism and transport in astrocytes, potentially leading to neuronal dysfunction and neurodegeneration. Microglial cells expressing APOE4 show an amplified pro-inflammatory response compared to those expressing APOE3, indicating a role for APOE isoforms in modulating immune responses in the CNS (Lee, S et al. 2023; Fonken, L. K. and Gaudet, A. D. 2022). Understanding the effects of APOE isoforms on different cell types in the CNS is crucial for studying neurological disorders.

In peripheral tissues, APOE is predominantly produced in the liver, with minor contributions from the adrenal gland and macrophages (Martínez-Martínez et al. 2020). Peripheral APOE is then incorporated into lipoprotein particles in the plasma. In plasma, APOE is specifically associated with VLDL, chyomicron remnants, and a subset of HDL particles (Getz, G. S. and Reardon, C. A. 2009). It is a high affinity ligand for the LDL receptor as well as its family members including the LDL receptor related protein (LRP1), VLDL receptor, and APOE2 receptor (LPR8). APOE interacts with these receptors promoting the endocytic clearance of plasma lipoproteins, especially VLDL and remnant lipoproteins (Getz, G. S. and Reardon, C. A. 2009). Peripheral APOE plays a critical role in regulating lipid metabolism and has profound implications for cardiovascular (CVD) function and systemic inflammation. Notably, APOE isoforms influence the binding of APOE to different lipoprotein particles, such as very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and HDLs, leading to variations in their metabolism (Williams, T et al. 2020). APOE4 exhibits a preference for binding to VLDLs and LDLs, while APOE3 shows a higher affinity towards HDLs (Phillips, M. C. 2014). Furthermore, genetic variation in the APOE
gene has been found to affect inflammation by influencing serum C-reactive protein (CRP) levels (Eriksson, U. K et al. 2011). The intricate interactions between APOE isoforms, lipoproteins, receptors, and inflammatory signalling pathways contribute to the complex regulation of lipid metabolism and inflammation in peripheral tissues. These interrelated processes have implications for the development and progression of CVD and the overall systemic inflammatory status (Dankner, R. et al. 2020).

Previous studies have reported that lipid markers such as LDL, HDL, small-density LDL (SDDL), and oxidized LDL (OXLDL) are affected by APOE polymorphism. APOE4 has been linked to higher levels of LDL and SDDL particles, while APOE2 has been associated with lower LDL and higher SDDL levels. Additionally, APOE4 is believed to correlate with lower levels of the cardioprotective, HDL, and APOA markers while APOE2 is associated with high levels of the same. APOE4 has also been associated with increased susceptibility to OXLDL-induced oxidative stress and inflammation (V. and Sing, C. F. 2002; Seet, W. T. et al. 2004). Further, inflammatory markers such as high-sensitivity C-reactive protein (HSCRP) and myeloperoxidase (MPO) have shown to be influenced by the APOE polymorphism. APOE4 has been linked to higher levels of HSCRP associated with CVD as well as MPO which is associated with oxidative stress and inflammation (Kahri, J. et al. 2006).

With evidence suggesting that the APOE polymorphisms play a significant role in the modulation of serological lipid and inflammatory markers, effective analysis must be carried out to elucidate the mechanism underlying these effects. However, due to the complexity and heterogeneity of the APOE genotypes, establishing a clear cause-and-effect relationship between the polymorphism and lipid and inflammatory profiles remains challenging. To address this gap in knowledge, our study aims to provide a comprehensive analysis of the APOE polymorphism and its effects on lipid and inflammatory markers. By analysing lipid metabolism and inflammatory markers, we hope to understand APOE4’s influence on diseases like CVD and AD. Understanding the interplay of the APOE polymorphism with lipid metabolism and inflammation will enable early risk assessment and the implementation of personalized interventions for mitigating the risk of CVD and AD.

2. Methods

2.1 Study population

This study population comprised 916 individuals who were tested for vital lipid and inflammatory biomarkers at Vibrant America Clinical Laboratory. The study was exempted from formal ethical reviews by Western IRB (Work order #1-1098539-1) (Washington, USA) since the study comprises the retrospective analysis of deidentified clinical data and test results.

2.1 APOE Genotyping

For genotyping, genomic DNA was isolated from blood samples, and genetic analysis was carried out by the PCR-RFLP method using Real-Time PCR System.
2.2 Determination of serum lipids and inflammatory markers

Blood samples were collected and processed to obtain serum. Total cholesterol levels were measured using an enzymatic assay catalyzed by cholesterol dehydrogenase and estimated on the Beckman Coulter AU680 analyzer. Serum levels of APOA, APOB, and Lp(a) were estimated using a particle-enhanced immunoturbidometric assay on the Roche Cobas 6000 C 501 analyzer. LDL, HDL, SDLDL, and triglyceride levels in serum were measured using an enzyme-based colorimetric assay on the Beckman Coulter AU680 analyzer. BNPNT levels were determined through an electrochemiluminescence immune assay. Total serum HOMOC levels were estimated using an enzyme-linked immunosorbent assay on the Beckman Counter AU series Analyzers. OXLDL levels were quantified using a sandwich technique that uses two monoclonal antibodies specific to antigenic determinants on the oxidized apolipoprotein B. The reaction was based on the reaction between the OXLDL in the serum with anti-oxidized LDL antibodies, the reaction is monitored spectrometrically at 450nm. Serum MPO was determined by a latex-enhanced immunoturbidimetric assay based on antigen-antibody interaction. The MPO in the serum binds to a specific anti-MPO antibody coated on the latex. This causes agglutination and the turbidity of agglutination is directly measured as the concentration of MPO in the serum. The in vitro quantification of PLAC is an enzymatic assay based on the hydrolysis of the sn-2 position of the substrate and produces a colored product 4-nitrophenol. The change in the absorbance with the production of 4-nitrophenol is measured as the Lp-PLAC activity.

2.3 Statistical analysis

Statistical analysis was performed using Microsoft Excel (version 2021). Descriptive statistics were used to summarize the characteristics of the study population, including mean and standard deviation for continuous variables. To examine the association between the APOE genotypes and the lipid and inflammatory profile, t-tests were performed. The means of the marker levels were compared among genotypes. Pearson correlation coefficients were calculated to examine the relationship between each lipid and inflammatory profile variable and the APOE genotypes. The level of statistical significance was set at p < 0.05. The results of the statistical analysis were reported as mean ± standard deviation, and correlation coefficients as appropriate.

3. Results

The current research aimed to study the association of APOE genotypes with circulating levels of vital lipid markers including total cholesterol, LDL, HDL, triglycerides, APOA, APOB, Lp(a), BNPNT, SDLDL, OXLDL, APOBAR, and LDLCAL, and inflammatory markers including HSCRP, HOMOC, MPO, and PLAC.

3.1 Baseline Characteristics of lipid and inflammatory markers
The present study evaluated the association of 12 lipids and 4 inflammatory markers. The overall study population included 64.84% female and 35.2% male subjects with the mean age of females being 50.89 ± 15.83 years while that for males was 50.90 ± 15.83 years. The frequency of the six genotypes analyzed in the study is E3/E3 (64.84%), E2/E2 (0.001%) E2/E3 (10.26%), E2/E4 (2.183%), E3/E4 (20.52%), E4/E4 (2.07%). The baseline characteristics of lipid and inflammatory markers of the subjects studied are given in Table 1. Among the lipid markers, elevated levels were observed for cholesterol (193.0699 ± 41.25673 mg/dL), while lower levels were found for APOBAR (0.602094 ± 0.187758 U/L). In terms of the inflammatory markers, higher levels were detected for MPO (1143.84 ± 995.1264 pmol/L), whereas lower levels were observed for HSCRP (2.791012 ± 6.386284 mg/dL).
<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender in percentage</strong></td>
<td></td>
</tr>
<tr>
<td>Female (N = 594)</td>
<td>64.84%</td>
</tr>
<tr>
<td>MALE (N = 322)</td>
<td>35.2%</td>
</tr>
<tr>
<td><strong>Age (Mean ± SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Female (N = 594)</td>
<td>50.89 ± 15.83</td>
</tr>
<tr>
<td>MALE (N = 322)</td>
<td>50.90 ± 15.83</td>
</tr>
<tr>
<td><strong>APOE genotype frequency</strong></td>
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</tr>
<tr>
<td>E3/E3 (N = 594)</td>
<td>64.84%</td>
</tr>
<tr>
<td>E2/E2 (N = 1)</td>
<td>0.001%</td>
</tr>
<tr>
<td>E2/E3 (N = 94)</td>
<td>10.26%</td>
</tr>
<tr>
<td>E2/E4 (N = 20)</td>
<td>2.183%</td>
</tr>
<tr>
<td>E3/E4 (N = 188)</td>
<td>20.52%</td>
</tr>
<tr>
<td>E4/E4 (N = 19)</td>
<td>2.074%</td>
</tr>
<tr>
<td><strong>Parameters (mmol/L)</strong></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>193.0699 ± 41.25673</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>124.4473 ± 36.66815</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>60.35721 ± 17.87262</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>97.05284 ± 47.60668</td>
</tr>
<tr>
<td>APOA (mg/dL)</td>
<td>168.2994 ± 35.19329</td>
</tr>
<tr>
<td>APOB (mg/dL)</td>
<td>97.44402 ± 24.03082</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>37.63487 ± 38.37169</td>
</tr>
<tr>
<td>HSCRP (mg/dL)</td>
<td>2.791012 ± 6.386284</td>
</tr>
<tr>
<td>HOMOC (µmol/L)</td>
<td>9.724662 ± 3.174314</td>
</tr>
<tr>
<td>BNPNT (pg/mL)</td>
<td>93.43146 ± 226.5025</td>
</tr>
<tr>
<td>SDLDL (mg/dL)</td>
<td>29.3387 ± 11.08218</td>
</tr>
<tr>
<td>OXLDL (U/L)</td>
<td>45.99441 ± 20.38091</td>
</tr>
</tbody>
</table>
### Characteristics

<table>
<thead>
<tr>
<th>Gender in percentage</th>
<th>MPO (pmol/L)</th>
<th>APOBAR (U/L)</th>
<th>LDLCAL (mg/dL)</th>
<th>PLAC (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1143.84 ± 995.1264</td>
<td>0.602094 ± 0.187758</td>
<td>113.2985 ± 35.09226</td>
<td>164.0889 ± 43.42017</td>
</tr>
</tbody>
</table>

### 3.2 Effects of APOE Polymorphism on the Lipid and Inflammatory Profile

The mean values for the lipid and inflammatory parameters in association with the APOE genotypes for the study population are presented together in Table 2. The box plot is created to represent the distribution of lipid and inflammatory markers studied with respect to APOE genotypes in Fig. 1, Fig. 2, and Fig. 3, respectively. On comparing with the most commonly occurring genotype, E3/E3, it was found that the individuals carrying the E2/E3 genotypes had higher levels of HDL, triglycerides, APOA, and HSCRP while the individuals carrying the E2/E4 genotype had higher levels of HDL, triglycerides, Lp(a), and BNPNT, comparatively. Individuals carrying the E3/E4 genotypes had higher levels of total cholesterol, LDL, Lp(a), HSCRP, SDLDL, OXLDL, MPO, LDL-CAL, PLAC, and APOB when compared to those carrying the E3/E3 genotype. Individuals in the E4/E4 group had higher concentrations for total cholesterol, LDL cholesterol, APOB, Lp(a), HSCRP, SDLDL, OXLDL, MPO, LDL-CAL, and PLAC compared with E3/E3 carriers.
Table 2
Baseline characteristics of serological lipids and inflammatory markers by APOE genotypes

<table>
<thead>
<tr>
<th>Parameters (mmol/L)</th>
<th>E3/E3</th>
<th>E2/E3</th>
<th>E2/E4</th>
<th>E3/E4</th>
<th>E4/E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>191.2 ± 40.64</td>
<td>182.957 ± 42.2</td>
<td>191.25 ± 28.56</td>
<td>202.36 ± 42.487</td>
<td>209.89 ± 38.76</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>123.5 ± 35.96</td>
<td>106.503 ± 36.7</td>
<td>114.55 ± 24.50</td>
<td>134.84 ± 35.601</td>
<td>151.789 ± 32.47</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>59.8 ± 17.47</td>
<td>62.18 ± 18.77</td>
<td>60.56 ± 21.028</td>
<td>60.7505 ± 18.41</td>
<td>59.189 ± 14.32</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>96.72 ± 47.58</td>
<td>107.9 ± 58.28</td>
<td>122.7 ± 74.797</td>
<td>90.231 ± 36.033</td>
<td>95.321 ± 43.98</td>
</tr>
<tr>
<td>APOA (mg/dL)</td>
<td>167.7 ± 35.36</td>
<td>174.78 ± 34.56</td>
<td>164.09 ± 34.28</td>
<td>167.568 ± 34.89</td>
<td>159.6468 ± 30.9</td>
</tr>
<tr>
<td>APOB (mg/dL)</td>
<td>97.2 ± 23.52</td>
<td>86.2947 ± 25.35</td>
<td>90.51 ± 12.518</td>
<td>103.617 ± 23.69</td>
<td>109.26 ± 20.96</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>37.3 ± 38.66</td>
<td>34.16 ± 34.39</td>
<td>38.15 ± 39.806</td>
<td>39.743 ± 38.566</td>
<td>40.229 ± 47.74</td>
</tr>
<tr>
<td>HSCRP (mg/dL)</td>
<td>2.65 ± 5.8708</td>
<td>3.5656 ± 7.58</td>
<td>1.717 ± 1.416</td>
<td>2.8986 ± 7.3807</td>
<td>3.27 ± 8.33</td>
</tr>
<tr>
<td>BNPNT (pg/mL)</td>
<td>94.9 ± 267.15</td>
<td>85.39 ± 99.762</td>
<td>135.9 ± 144.63</td>
<td>90.23 ± 130.412</td>
<td>75.77 ± 78.86</td>
</tr>
<tr>
<td>SDLDL (mg/dL)</td>
<td>29.2 ± 10.922</td>
<td>26.349 ± 11.19</td>
<td>28.36 ± 8.019</td>
<td>30.867 ± 11.569</td>
<td>32.889 ± 10.74</td>
</tr>
<tr>
<td>OXLDL (U/L)</td>
<td>45.8 ± 20.561</td>
<td>44.8 ± 17.67</td>
<td>42.36 ± 18.072</td>
<td>46.74 ± 20.999</td>
<td>51.585 ± 24.36</td>
</tr>
<tr>
<td>MPO (pmol/L)</td>
<td>1141.8 ± 1013.</td>
<td>1156.6 ± 927.7</td>
<td>953.9 ± 754.05</td>
<td>1160.3 ± 1012.1</td>
<td>1215.8 ± 879.4</td>
</tr>
<tr>
<td>APOBAR (U/L)</td>
<td>0.60 ± 0.1808</td>
<td>0.51377 ± 0.18</td>
<td>0.576 ± 0.151</td>
<td>0.644 ± 0.199</td>
<td>0.7060 ± 0.18</td>
</tr>
<tr>
<td>LDLCAL (mg/dL)</td>
<td>112.01 ± 34.07</td>
<td>99.1957 ± 36.9</td>
<td>106.11 ± 22.58</td>
<td>123.56 ± 35.306</td>
<td>131.67 ± 33.96</td>
</tr>
<tr>
<td>PLAC (ng/mL)</td>
<td>162.68 ± 42.28</td>
<td>147.26 ± 38.29</td>
<td>155.54 ± 40.44</td>
<td>175.66 ± 45.72</td>
<td>189.81 ± 44.46</td>
</tr>
</tbody>
</table>
3.3 Pearson correlation of APOE genotypes with vital lipid and inflammatory biomarkers

The current study used Pearson correlation coefficients to examine the associations between different genotypes and the E3/E3 genotype in terms of the biomarker profile. The results are presented in Table 3 and the heat map is plotted to visualize the correlations in Fig. 4. Here we discuss the correlation of the ε2 alleles with the studied biomarker profile. The E2/E2 genotype was excluded from statistical analysis primarily due to the limited number of participants (n = 1) exhibiting this genotype in our study population. For the E2/E3 genotype, a weak negative correlation among triglycerides, HDL, APOA, SDLDL, APOBAR, PLAC, and Apo-B was observed. Conversely, there was a weak positive correlation among LDLCAL, MPO, OXLDL, BNPNT, HOMOC, HSCRP, LPA, LDL, and cholesterol. E2/E4 carriers exhibited a weak positive correlation with BNPNT, SDLDL, OXLDL, LDLCAL, and PLAC and exhibited a moderate positive correlation with HSCRP and MPO. The lipid parameters LDL, HDL, APOB, LPA, and HOMOC showed weak negative correlations with E2/E4 carriers, while triglycerides, APOBAR, and APOA showed a weak positive correlation.

The assessment of the ε3 genotypes revealed that E3/E4 carriers had a weak negative correlation with total cholesterol, LDL, HDL, triglycerides, Lp(a), HSCRP, BNPNT, SDLDL, ox-LDL, MPO, APOBAR, LDL-CAL, APOA, and Apo-B. The analysis also showed a weak positive correlation with HOMOC and PLAC.

On assessing the ε4 genotypes, E4/E4 carriers were found to have a moderate negative correlation with HOMOC and a weak negative correlation with markers including, total cholesterol, LDL, triglycerides, BNPNT, SDLDL, PLAC, and Apo-B. Conversely, the genotype observed a weak positive correlation with HDL, APOA, Lp(a), HSCRP, OXLDL, APOBAR, and LDL-CAL levels. Additionally, E4/E4 carriers exhibited an increase in inflammatory levels with a moderate positive correlation with MPO levels.

Based on the statistical analysis conducted in our study, we observed significant differences in LDL levels among individuals with the E4/E4 genotype and APOB levels among individuals with the E2/E3 genotype. Specifically, we found that LDL levels in E4/E4 carriers showed significant differences than E3/E3 genotype (p = 0.02). Similarly, APOB levels in E2/E3 carriers showed statistically significant differences compared to E3/E3 genotypes (p = 0.00). However, the analysis did not reveal significant associations for the other lipid and inflammatory markers studied.
### Table 3
Pearson correlation of APOE genotypes with vital lipid and inflammatory biomarkers

<table>
<thead>
<tr>
<th>Parameters (mmol/L)</th>
<th>E2/E3</th>
<th>E2/E4</th>
<th>E3/E4</th>
<th>E4/E4</th>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.104</td>
<td>0.576</td>
<td>-0.134</td>
<td>1.000</td>
</tr>
<tr>
<td>LDL</td>
<td>0.032</td>
<td>0.194</td>
<td>-0.176</td>
<td>0.413</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.048</td>
<td>0.723</td>
<td>-0.335</td>
<td>0.922</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.236</td>
<td>0.557</td>
<td>0.377</td>
<td>0.250</td>
</tr>
<tr>
<td>APOA</td>
<td>-0.024</td>
<td>0.575</td>
<td>-0.457</td>
<td>0.931</td>
</tr>
<tr>
<td>APOB</td>
<td>-0.050</td>
<td>0.000</td>
<td>-0.187</td>
<td>0.323</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.052</td>
<td>0.907</td>
<td>-0.046</td>
<td>0.955</td>
</tr>
<tr>
<td>HSCRP</td>
<td>0.075</td>
<td>0.708</td>
<td>0.429</td>
<td>0.537</td>
</tr>
<tr>
<td>HOMOC</td>
<td>0.193</td>
<td>0.973</td>
<td>-0.219</td>
<td>0.403</td>
</tr>
<tr>
<td>BNPNT</td>
<td>0.053</td>
<td>0.894</td>
<td>0.118</td>
<td>0.594</td>
</tr>
<tr>
<td>SDLDL</td>
<td>-0.184</td>
<td>0.460</td>
<td>0.268</td>
<td>0.788</td>
</tr>
<tr>
<td>OXLDL</td>
<td>0.012</td>
<td>0.877</td>
<td>0.038</td>
<td>0.613</td>
</tr>
<tr>
<td>MPO</td>
<td>0.056</td>
<td>0.966</td>
<td>0.562</td>
<td>0.556</td>
</tr>
<tr>
<td>APOBAR</td>
<td>-0.260</td>
<td>0.184</td>
<td>-0.357</td>
<td>0.997</td>
</tr>
<tr>
<td>LDLCAL</td>
<td>0.015</td>
<td>0.316</td>
<td>0.111</td>
<td>0.568</td>
</tr>
<tr>
<td>PLAC</td>
<td>-0.079</td>
<td>0.469</td>
<td>0.133</td>
<td>0.629</td>
</tr>
</tbody>
</table>

### 4. Discussion

APOE is a multifunctional protein that is synthesized and secreted by various mammalian tissues. While hepatocytes contribute to the majority of the peripheral pool, APOE can also be expressed in adipose tissues, the kidney, and the adrenal glands. In the brain, the glia primarily synthesizes APOE, distinguishing the peripheral and brain APOE pools (D’Alonzo Z. J. et al. 2023). Human APOE is polymorphic, with three major alleles (ε2, ε3, and ε4), which significantly alter its structure and function. APOE plays a crucial role in lipid metabolism and cholesterol transport in both the brain and the periphery (Huebbe, P. and Rimbach, G. 2017). It is implicated as a key factor in both CVD and AD, with a wide range of parameters involved (Xu, C. et al. 2022). The ε4 allele of APOE, particularly, is associated with altered lipid profiles, increased LDL cholesterol levels, decreased HDL levels, and disrupted cholesterol metabolism. These abnormalities contribute to the development of atherosclerosis in CVD and the
accumulation of cholesterol in the brain in the case of AD (Jabeen, K et al. 2022). Additionally, APOE isoforms, especially ε4, influence inflammatory markers, such as CRP leading to heightened inflammation (Asante, I. et al. 2022). The interplay between APOE gene polymorphism, lipid abnormalities, and inflammation connects the risk of both CVD and AD (Miao, G. et al. 2023).

In the case of CVD, atherosclerosis plays a significant role (Frąk, W. et al. 2022). Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in large arteries, leading to impaired endothelial function (Frąk, W. et al. 2022). A key player in this process is APOE, whose production in atherosclerotic lesions is beneficial as it contributes to reverse cholesterol transport, inhibits the proliferation of smooth muscle cells, prevents lipid oxidation, and restricts platelet aggregation (Golden, L.R. and Johnson, L.A. 2022). Its absence or dysfunction can result in hyperlipidemia and the formation of atherosclerotic lesions (Golden, L.R. and Johnson, L.A. 2022). APOE4 carriers, however, face increased risks in CVD due to higher levels of non-HDL lipoproteins, primarily caused by reduced clearance of very low-density lipoprotein (VLDL) cholesterol. This reduced clearance may occur due to the downregulation of LDL receptors or enhanced sequestration of VLDL in hepatocytes (Marais, A. D. et al. 2014). Furthermore, APOE4 has been found to induce endoplasmic reticulum stress in macrophages, leading to mitochondrial malfunction and contributing to inflammation (Waigi, E. W. et al. 2023). Combining the effects of APOE4 and its associated increase in fasting LDL levels, individuals with this allele face an increased risk of CVD and atherosclerosis. The interplay between atherosclerosis, APOE, lipid abnormalities, and inflammation underlines the complex nature of CVD development and progression (Dergunov, A. D. 2011).

AD involves multiple hallmarks that contribute to its progression, including synapse loss, neuronal death, amyloid β peptide (Aβ) deposition, tau protein aggregation, neuroinflammation, and disruption of the blood-brain barrier (BBB) (Liu, C. C. et al. 2013). Lipid metabolism, particularly LDL, plays a crucial role in AD as the brain relies on a well-regulated lipid balance, encompassing phospholipids, sphingolipids, ceramides, and cholesterol, for optimal function. Maintaining this balance is vital for neuronal health, and glial cells play a significant role in this process (Huang, Y. and Mahley, R. W. 2014). Transporters like ApoE facilitate lipid transfer between neurons and glia, while microglia, through receptors such as LDLR, LRP1, and TREM2, are responsible for clearing cholesterol. Any disruption in lipid regulation can trigger inflammation, which is a major hallmark of AD (Riddell, D. R. et al. 2008). Moreover, it has been shown that APOE plays a role in regulating the integrity of the BBB, with specific effects depending on the isoforms of APOE. APOE4 is associated with BBB dysfunction, while APOE2 and APOE3 are linked to improved barrier function (Jackson, R. J. et al. 2022). Thus, the role of APOE in AD is clearly multifactorial, impinging upon not only the amyloid cascade hypothesis but also many of the other major hallmarks of the disease (Chukwu, L. C. et al. 2023).

In the present study, the marker profile was assessed using the marker levels for the E3/E3 genotype as a reference due to its dominant prevalence in the general population. The results revealed that individuals with the E2/E3 genotype exhibited high levels of triglycerides, which is a major risk factor for CVD and AD. The genotype was also associated with high levels of cardioprotective and neuroprotective markers
such as HDL and APOA. However, this genotype was also associated with elevated levels of inflammatory markers including HSCRP and MPO. Although there was variability in the marker levels, the E2/E3 genotype demonstrated negative associations with most lipid and inflammatory markers related to CVD and AD risk.

Similarly, the E2/E4 genotype was found to be associated with high levels of HDL, triglycerides, Lp(a), and BNPT, while being associated with low levels of most risk markers. Individuals with the E2/E4 genotype exhibited lower levels of various lipid markers, including cholesterol, LDL, APOA, APOB, SDLDL, OXLDL, APOBAR, and LDLCAL. Additionally, all assessed inflammatory markers were lower in individuals with the E2/E4 genotype. These findings align with previous studies (Lumsden, A. L. et al. 2020) that reported the protective effects of the E2/E3 and E2/E4 genotypes against hypercholesterolemia, evidenced by lower levels of "bad" cholesterol indicators such as total cholesterol, LDL, and APOB. The E2/E3 genotype was also associated with increased levels of "good" cholesterol indicators like HDL-cholesterol and APOA, which corresponded to a reduced risk of AD and CVD.

The role of the apo ε2 allele in CVD and AD has been a topic of debate. Although it has been linked to lower LDL cholesterol levels, it is frequently associated with hypertriglyceridemia, as observed for the E2/E3 and E2/E4 genotypes in this study. Previous studies (Pablos-Méndez, A. et al. 1997) have demonstrated that APOE polymorphisms significantly influence postprandial triglyceride levels, with the ε2 allele exhibiting a more pronounced response to a fat load. This response may be attributed to the dysregulated breakdown of triglyceride-rich lipoproteins, resulting in their accumulation and potentially higher triglyceride levels in individuals with the E2/E4 genotype (Raffai, R. L. 2015). It has been suggested that the association between the apo ε2 allele and triglycerides could counteract the potential protective effects of lower LDL cholesterol levels (Zurnić, I. et al. 2014).

Overall, these findings support the notion that specific genotypes, such as E2/E3 and E2/E4, may confer protective effects against CVD and AD by promoting efficient lipid metabolism and reducing cholesterol accumulation in arteries and the brain, respectively. These genotypes enhance the clearance of LDL cholesterol, preventing its deposition in blood vessels and amyloid plaques in the brain, thus lowering the risk of atherosclerosis and dementia. However, the association between the apo ε2 allele and triglycerides highlights the need for a nuanced understanding to fully comprehend its impact on CVD and AD risk. Further research and mechanistic investigations are necessary to elucidate the underlying mechanisms and implications of these genetic associations. Another study also indicated that APOE2 carriers, when compared with APOE3 carriers, have higher concentrations of HSCRP, a well-established marker of inflammation and a recognized risk factor for CVD and AD (Civeira-Marín, M. et al. 2022). However, the precise mechanism behind this association still requires further investigation.

In the current study, it was observed that the E3/E4 genotype is associated with elevated levels of several lipid and inflammatory markers, including cholesterol, LDL, HDL, APOB, Lp(a), SDLDL, OXLDL, APOBAR, LDLCAL, HSCRP, HOMOC, MPO, and PLAC. However, individuals with this genotype exhibited lower levels of triglycerides, APOA, and BNPNT. These findings indicate that E3/E4 carriers are more likely to have
higher levels of lipid and inflammatory markers associated with CVD and AD risk. Although there was a marginal increase in HDL levels in E3/E4 carriers compared to the reference genotype E3/E3, with a mean of $60.56 \pm 21.028$ mg/dL versus $59.8 \pm 17.47$ mg/dL, this increase may not provide significant cardioprotective or neuroprotective effects.

Conversely, the E4/E4 genotype, found to be relatively less prevalent in the population, exhibits a strong association with CVD and AD. Statistical analysis of this genotype in the current study revealed elevated levels of lipid markers, including cholesterol, LDL, APOB, Lp(a), SDLDL, OXLDL, APOBAR, and LDLCAL, as well as all inflammatory markers HSCRP, HOMOC, MPO, and PLAC. Interestingly, E4/E4 carriers displayed low levels of triglycerides and BNPNT markers associated with CVD and AD risk. As anticipated, the genotype was associated with low levels of cardio and neuro-protective markers, HDL and APOA. Overall, these observations suggest that individuals carrying the E3/E4 genotype are prone to having higher levels of risk markers linked to CVD and AD. Likewise, the E4/E4 genotype is also characterized by elevated CVD and AD risk markers, alongside lower levels of specific markers associated with both risk factors and overall systemic protection.

These findings are consistent with previous research (Villeneuve, S. et al. 2015) indicating that individuals with the ε4 allele tend to have higher concentrations of LDL and cholesterol. The impaired clearance of cholesterol from the bloodstream and brain, influenced by the APOE protein, may underlie this observation. As a result, individuals carrying the ε4 allele face an increased risk of developing CVD and AD due to elevated total cholesterol levels. It is important to note that cholesterol itself is not inherently problematic, but rather the accumulation of cholesterol in the arterial intima leading to atherosclerotic plaque in CVD and accumulation of amyloid-beta plaques in AD. Therefore, the regulation of cholesterol transport and localization within the body, mediated by apolipoproteins like APOE, plays a crucial role in CVD and AD (Villeneuve, S. et al. 2015). Moreover, since a significant portion of APOB and Lp(a) in plasma is typically bound to LDL, it is not surprising that individuals carrying the ε4 allele exhibit the highest levels of APOB (Welty, F. K. et al. 2000; Moriarty, P. M. et al. 2017). These findings align with previous studies suggesting alterations in APOB, Lp(a), and LDL levels in both AD and CVD. Further investigation is needed to understand the involvement of APOB and Lp(a) in cellular pathways associated with disease pathogenesis (Solfrizzi, V. et al. 2002; Qiao, S. Y. et al. 2022).

Previous research has established that LDL itself is not directly responsible for cholesterol accumulation in monocytes/macrophages or the brain. Instead, the uptake of modified forms of LDL, particularly ox-LDL and SDLDL, plays a crucial role in cholesterol accumulation among individuals carrying the ε4 allele (Qiao, S. Y. et al. 2022). Cholesterol accumulation in macrophages is associated with CVD, while its accumulation in the brain is linked to AD (Xu, L. et al. 2022). In the context of CVD, it is believed that oxidative stress promotes the formation of ox-LDL and SDLDL within the vascular wall, where they have a significant impact on atherosclerotic plaque development (Blagov, A. V. et al. 2023). Similarly, in AD, SDLDL and OXLDL may have a greater tendency to cross the BBB and be taken up by brain cells, potentially contributing to cholesterol accumulation and the formation of amyloid-beta plaques (Bacchetti, T. et al. 2015). Another noteworthy marker, HOMOC, has been identified as a potential
inflammatory marker in both CVD and AD (Henderson, H. E. et al. 1999). Elevated HOMOC levels among individuals carrying the ε4 allele are associated with increased inflammation, oxidative stress, and endothelial dysfunction, all of which contribute to the progression of CVD and AD (Akhabue, E. et al. 2014).

To our knowledge, the present study is the first to determine the levels of APOBAR, LDL-CAL, and PLAC in the blood serum of different APOE genotypes. It was found that the 4 allele-carrying individuals typically had higher levels of APOBAR, LDL-CAL, and PLAC in their blood serum, with the descending order being E4/E4 > E3/E4 > E3/E3 > E2/E4 > E2/E3. Previous studies suggest that the elevated APOBAR is indicative of impaired LDL clearance and a higher abundance of LDL particles contributing to cholesterol accumulation. Higher LDL-CAL levels reflect impaired LDL cholesterol clearance, while increased PLAC levels indicate a propensity for plaque development (Borén, J. and Williams, K. J. 2016). These findings reveal that the 4 allele may be associated with impaired lipid metabolism and an elevated risk of CVD and AD. Further research is needed to understand the underlying mechanisms and their contribution to disease development.

Based on the statistical analysis conducted in our study, we observed that individuals with the E4/E4 genotype had significantly higher levels of LDL when compared to the E3/E3 genotype (P = 0.02). Similarly, the E2/E3 genotype had significantly lower APOB levels in comparison to the E3/E3 genotype (P = 0.00). The significant associations of the E4/E4 genotype with high levels of the risk factor, LDL, and the E2/E3 genotype with low levels of the risk factor APOB, signify important relationships between the APOE polymorphisms and lipid alterations. These findings strengthen the understanding that APOE4 confers disease risk while APOE2 is disease protective, in terms of CVD and AD. While our study identified significant associations between APOE genotype and LDL levels for APOE4 individuals and APOB levels for APOE2 individuals, the non-significant associations observed for the other lipid and inflammatory markers suggest that these relationships may be more nuanced and influenced by multiple factors. Further research is necessary to elucidate the underlying mechanisms and explore the broader implications of the APOE genotype on lipid and inflammatory markers.

The correlation analysis conducted in the current study revealed several findings regarding the relationship between APOE polymorphism and the studied lipid and inflammatory markers. Regarding HDL cholesterol levels, a weak negative correlation was observed in the ε2 and ε3 groups, while a weak positive correlation was found in the ε4 group. These results align with a previous study (Wei, S. et al. 2020), which reported a positive correlation between the ε4 group and lower levels of HDL. This suggests that APOE exerts isoform-specific effects on HDL metabolism. In terms of total cholesterol levels, a weak negative correlation was observed in the ε3 and ε4 groups. Among the ε2 groups, the E2/E4 genotype showed a weak negative correlation, while the E2/E3 genotype showed a weak positive correlation. These findings are consistent with previous studies (Kang, et al. 2016), which indicated a positive correlation between e2e3 and decreased total cholesterol levels.
For LDL levels, a weak negative correlation was found in the ε2 groups, with the E2/E4 genotype showing a negative correlation and the E2/E3 genotype showing a positive correlation. Among the ε3 and ε4 groups, both the E3/E4 and E4/E4 genotypes exhibited a weak negative correlation. These results are in line with previous studies (Wang, C. et al. 2019), which demonstrated a negative correlation indicating increased LDL levels in individuals with E3/E4 and E4/E4 genotypes compared to those with the E3/E3 genotype. Furthermore, a weak negative correlation was observed for APOB across all genotypes, consistent with previous studies (Fallaize, R. et al. 2017) indicating increased APOB levels in individuals with the E2/E3 genotype compared to those with the E3/E3 genotype. The current study also investigated the correlation between APOE polymorphism and additional lipid and inflammatory markers. It was found that triglycerides and PLAC exhibited a weak negative correlation, while LDLCAL and OXLDL showed a weak positive correlation. Additionally, HSCRP and MPO demonstrated a moderate positive correlation. Notably, this study is the first to examine the correlation of APOE polymorphism with MPO, APOBAR, LDLCAL, and PLAC.

The findings from this study suggest that the APOE4 genotype confers an increased risk for CVD and AD complications through dysregulated lipid metabolism and abnormal inflammatory profiles. Genetic APOE testing can be used as an early screening tool for CVD and AD risk assessment. The feasibility of genetic testing in early risk assessment makes it an attractive prospect for CVD and AD screening as it can be done from the comfort of one’s home. Saliva or Dried Blood Spot kits are available for easy sample collection and extraction of DNA for genetic assessment by diagnostic laboratories (Galluzzi, S. et al. 2022). The convenience related to the accessibility and feasibility of APOE testing via test kits might help individuals get familiarized with the test kits as early tools for disease risk assessment. This may prospectively lead to increased early CVD and AD risk screening which may enable early diagnosis and the implementation of personalized interventions, including targeted dietary and lifestyle modifications (Civeira-Marín, M. et al. 2022). For APOE4 carriers, a rigorous intervention involving dietary, supplement, and lifestyle practices can be implemented to manage lipid abnormalities and inflammation (Marais, A. D. 2019). This may include following a Mediterranean diet, incorporating omega-3 fatty acids, increasing fiber intake, avoiding smoking, and exercising regularly (Román, G. C., 2019). Conversely, APOE2 carriers, who are at lower risk, can focus on consuming a balanced diet and adopting healthy lifestyle practices to optimize their health.

This research emphasizes the significance of understanding the role of APOE polymorphisms in lipid metabolism, inflammation, and disease susceptibility. While most of the markers did not yield significant results, the study successfully identified changing trends in lipid and inflammatory marker profiles associated with APOE polymorphism. However, it is important to acknowledge the limitations of our study. One limitation is the unequal sample sizes among the genotypes, which may have hindered our ability to observe robust correlations and could have contributed to the lack of statistical significance by reducing our statistical power. We also recognize that our results may have been influenced by other unmeasured or uncontrolled factors. Factors like lifestyle choices (e.g., diet, exercise) and environmental influences (e.g., pollution, stress levels) have the potential to impact lipid and inflammatory levels. Furthermore, genetic variations beyond the APOE genotype, which were not considered in our study, could
have influenced the observed weak correlations or lack of non-significance results thereof. The absence of ethnic diversity in our study is another limitation that could account for the non-significant results. Additionally, as our study was a one-time assessment, it lacks follow-up data that could provide further support for the associations identified. To strengthen the results obtained from this study, follow-up analyses with larger samples must be conducted.

5. Conclusion

In conclusion, the present study provides compelling evidence linking APOE gene polymorphisms to abnormal serum lipid and inflammatory profiles. Mutations in the gene change the three-dimensional structure of the APOE protein and its lipid-carrying capability. Specifically, individuals carrying the e4 alleles exhibited dysregulated lipid metabolism and abnormal inflammatory markers profile which tended towards increased CVD and AD risk, while e2 alleles tended towards decreased risk. These findings underline the importance of early detection, prompt diagnosis, and standardized therapeutic approaches to mitigate the risk of diseases related to abnormal lipid and inflammatory profiles. APOE testing via simple at-home collection using saliva or dried blood spot samples is a convenient way of assessing risk for CVD and AD. By prioritizing proactive measures in at-risk individuals, we can effectively reduce the incidence and severity of lipid and inflammation-related disorders.

Declarations

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Disclosure

The data and materials in this manuscript have not been published elsewhere and are not under consideration by another journal.

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Conflicts of Interest

The authors have read the journal’s policy and the authors of this manuscript have the following competing interests: Rajavelu, Reddy, Pereira, and Song are paid employees of Vibrant America LLC. Jayaraman, Krishna, Wang, Bei, Rajasekaran, and Krishnamurthy are paid employees of Vibrant Sciences
Vibrant Sciences or Vibrant America is a commercial lab that performs serological testing for serum lipid and inflammatory biomarkers and genetic testing for APOE polymorphisms. Vibrant Sciences or Vibrant America could benefit from increased testing based on the results. There are no patents, products in development, or marketed products to declare. This does not alter our adherence to Biochemical Genetics policies on sharing data and materials.

Author Contributions

Karthik Krishna and Tianhao Wang performed the research. Hari Krishnan Krishnamurthy, John J. Rajasekaran, and Vasanth Jayaraman designed the study. Qi Song, Kang Bei, Imbaasree Rajavelu, and Swarnkumar Reddy analyzed the data. Hari Krishnan Krishnamurthy, Imbaasree Rajavelu, Swarnkumar Reddy, and Michelle Pereira wrote the article.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Informed Consent

Not applicable

Consent for publication

Not applicable

Ethical Compliance

The study was carried out in de-identified clinical samples, hence exempted from formal ethical clearance. IRB exemption (work order #1-1098539-1) was determined by the Western Institutional Review Board (WIRB) for Vibrant America Biorepository to use de-linked and deidentified remnant human specimens and medical data for research purposes.

Institutional Review Board Statement

The study comprises a retrospective analysis exempted by the Western Institutional Review Board.

References


Figures

Figure 1

Figure 2


Figure 3
Distribution of serum biomarkers with respect to Apo E genotype. a. Myeloperoxidase, b. APOBAR, c. LDLCAL, d. Lp-PLA2

Figure 4

Heat map for Pearson correlation of serum lipids and inflammatory markers with Apo E genotypes.