**Original Article**

**Polymorphisms of the ApoE (Apolipoprotein E) Gene and Their Influence on Dyslipidemia in HIV-1-Infected Individuals**

Tanida Suwalak1,2, Pornpen Srisawasdii, Apichaya Puangpetch1, Siwalee Santon1, Napatrupron Koomdee1, Montri Chamnanphon1, Angkana Charoenyingwattana3, Wasun Chantratita4, and Chonlaphat Sukasem1*

*Division of Pharmacogenomics and Personalized Medicine, 2Division of Clinical Chemistry, and 3Division of Virology, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University; and 4Division of Macrogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University; and Thailand Center of Excellence for Life Sciences, Ministry of Science and Technology, Bangkok, Thailand

**SUMMARY:** The purpose of this retrospective case-control study was to investigate the frequency of Apolipoprotein E (ApoE) polymorphisms and their influence on antiretroviral therapy (ART)-induced lipodystrophy or dyslipidemia in HIV-infected Thai patients. The clinical characteristics and frequencies of ApoE genotypes were compared between the case (moderate to severe lipodystrophy, \( n = 67 \)) and control (absent to mild lipodystrophy, \( n = 18 \)) groups. The ApoE genotype frequencies among the 85 participants were 2.35\% (\( n = 2 \)) for \( E2/E2 \), 20\% (\( n = 17 \)) for \( E2/E3 \), 9.41\% (\( n = 8 \)) for \( E2/E4 \), 36.47\% (\( n = 31 \)) for \( E3/E3 \), 30.59\% (\( n = 26 \)) for \( E3/E4 \), and 1.18\% (\( n = 1 \)) for \( E4/E4 \). None of the ApoE genotypes showed association with ART-induced lipodystrophy. However, the levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-cholesterol), and ApoB were lower in patients carrying the \( E2 \) allele but higher in \( E4 \) carriers. Interestingly, the ratios between TC and high-density lipoprotein (TC/HDL cholesterol ratio) and ApoB/ApoA-I ratio were significantly higher in the case group. Patients carrying the \( E2 \) allele displayed protective lipid profile, while those carrying \( E4 \) appeared to be at higher risk of dyslipidemia. In conclusion, ApoE polymorphisms were not associated with lipodystrophy in patients undergoing antiretroviral therapy but influenced lipid alteration.

**INTRODUCTION**

Antiretroviral therapy (ART) has been available and effectively used in patients infected with the human immunodeficiency virus (HIV) (1,2). Moreover, highly active antiretroviral therapy (HAART) has the potential to reduce the rates of mortality and morbidity among HIV-infected individuals and improve their quality of life. Certain antiretroviral drugs, however, have been shown to be associated with the development of lipodystrophy (3,4), an adverse effect resulting in poor quality of life among HIV-infected patients (5). The main clinical features associated with lipodystrophy are peripheral fat loss (lipoatrophy) in the face, limbs, and buttocks and central fat accumulation in the abdomen, breasts, and dorso-cervical spine (6). Facial lipoatrophy is the most disgracing feature of HIV-associated lipodystrophy as the face cannot be masked by clothes and serves as an indicator of the health status of individuals (6).

Lipodystrophy does not occur in all HIV-infected patients undergoing antiretroviral therapy, but has been diagnosed in 20–80\% of patients depending on race, the drug employed, and treatment duration (6). Moreover, the pharmacogenetic study revealed that susceptibility to lipodystrophy is dependent on genetic factors. Evidence of association between lipodystrophy and genetic variation has been reported in several studies (7–10). Recent studies have shown that variations in TNF-α (tumor necrosis factor alpha) and HLA-B*4001 are strong genetic risk factors for stavudine-associated lipodystrophy among HIV-infected patients (7–9). Moreover, variations in Apolipoprotein C3 (ApoC3), which encodes for apolipoprotein CIII that plays a role in the transport and clearance of lipoprotein remnants from the bloodstream, were found to be associated with the development of HIV-associated lipodystrophy (10,11). In addition, several genes involved in lipid metabolism, storage, and clearance, such as ApoC3, SREBP-1 (sterol response element-binding protein-1), FAS (fatty acid synthase), and ApoA5 are also associated with antiretroviral-induced lipodystrophy and/or dyslipidemia (10–13).

ApoE, a gene possibly associated with lipodystrophy, is located on the long (q) arm of chromosome 19 at position 13.2. ApoE exhibits genetic polymorphism, and its 3 common alleles designated \( E2 \), \( E3 \), and \( E4 \) allow for 6 different genotypes, as follows: \( E2/E2 \), \( E2/E3 \), \( E2/E4 \), \( E3/E3 \), \( E3/E4 \), and \( E4/E4 \) (14). ApoE polymorphism is a major risk factor for the development of cardiovascular diseases (CVD) (15,16). Individuals with \( E3/E4 \) and \( E4/E4 \) genotypes show reduced activity of the low-density lipoprotein (LDL) receptor, which in turn results in increased concentrations of total and LDL cholesterol (17). Moreover, subjects harboring the \( E4 \) allele have enhanced postprandial lipemia, which could contrib...
ute to increased risk of CVD (16). Patients with ART-associated lipodystrophy exhibit abnormal lipid distribution, hypertriglyceridemia, high LDL cholesterol, and low high-density lipoprotein (HDL) cholesterol levels characteristic of individuals at risk of CVD (10,11). This observation supports the hypothesis that ApoE polymorphism plays a role in the development of lipodystrophy and/or dyslipidemia in HIV-infected individuals.

To our knowledge, little information is available on the association between ART-induced lipodystrophy and ApoE polymorphism (18). The present study investigates the frequency of ApoE polymorphisms (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4) among HIV-1-infected Thai patients as well as the influence of these variants on stavudine-based HAART-induced lipodystrophy and dyslipidemia.

MATERIALS AND METHODS

Study population: This is a retrospective case-control study conducted on 85 HIV-infected patients who visited the Infectious Diseases Clinic of Ramathibodi Hospital, Mahidol University, in Bangkok, Thailand between March 2006 and February 2007; these patients were from a previous study (7,19–21). The inclusion criteria were as follows: the HIV-infected patients should be adults (≥15 years old) who were maintained on antiretroviral regimens. Patients were subjected to evaluation and physical examination for lipodystrophy. For all patients, baseline data collected included demographic, time elapsed since the initial diagnosis of HIV infection, antiretroviral therapy, and duration of the infection being incorrectly attributed to the syndrome, a previous AIDS-defining illness, time elapsed since the initial diagnosis of HIV (or first positive antibody test), a previous AIDS-defining illness, and site of lipoatrophy or dyslipidemia for each region was rated as absent, mild (noticeable on close inspection), moderate (readily noticeable by close operator). Scans were performed for assessing total fat mass, fat-free mass, and bone mass, as described in the Lipodystrophy Case Definition Study (25). Bioelectrical impedance analysis was performed using multi-frequency impedance analyzer (InBody 720; Biospace, Cerritos, CA, USA), as described by the manufacturer for the determination of body fat mass and visceral fat area.

Lipid profile was measured on Dimension RxL Max (Siemens, Malven, PA, USA) using commercially available enzymatic methods as per the manufacturers’ recommendations. The method employed for the analysis of lipids and lipoproteins was standardized as per the Lipid Standardization Program of the National Heart Lung and Blood Institute, Center for Disease Control and Prevention (CDC). The accuracy and precision of the measurements in this study fell within the acceptable criteria of the National Cholesterol Education Program.

DNA isolation: Genomic DNA was isolated using a standard phenol-chloroform extraction protocol, resuspended in Tris-HCL buffer (pH 8.5) (19), and quantitated using UV spectrophotometer ND-1000 (Nano Drop Technologies, Rockland, DE, USA). The quality of the isolated DNA was determined by calculating the ratio of the absorbance at 260 and 280 nm (A260/A280).

ApoE polymorphisms: Polymorphisms in human ApoE were determined using real-time PCR with hybridization probes, using Lightcycler® 1.x/2.0 instrument and the LightMix® Kit ApoE C112R and R158C (rs429358 and rs7412), respectively. A 228 bp fragment of the human ApoE gene was amplified using specific primers. The PCR fragments were analyzed using a
**RESULTS**

Clinical characteristics of the study population: A total of 85 HIV-infected patients receiving stavudine-based therapy were included in this pharmacogenetic association study after informed consent was obtained in writing. The mean (±SD) age of patients was 43.20 ± 8.10 years. Thirty-eight patients were male (44.70%), while 47 were female (55.30%). The mean time elapsed since the diagnosis of HIV infection was 105.3 ± 41.1 months, and the duration of antiretroviral therapy, 81.0 ± 19.2 months. The case group comprised 67 patients, which included 29 and 38 patients with moderate and severe lipodystrophy, respectively. On the other hand, the control group comprised 18 patients, including 10 patients without lipodystrophy and 8 patients with mild lipodystrophy. The clinical characteristics of patients from the case and control groups are summarized in Table 1. Statistical analysis revealed that all baseline characteristics were similar in both groups, with the exception that the case group had a higher proportion of male patients compared to the control group (P < 0.001). Anthropometric measurements revealed that the case group had a significantly lower BMI at baseline (P = 0.0027) and hip circumference (P = 0.025). Moreover, the CD4+ T-cell count (cell/mm³) at the time of treatment initiation was significantly lower in the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case (n = 67)</th>
<th>Control (n = 18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males, n (%)</td>
<td>34.00 (50.70)</td>
<td>4.00 (22.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>33.00 (49.30)</td>
<td>14.00 (77.80)</td>
<td>0.006</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.04 (8.40)</td>
<td>40.28 (6.25)</td>
<td>0.080</td>
</tr>
<tr>
<td>Weight at ART initiation (kg)</td>
<td>55.53 (10.61)</td>
<td>58.79 (16.22)</td>
<td>0.308</td>
</tr>
<tr>
<td>Weight at baseline (kg)</td>
<td>57.33 (10.53)</td>
<td>60.77 (16.00)</td>
<td>0.278</td>
</tr>
<tr>
<td>Body mass index at ART initiation (kg/m²)</td>
<td>21.16 (3.38)</td>
<td>23.41 (6.19)</td>
<td>0.042</td>
</tr>
<tr>
<td>Body mass index at baseline (kg/m²)</td>
<td>21.77 (3.31)</td>
<td>24.20 (6.15)</td>
<td>0.027</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>80.47 (76.00–89.97)</td>
<td>83.53 (78.58–94.03)</td>
<td>0.216</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>90.43 (6.15)</td>
<td>94.62 (9.34)</td>
<td>0.025</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.91 (0.06)</td>
<td>0.91 (0.07)</td>
<td>0.910</td>
</tr>
<tr>
<td>Duration of known HIV infection (months)</td>
<td>98.62 (74.17–130.52)</td>
<td>91.97 (78.36–112.32)</td>
<td>0.616</td>
</tr>
<tr>
<td>Duration of ART (months)</td>
<td>82.55 (19.63)</td>
<td>75.05 (16.71)</td>
<td>0.142</td>
</tr>
<tr>
<td>Duration of stavudine treatment (months)</td>
<td>49.65 (20.98)</td>
<td>46.08 (25.39)</td>
<td>0.542</td>
</tr>
<tr>
<td>CD4+ cell count at ART initiation</td>
<td>60.00 (11.75–164.50)</td>
<td>212.50 (53.75–268.25)</td>
<td>0.023</td>
</tr>
<tr>
<td>CD4+ percentage</td>
<td>7.13 (6.10)</td>
<td>9.83 (5.75)</td>
<td>0.097</td>
</tr>
<tr>
<td>CD4+ cell count at baseline</td>
<td>512.97 (224.14)</td>
<td>600.11 (227.18)</td>
<td>0.149</td>
</tr>
<tr>
<td>CD4+ percentage</td>
<td>22.40 (7.90)</td>
<td>26.22 (6.29)</td>
<td>0.062</td>
</tr>
<tr>
<td>DEXA scans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm</td>
<td>23.35 (11.70)</td>
<td>32.16 (10.40)</td>
<td>0.008</td>
</tr>
<tr>
<td>Leg</td>
<td>21.35 (9.94)</td>
<td>27.52 (11.19)</td>
<td>0.035</td>
</tr>
<tr>
<td>Trunk</td>
<td>27.07 (9.03)</td>
<td>30.62 (5.82)</td>
<td>0.142</td>
</tr>
<tr>
<td>Total</td>
<td>27.93 (8.54)</td>
<td>33.78 (5.45)</td>
<td>0.011</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm (1)</td>
<td>0.69 (0.41–0.94)</td>
<td>0.78 (0.69–1.43)</td>
<td>0.021</td>
</tr>
<tr>
<td>Leg (2)</td>
<td>2.13 (1.67)</td>
<td>3.16 (1.39)</td>
<td>0.026</td>
</tr>
<tr>
<td>Trunk (2)</td>
<td>7.67 (3.82)</td>
<td>10.01 (5.30)</td>
<td>0.050</td>
</tr>
<tr>
<td>Total (2)</td>
<td>16.30 (6.26)</td>
<td>21.00 (7.12)</td>
<td>0.012</td>
</tr>
<tr>
<td>Total lean mass (kg)</td>
<td>41.88 (9.14)</td>
<td>41.12 (11.79)</td>
<td>0.784</td>
</tr>
<tr>
<td>Total mass (kg)</td>
<td>58.18 (11.21)</td>
<td>62.12 (16.98)</td>
<td>0.270</td>
</tr>
</tbody>
</table>

1: Data are expressed as median (interquartile range).
2: Data are expressed as mean (standard deviation).
ART, antiretroviral therapy; kg, kilogram; m, meter; cm, centimeter; mm, millimeter. DEXA, Dual-energy X-ray absorptiometry; CD, cluster of differential.
Among these patients, followed by the cally significant differences in the fat mass (% of arm, legs, trunk, and total fat) were observed in all sites of measurement in the case group (arm, $P = 0.0021$; leg, $P = 0.0026$; trunk, $P = 0.050$; total fat, $P = 0.012$).

**Frequency of various ApoE genotypes:** The ApoE polymorphisms C112R and R158C were investigated in all 85 patients for the determination of their ApoE genotypes. The polymorphisms 112C/158C, 112C/158R, and 112R/158R correspond to the E2, E3, and E4 alleles of the gene. Allele frequency data (Table 2) revealed that the E3 allele (61.76%) was predominant among these patients, followed by the E4 (21.18%) and E2 (17.06%) alleles. Moreover, the frequencies of the various ApoE genotypes were 2.35% ($n = 2$) for E2/E2, 20% ($n = 17$) for E2/E3, 9.41% ($n = 8$) for E2/E4, 36.47% ($n = 31$) for E3/E3, 30.59% ($n = 26$) for E3/E4, and 1.18% ($n = 1$) for E4/E4.

**Association of lipid markers with lipodystrophy:** The average levels of lipid markers (triglycerides, TC, LDL cholesterol, HDL cholesterol, and Lipoprotein [a]) and lipid ratios (TC/HDL cholesterol and ApoB/ApoA-I ratios) were compared between the case and control groups (Table 3). The level of serum ApoB was significantly lower in the case group ($P = 0.050$), while statistically significant differences were not observed in the other lipid parameters. Both TC/HDL cholesterol ($P = 0.017$) and ApoB/ApoA-I ratios ($P = 0.026$) lipid ratios were significantly lower in the case group.

**Association between ApoE genotypes and lipid parameters:** The lipid parameters of all ApoE genotypes were compared and tabulated (Table 4), which failed to reveal any association between these genotypes and lipid parameters. The lipid parameters of each ApoE genotype was subsequently compared with a common genotype E3/E3, and visualized using Box and Whisker diagram (Figure 1A–I). Patients carrying the ApoE genotype E2/E2 and E2/E3 had significantly lower levels of TC (Figure 1A) and LDL cholesterol (Figure 1B) compared to the E3/E3 genotype. Moreover, patients with the E2/E2 genotype had significantly lower level of ApoB (Figure 1G), the TC/HDL cholesterol (Figure 1H), and ApoB/ApoA-I ratios (Figure 1I) compared to patients with the E3/E3 genotype. On the other hand, patients carrying the E4/E4 genotype displayed a trend towards higher ApoB (Figure 1H) levels compared to patients with the E3/E3 genotype.

**Association between ApoE alleles/genotypes and lipodystrophy:** The association of ApoE genotype and alleles frequencies with lipodystrophy was analyzed using chi-square or Fisher’s exact tests (Table 3); however, statistically significant association was not observed.

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**Table 2. The association of ApoE allele frequencies and lipodystrophy**

<table>
<thead>
<tr>
<th>ApoE alleles</th>
<th>Total (n = 85)</th>
<th>Case (n = 67)</th>
<th>Control (n = 18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>29 (17.06%)</td>
<td>24 (17.91%)</td>
<td>5 (13.89%)</td>
<td>0.804(1)</td>
</tr>
<tr>
<td>E3</td>
<td>105 (61.76%)</td>
<td>81 (60.45%)</td>
<td>24 (66.67%)</td>
<td></td>
</tr>
<tr>
<td>E4</td>
<td>36 (21.18%)</td>
<td>29 (21.64%)</td>
<td>7 (19.44%)</td>
<td></td>
</tr>
<tr>
<td>ApoE genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2/E2</td>
<td>2 (2.35%)</td>
<td>2 (2.99%)</td>
<td>0 (0.00%)</td>
<td>0.962(2)</td>
</tr>
<tr>
<td>E2/E3</td>
<td>17 (20.00%)</td>
<td>14 (20.90%)</td>
<td>3 (16.67%)</td>
<td></td>
</tr>
<tr>
<td>E2/E4</td>
<td>8 (9.41%)</td>
<td>6 (8.90%)</td>
<td>2 (11.11%)</td>
<td></td>
</tr>
<tr>
<td>E3/E3</td>
<td>31 (36.47%)</td>
<td>23 (34.33%)</td>
<td>8 (44.44%)</td>
<td></td>
</tr>
<tr>
<td>E3/E4</td>
<td>26 (30.59%)</td>
<td>21 (31.34%)</td>
<td>5 (27.78%)</td>
<td></td>
</tr>
<tr>
<td>E4/E4</td>
<td>1 (1.18%)</td>
<td>1 (1.49%)</td>
<td>0 (0.00%)</td>
<td></td>
</tr>
</tbody>
</table>

Data express by Frequency (%).
\(1\): P-value derived from Chi-square test.
\(2\): P-value derived from Fisher’s exact test.

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**Table 3. Association of lipid markers with lipodystrophy in HAART treated HIV-infected patients**

<table>
<thead>
<tr>
<th>Lipid marker</th>
<th>Case (n = 67)</th>
<th>Control (n = 18)</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid (mg/dL)(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>211.40 (44.82)</td>
<td>199.39 (35.07)</td>
<td>1.108</td>
<td>0.296</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>196.16 (150.61)</td>
<td>136.89 (83.03)</td>
<td>2.563</td>
<td>0.113</td>
</tr>
<tr>
<td>LDL-C</td>
<td>130.79 (37.52)</td>
<td>120.67 (30.40)</td>
<td>1.112</td>
<td>0.140</td>
</tr>
<tr>
<td>HDL-C</td>
<td>54.33 (15.11)</td>
<td>60.22 (14.01)</td>
<td>2.222</td>
<td>0.295</td>
</tr>
<tr>
<td>Lipoprotein (a)</td>
<td>21.31 (20.72)</td>
<td>12.63 (14.42)</td>
<td>2.794</td>
<td>0.098</td>
</tr>
<tr>
<td>Apolipoprotein (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoA-I</td>
<td>140.13 (26.33)</td>
<td>149.32 (33.71)</td>
<td>1.525</td>
<td>0.220</td>
</tr>
<tr>
<td>ApoB</td>
<td>93.91 (26.77)</td>
<td>80.45 (19.52)</td>
<td>3.967</td>
<td>0.050</td>
</tr>
<tr>
<td>ApoB/apoA-I</td>
<td>0.69 (0.26)</td>
<td>0.55 (0.13)</td>
<td>5.123</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Data express by mean (SD).
\(1\): All biochemical measures are given in conventional units; conversions to Systeme International units are as follows: cholesterol (mmol/L), multiply by 0.0259, triglycerides (mmol/L), multiply by 0.0113.
LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; TC, total cholesterol.
ApoE Gene and Dyslipidemias in HIV Infections

Table 4. Relationship between ApoE genotypes and plasma lipid level

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>156.50 (138.00–175.00)</td>
<td>186.00 (168.00–211.00)</td>
<td>190.50 (153.50–253.00)</td>
<td>210.00 (194.50–239.00)</td>
<td>212.50 (203.00–263.00)</td>
<td>211.00</td>
<td>0.075</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>237.50 (164.00–311.00)</td>
<td>165.00 (86.00–210.00)</td>
<td>150.50 (117.00–286.00)</td>
<td>138.00 (89.50–234.50)</td>
<td>101.50 (89.00–183.00)</td>
<td>150.00</td>
<td>0.654</td>
</tr>
<tr>
<td>HDL-C</td>
<td>59.50 (50.00–86.00)</td>
<td>65.00 (39.00–61.00)</td>
<td>44.50 (37.00–56.00)</td>
<td>52.00 (45.50–61.50)</td>
<td>59.50 (47.00–65.00)</td>
<td>54.00</td>
<td>0.339</td>
</tr>
<tr>
<td>LDL-C</td>
<td>82.50 (69.00–96.00)</td>
<td>104.00 (90.00–137.00)</td>
<td>105.50 (90.00–172.00)</td>
<td>129.00 (114.00–148.50)</td>
<td>134.50 (117.00–156.00)</td>
<td>135.00</td>
<td>0.100</td>
</tr>
<tr>
<td>Lp (a)</td>
<td>12.78 (3.35–2.22)</td>
<td>13.80 (4.21–26.70)</td>
<td>6.20 (0.39–23.3)</td>
<td>11.70 (5.73–34.0)</td>
<td>14.95 (2.39–34.50)</td>
<td>3.35</td>
<td>0.953</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>113.15 (99.30–127.00)</td>
<td>138.00 (123.00–148.00)</td>
<td>115.50 (108.50–139.50)</td>
<td>146.00 (130.50–161.00)</td>
<td>141.00 (130.00–170.00)</td>
<td>162.00</td>
<td>0.090</td>
</tr>
<tr>
<td>ApoB</td>
<td>39.75 (39.00–40.50)</td>
<td>78.00 (70.60–88.20)</td>
<td>80.45 (70.75–124.50)</td>
<td>88.90 (72.30–113.50)</td>
<td>91.60 (83.50–113.00)</td>
<td>98.90</td>
<td>0.078</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>2.62 (2.38–2.87)</td>
<td>3.57 (3.00–4.30)</td>
<td>4.13 (3.71–5.64)</td>
<td>3.56 (3.25–4.38)</td>
<td>3.75 (3.34–4.47)</td>
<td>2.78</td>
<td>0.174</td>
</tr>
<tr>
<td>ApoB/ApoA-I</td>
<td>0.36 (0.32–0.39)</td>
<td>0.62 (0.53–0.67)</td>
<td>0.68 (0.61–1.00)</td>
<td>0.58 (0.51–0.74)</td>
<td>0.66 (0.53–0.78)</td>
<td>0.61</td>
<td>0.286</td>
</tr>
</tbody>
</table>

(1) Only 1 sample was E4/E4 genotype median and P-value cannot be calculated.
(2) Statistical significant was indicated by a Kruskal-Wallis test.

DISCUSSION

The potential influence of ApoE polymorphisms on the development of dyslipidemia and ART-induced lipodystrophy was investigated by determining ApoE genotypes in a cohort of 85 HIV-1-infected Thai patients. The prevalence of ApoE variants was also investigated in the current study. E3/E3 was determined to be the most common genotype followed by E3/E4 and E2/E3, while the genotypes E4/E4, E2/E2, and E2/E4 were less prevalent in the study population. Importantly, the present study revealed that patients from the case group exhibited significantly higher levels of ApoB and TC/HDL cholesterol and ApoB/ApoA-I ratios. However, statistically significant association was not observed between ApoE polymorphisms and the development of lipodystrophy. This observation is in agreement with another study where no correlation was observed between ApoE gene polymorphisms and trunk fat accumulation among white HIV-infected patients (18).

There are several possible explanations for the occurrence of dyslipidemia in HIV-infected patients receiving combination ART. ApoB is a major structural component of chylomicrons, VLDL (very-low-density lipoprotein), and LDL, which are atherogenic lipoproteins. One molecule of ApoB is present on each lipoprotein particle; thus, the level of total ApoB reflects the total number of atherogenic particles and therefore, the risk of developing atherosclerosis (26). Increasing serum ApoB levels in HIV patients receiving antiretroviral therapy has been reported elsewhere (27, 28), and is supported by the results of the present study. Patients undergoing antiretroviral therapy, particularly those presenting lipodystrophy, are at risk of developing atherosclerosis. ApoB levels and lipid ratios, such as ApoB/ApoA-I and TC/HDL cholesterol ratios, reflect the same phenomenon.

The ApoE protein is a major component of VLDL cholesterol, a specific type of lipoprotein that has a role in the removal of excess cholesterol from the blood and its transport to the liver for processing (29). Analysis of the association between ApoC3 polymorphisms and dyslipidemia/lipodystrophy in patients receiving HAART revealed significantly higher levels of serum ApoE as well as ApoC3 in patients with lipodystrophy (11). This finding supported the hypothesis that ApoE polymorphism could contribute to the differential risk of developing lipodystrophy among various HIV-infected individuals.

The effect of ApoE polymorphisms on dyslipidemia and lipodystrophy in HIV-infected patients has been sparsely studied to date (18). In an Italian cohort of 151 HIV-1-infected patients, Marzocchetti et al. showed that patients with the ApoE E3/E3 genotype were at lower risk of developing hypertriglyceridemia following the initiation of ART. The role of ApoE in the transport and clearance of lipoprotein remnants from the bloodstream (18,29) suggests the functional association of ApoE polymorphisms with dyslipidemias.

Similar to a previous publication (18), the present study failed to reveal association of ApoE polymorphisms with lipodystrophy; however, individuals carrying the E2 allele (E2/E2 and E2/E3 genotypes) were observed to have lower atherogenic lipid parameters, including TC, LDL cholesterol, and ApoB, compared to the E3/E3 genotype, reflecting the protective role of the E2 allele. On the other hand, patients with the E4/E4 genotype displayed a trend towards higher ApoB level compared to any of the other genotypes. The E4/E4 genotype was observed in a single patient; statistical analysis could therefore not be performed. As mentioned previously, increased level of ApoB reflects increased numbers of atherogenic particles; thus, the patient with the E4/E4 genotype is likely to be more susceptible to dyslipidemia compared to the other genotypes. This observation is in agreement with a previous finding on the association of E4 allele with risk of CVD (30–31). The present study revealed statistically significant differences in certain lipid parameters (ApoB, TC/HDL cholesterol, and ApoB/ApoA-I ratios) between the case and control groups.

The present study has certain limitations; in particular, it is a retrospective study with a small sample size. Future prospective studies with larger sample sizes are required for replicating the findings of the present study, and for exploring similar genetic markers to allow a more precise definition of the association between ApoE polymorphisms and lipodystrophy. In addition, patients of the present study were HIV-infected Thai patients, and the possibility exists that different genetic predictors of dyslipidemia are present in other populations. Thus, the results of the present study are not applicable to other races until validated through a prospect-
Fig. 1. ApoE genotypes and lipid parameters. $P$-value displayed in each bar was derived from Mann-Whitney U test compared between each ApoE genotype and E3/E3 genotype. Only 1 sample was E4/E4 genotype median and $P$-value cannot be calculated. (A) Total cholesterol; (B) LDL-C; (C) Triglyceride; (D) HDL-C; (E) Lp (a); (F) ApoA-I; (G) ApoB; (H) TC/HDL ratio; and (I) ApoB/apoA-I ratio.

tive study involving non-Asian patients. Another limitation of the present study is the possibility that differences in clinical characteristics (gender, BMI, and CD4+ T-cell counts) between the case and control groups are confounding factors that masked the influence of genetic polymorphisms.

In conclusion, polymorphism in the ApoE gene is not associated with lipodystrophy in HAART-treated patients. However, the present study revealed significant effect of ApoE polymorphisms on HAART associated fat metabolism and dyslipidemia in a cohort of HIV-1-infected Thai patients. Further studies are required to validate this finding in independent cohorts.
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Acknowledgments  This study was supported by research grants from (i) New Researchers Grant (MRG 5480136), The Mahidol University (MU)/The Thailand Research Fund and Office of the Higher Education Commission, (ii) Pharmacogenomics Project, Thailand Center of Excellence for Life Science (TCELS) and Mahidol University (MU). We are also grateful to all participants who contributed to the study.

Conflict of interest  None to declare.

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