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**Original Article**

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

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**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-$\alpha$ (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 lipidemia, which could contrib-

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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 ($\geq$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 demographics, time elapsed since the initial diagnosis of HIV (or first positive antibody test), a previous AIDS-defining illness, $CD4^+$ T-cell count, HIV RNA levels, past and current antiretroviral therapy, and duration of the antiretroviral therapy.

**Evaluation of lipodystrophy syndrome**: The existence of lipodystrophy was independently assessed by patients and the investigator. Patients completed a lipodystrophy-specific questionnaire and underwent a standardized, lipodystrophy-specific physical examination by the same infectious disease specialist throughout the study (data collection form available from the investigator. Patients and investigators independently assessed and recorded the presence and site of lipoatrophy or diffuse fat accumulation in the faces, necks, dorso-cervical areas, arms, breasts, abdomen, buttocks, and legs. The degree of lipoatrophy and diffuse fat accumulation in each region was rated as absent, mild (noticeable on close inspection), moderate (readily noticeable by patient and/or the physician), or severe (readily noticeable to casual observer), and scored as 0, 1, 2, or 3, respectively (22,23).

To reduce the possibility of a mild physical manifestation being incorrectly attributed to the syndrome, a lipodystrophy group termed ''moderate to severe lipodystrophy'' was defined on the basis of the number and severity of physical manifestations in 3 affected areas: sunken cheeks, thinning of extremities, and thinning of hips or buttocks. Patients with any ''severe'' signs were included in the case group, as were patients with a ''moderate'' sign in the presence of at least 1 additional sign (mild or moderate). On the other hand, patients with a single moderate sign (no additional signs) and those with 1, 2, or 3 mild signs were assigned to the control (absent to mild lipodystrophy) group. The presence of moderate or severe isolated abdominal obesity was an exclusion criterion in order to minimize the misclassification bias that would occur if patients with age-related central obesity were included. Patients exhibiting no signs of lipodystrophy were grouped together with patients whose signs were too mild (''absent to mild'' lipodystrophy group). Mild lipodystrophy is likely to have lesser cosmetic and clinical impact on the compliance of patients to antiretroviral therapy; thus, patients were categorized into ''moderate to severe'' and ''absent to mild'' lipodystrophy groups for further analysis of association (22,23). The ''moderate to severe'' lipodystrophy group was defined as the ''case group'', and the ''absent to mild'' lipodystrophy group, as the ''control group''.

Anthropometric measurements were performed by the same dietician throughout the study. The height, body weight, circumference of neck, chest, mid-arm, mid-thigh, waist, and hip, and the skinfold thickness in 4 sites (scapular, biceps, triceps, and suprailiac) were measured according to the recommendations of the World Health Organization (24).

Skinfold thickness and circumference were measured using skinfold caliper and the anthropometric tape, respectively. Body mass indices (BMIs) and waist-hip ratios were calculated from the anthropometric data. Whole-body dual-energy X-ray absorptiometry (DEXA) scans (Hologic Discovery A, version 12.6.1; Hologic, Bedfold, MA, USA) were conducted by a single 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 manufacture 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 manufactures' 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 ($A_{260/280}$).

***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

SimpleProbe® probe (*ApoE* C112R, detected in channel 530) or probes labeled with LightCycler® Red 640 (*ApoE* R158C, detected in channel 640).

**Statistical analyses**: Continuous data with normal distribution have been represented as mean (± standard deviation or SD), and those with non-normal distribution, as median (interquartile range). Depending on data distribution, independent t-test or the Mann-Whitney U test were employed for data comparison between the HIV-infected case and control groups. The Chi-square or Fisher's exact tests were employed for evaluating associations between *ApoE* genotypes and the incidence of lipodystrophy and particular lipid parameters. A Kruskal-Wallis and Mann-Whitney U tests were employed for testing the significance of differences in lipid parameters observed between various *ApoE* genotypes.

## 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/$mm^3$) at the time of treatment initiation was significantly lower in the

Table 1. Clinical characteristics of HIV-infected patients classified according to lipodystrophy

| Variable | Case ($n$ = 67) | Control ($n$ = 18) | $P$-value |
|---|---|---|---|
| Males, $n$ (%) | 34.00 (50.70) | 4.00 (22.20) | <0.001 |
| Female, $n$ (%) | 33.00 (49.30) | 14.00 (77.80) | 0.006 |
| Age[2] (years) | 44.04 (8.40) | 40.28 (6.25) | 0.080 |
| Weight at ART initiation (kg)[2] | 55.53 (10.61) | 58.79 (16.22) | 0.308 |
| Weight at baseline (kg)[2] | 57.33 (10.53) | 60.77 (16.00) | 0.278 |
| Body mass index at ART initiation (kg/$m^2$)[2] | 21.16 (3.38) | 23.41 (6.19) | 0.042 |
| Body mass index at baseline (kg/$m^2$)[2] | 21.77 (3.31) | 24.20 (6.15) | 0.027 |
| Waist circumference (cm)[1] | 80.47 (76.00–89.97) | 83.53 (78.58–94.03) | 0.216 |
| Hip circumference (cm)[2] | 90.43 (6.15) | 94.62 (9.34) | 0.025 |
| Waist/hip ratio[2] | 0.91 (0.06) | 0.91 (0.07) | 0.910 |
| Duration of known HIV infection (months)[1] | 98.62 (74.17–130.52) | 91.97 (78.36–112.32) | 0.616 |
| Duration of ART (months)[2] | 82.55 (19.63) | 75.05 (16.71) | 0.142 |
| Duration of stavudine treatment (months)[2] | 49.65 (20.98) | 46.08 (25.39) | 0.542 |
| CD4+ cell count at ART initiation | | | |
| CD4+ (cells/$mm^3$)[1] | 60.00 (11.75–164.50) | 212.50 (53.75–268.25) | 0.023 |
| CD4+ percentage[2] | 7.13 (6.10) | 9.83 (5.75) | 0.097 |
| CD4+ cell count at baseline | | | |
| CD4+ (cells/$mm^3$)[1] | 512.97 (224.14) | 600.11 (227.18) | 0.149 |
| CD4+ percentage[2] | 22.40 (7.90) | 26.22 (6.29) | 0.062 |
| DEXA scans | | | |
| Fat mass (%)[2] | | | |
| Arm | 23.35 (11.70) | 32.16 (10.40) | 0.008 |
| Leg | 21.35 (9.94) | 27.52 (11.19) | 0.035 |
| Trunk | 27.07 (9.03) | 30.62 (5.82) | 0.142 |
| Total | 27.93 (8.54) | 33.78 (5.45) | 0.011 |
| Fat mass (kg) | | | |
| Arm[1] | 0.69 (0.41–0.94) | 0.78 (0.69–1.43) | 0.021 |
| Leg[2] | 2.13 (1.67) | 3.16 (1.39) | 0.026 |
| Trunk[2] | 7.67 (3.82) | 10.01 (5.30) | 0.050 |
| Total[2] | 16.30 (6.26) | 21.00 (7.12) | 0.012 |
| Total lean mass (kg)[2] | 41.88 (9.14) | 41.12 (11.79) | 0.784 |
| Total mass (kg)[2] | 58.18 (11.21) | 62.12 (16.98) | 0.270 |

[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.

case group ($P$ = 0.023). DEXA scans revealed statistically significant differences in the fat mass (%) of the arms ($P$ = 0.008), legs ($P$ = 0.035), and total fat ($P$ = 0.011) in the case group. Moreover, significantly lower fat mass (kg) was 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*.

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

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

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

**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 ($P$-value = 0.05), while statistically significant differences were not observed in the other lipid parameters. Both TC/HDL cholesterol ($P$-value = 0.017) and ApoB/ApoA-I ratios ($P$-value = 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.

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

| Lipid marker | Case ($n$ = 67) | Control ($n$ = 18) | F-test | $P$-value |
|---|---|---|---|---|
| Lipid (mg/dL)[1)] | | | | |
| Total cholesterol | 211.40 (44.82) | 199.39 (35.07) | 1.108 | 0.296 |
| Triglycerides | 196.16 (150.61) | 136.89 (83.03) | 2.563 | 0.113 |
| LDL-C | 130.79 (37.52) | 120.67 (30.40) | 1.112 | 0.140 |
| HDL-C | 54.33 (15.11) | 60.22 (14.01) | 2.222 | 0.295 |
| Lipoprotein (a) | 21.31 (20.72) | 12.63 (14.42) | 2.794 | 0.098 |
| Apolipoprotein (mg/dL) | | | | |
| ApoA-I | 140.13 (26.33) | 149.32 (33.71) | 1.525 | 0.220 |
| ApoB | 93.91 (26.77) | 80.45 (19.52) | 3.967 | 0.050 |
| Ratio | | | | |
| TC/HDL-C | 4.08 (1.11) | 3.41 (0.71) | 5.953 | 0.017 |
| ApoB/apoA-I | 0.69 (0.26) | 0.55 (0.13) | 5.123 | 0.026 |

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.

Table 4. Relationship between *ApoE* genotypes and plasma lipid level

| Lipid parameter | *ApoE* genotype | | | | | | |
|---|---|---|---|---|---|---|---|
| | *E2/E2* | *E2/E3* | *E2/E4* | *E3/E3* | *E3/E4* | *E4/E4*[1)] | *P*-value[2)] |
| Total cholesterol | 156.50 (138.00–175.00) | 186.00 (168.00–211.00) | 190.50 (153.50–253.00) | 210.00 (194.50–239.00) | 212.50 (203.00–263.00) | 211.00 | 0.075 |
| Triglyceride | 237.50 (164.00–311.00) | 165.00 (86.00–210.00) | 150.50 (117.00–286.00) | 138.00 (89.50–234.50) | 101.50 (89.00–183.00) | 150.00 | 0.654 |
| HDL-C | 59.50 (58.00–61.00) | 55.00 (39.00–61.00) | 44.50 (37.00–56.00) | 52.00 (45.50–61.50) | 59.50 (47.00–65.00) | 54.00 | 0.339 |
| LDL-C | 82.50 (69.00–96.00) | 104.00 (90.00–137.00) | 105.50 (90.00–172.00) | 129.00 (114.00–148.50) | 134.50 (117.00–156.00) | 135.00 | 0.100 |
| Lp (a) | 12.78 (3.35–2.22) | 13.80 (4.21–26.70) | 6.20 (0.39–23.3) | 11.70 (5.73–34.00) | 14.95 (2.39–34.50) | 3.35 | 0.953 |
| ApoA-I | 113.15 (99.30–127.00) | 138.00 (123.00–148.00) | 115.50 (108.50–139.50) | 146.00 (130.50–161.00) | 141.00 (130.00–170.00) | 162.00 | 0.090 |
| ApoB | 39.75 (39.00–40.50) | 79.00 (70.60–88.20) | 80.45 (70.75–124.50) | 88.90 (72.30–113.50) | 91.60 (83.50–113.00) | 98.90 | 0.078 |
| TC/HDL-C | 2.62 (2.38–2.87) | 3.57 (3.00–4.30) | 4.13 (3.71–5.64) | 3.56 (3.25–4.38) | 3.75 (3.33–4.47) | 2.78 | 0.174 |
| ApoB/ApoA-I | 0.36 (0.32–0.39) | 0.62 (0.53–0.67) | 0.68 (0.61–1.00) | 0.58 (0.51–0.74) | 0.66 (0.53–0.78) | 0.61 | 0.286 |

[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 lipodistrophy, 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/lipoatrophy in patients receiving HAART revealed significantly higher levels of serum ApoE as well as ApoC3 in patients with lipodystrophy (11). This finding promoted 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 hypertriglyceremia 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 hyperlipidemia.

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 artherogenic 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 AopB/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 prospec-

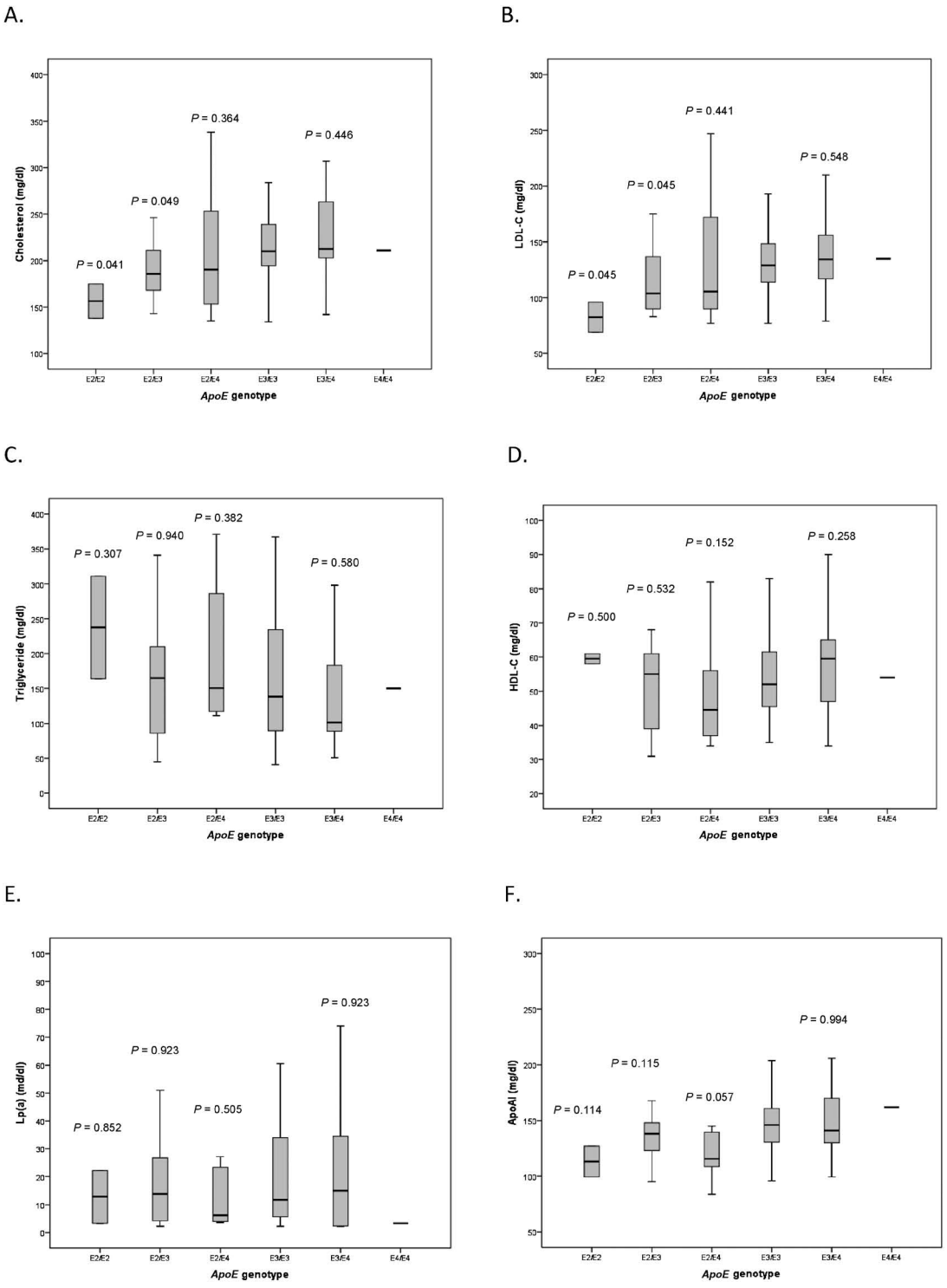


Fig. 1. *ApoE* genotypes and lipid parameters. *P*-value displayed in each bar was derived from Mann-Whitney U test compared between each *ApoE* genotype and *E3/E3* genotype. Only 1 sample was *E4/E4* genotype median and *P*-value cannot be calculated. (A) Total cholesterol; (B) LDL-C; (C) Triglyceride; (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.

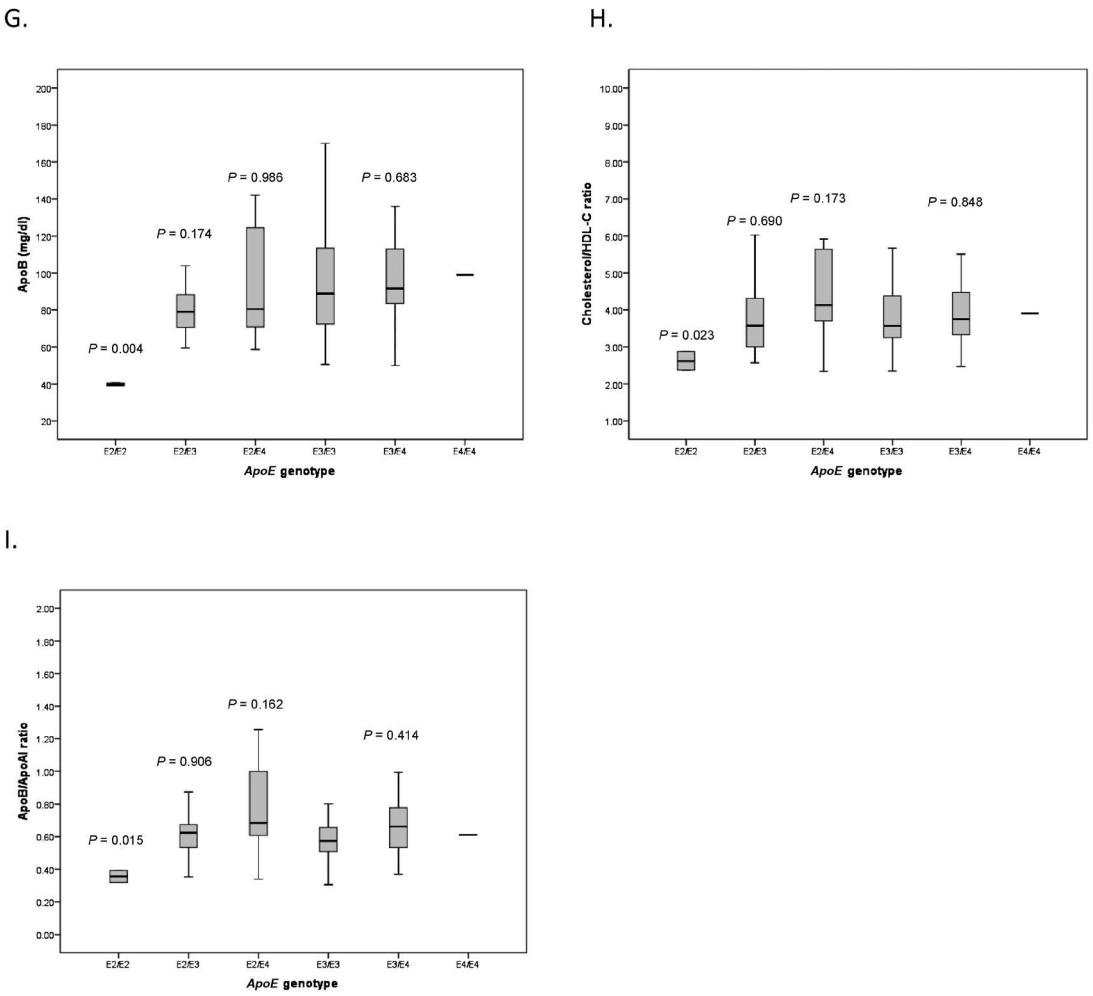


Fig. 1. Continued

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**Conflict of interest** None to declare.

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