Polymorphisms in *Fas* Gene Is Associated with HIV-Related Lipoatrophy in Thai Patients

Sirirat Likanonsakul,¹ Tippawan Rattanatham,¹ Siriluk Feangvad,¹ Sumonmal Uttayamakul,¹ Wisit Prasithsirikul,¹ Somkid Srisopha,¹ Ravee Nitiyanontakij,¹ Pimrapat Tengtrakulcharoen,² Maciej Tarkowski,³ Agostino Riva,³ Emi E. Nakayama,⁴ and Tatsuo Shioda⁴

Abstract

The present study aimed to evaluate the role of genetic polymorphisms in the emergence of lipoatrophy or lipodystrophy in HIV-infected patients with antiretroviral therapy (ART) in Thailand. Position 455 upstream of the *Apolipoprotein C3* gene (*ApoC3* T-455C, rs2854116), codon 64 of the *Beta3 adrenergic receptor* gene (*ARβ3* Tcod64C, rs4994), and position 670 upstream of the *Fas* gene (*Fas* A-670G, rs1800682) were genotyped in 829 HIV-infected Thai patients who had started ART. Crude and adjusted incidence rate ratios (IRR) were calculated using Poisson regression. The serum levels of cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were also analyzed. Multivariate analysis revealed an association between the *Fas* -670AA genotype, but not the *ApoC3* -455 or *ARβ3* cod64 genotypes, with the incidence of lipoatrophy after adjusting for gender and stavudine (d4T)-containing regimens (IRR=1.72, 95% CI=1.20–2.45, $p=0.003$). However, *ApoC3* -455C homozygous patients showed elevated serum levels of triglycerides, while this genotype did not affect serum total cholesterol, HDL, or LDL levels in patients with lipoatrophy or lipodystrophy. In contrast, the *ARβ3* cod64 genotype did not show any significant association with the serum levels of cholesterol, triglycerides, HDL, or LDL. In conclusion, *Fas* -670AA affected the incidence of lipoatrophy in HIV-1-infected Thai patients, while the *ApoC3* -455C allele affected the serum levels of triglycerides. These results confirmed the role of genetics in the development of ART-related metabolic disorders.

Introduction

METABOLIC AND MORPHOLOGICAL alterations observed in human immunodeficiency virus (HIV)-infected patients represent a significant health problem related both to the possible long-term consequences and to the behavioral and psychological well-being of patients. Since the introduction of highly active antiretroviral therapy (HAART), the life expectancy of patients infected with HIV type 1 (HIV-1) has increased remarkably. Despite the clinical benefits, long-term HAART is associated with a complex spectrum of morphological alterations, characterized by body changes that may stigmatize patients and compromise their compliance with therapy, as well as metabolic effects, including dyslipidemia and insulin resistance, which lead to an elevated risk of developing diabetes mellitus and myocardial infarction.^{1–3}

Morphological alterations are collectively named lipodystrophy (LD); however, in recent years it has been recognized that fat loss and fat gain represent distinct entities. Clinical features of fat loss include loss of subcutaneous fat in the face, arms, legs, and buttocks, while fat accumulation is characterized by excess fat deposition in visceral adipose tissue in the abdomen, breasts, dorsocervical region, and trunk.⁴

The underlying mechanism of antiretroviral therapy (ART)-related lipoatrophy (LA) has not been fully elucidated. Many mechanisms have been suggested, including mitochondrial toxicity by nucleoside reverse transcriptase inhibitors (NRTIs), inhibition of adipocyte differentiation by protease inhibitors (PIs), increased levels of inflammatory cytokines, and HIV itself.^{5–7} Mitochondrial toxicity has been clearly shown to play a pivotal role; in particular, thymidine analogs are strong inhibitors of DNA polymerase- γ and lead

¹Bamrasnaradura Infectious Diseases Institute, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand.

²Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

³Luigi Sacco Hospital, University of Milan, Milan, Italy.

⁴Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

to mitochondrial depletion and dysfunction⁸; mainly stavudine (d4T), but also zidovudine (AZT) and didanosine (ddI) are strongly related to LA.^{9,10} In previous *in vitro* and clinical studies, newer nucleoside and nucleotide agents, such as lamivudine (3TC), emtricitabine, abacavir, and tenofovir, appear to be much weaker inhibitors of mitochondrial DNA polymerase- γ or other mitochondrial functions, and appear to be associated with a lower risk of events thought to be related to mitochondrial toxicity.¹¹ However, a certain degree of mitochondrial toxicity is still present and will probably still affect HIV patients under ART, although to a lesser extent.^{12–14} In fact, switch studies from d4T to other NRTIs and complete switch off of thymidine analogs showed modest even if consistent increases in limb fat.^{15–17}

The pathogenesis of lipohypertrophy appears to be multifactorial, with age, sex, HIV itself, immune depression, and duration and type of ART related to its appearance.^{18–20} PIs were suggested to be involved in lipohypertrophy; however, visceral fat accumulation also occurs in the absence of PIs.^{21,22} A compartment-specific effect of mitochondrial toxicity within the adipose tissue may be related to lipohypertrophy²³ as well as dysregulation of fatty acid metabolism and altered expression of adipokines.^{24–26}

However, LA and lipohypertrophy do not occur in all treated patients, and there is a very large degree of inter-individual variability in the timing of emergence and severity of symptoms. A study of identical twins showed that genetic factors are involved in the accumulation of visceral adipose tissue.²⁷ These data suggest that host genetic factors may play a role, and inherited predispositions may have a significant influence on the appearance of LD and metabolic alterations as well as on the viroimmunological response to the drugs.

Therefore, host genetic polymorphisms may have a significant effect on the response to ART in terms of metabolism and immune response.²⁸ A study of Italian HIV patients suggested that single nucleotide polymorphisms (SNPs) in genes involved in apoptosis and lipid metabolism mediate the development of LA.²⁹ In particular, the TT and CT genotypes at position –455 of the *Apolipoprotein C3* (*ApoC3*) gene (*ApoC3* T–455C, rs2854116), nonsynonymous (W to R) CC and CT genotypes at codon 64 of the *Beta3 adrenergic receptor* (*AR β 3*) gene (*AR β 3* Tcod64C, rs4994), and the AA genotype at position –670 of the *Fas* gene (*Fas* A–670G, rs1800682) were associated with increased incidence rates of LA.²⁹ *ApoC3* protein, localized mainly in very low-density lipoprotein and high-density lipoprotein (HDL), is involved in fat metabolism and may delay the catabolism of triglyceride-rich particles. *ApoC3* inhibits lipoprotein lipase and hepatic lipase and decreases the chylomicrons in hepatic cells.^{30,31} *AR β 3* protein, expressed mainly in visceral adipose tissue,^{32,33} contributes to both lipolysis and the delivery of free fatty acids. *Fas* is the main gene that controls cell death by inducing apoptosis. A previous study indicated that *Fas* and *Fas* ligand influenced the immune reconstitution induced by ART.³⁴

In Thailand, the National Access to Antiretroviral Care program started in 2002 using standard regimens with GPO-VIR (a combination of d4T, 3TC, and nevirapine) as the first line of ART. The present study was performed to determine whether these genetic polymorphisms found in the Italian cohort also mediate the emergence of LA or LD in HIV-infected patients starting ART in Thailand in consideration of the relevance of these side effects in the currently used regi-

mens and of the differences in ethnicity, alimentation, and way of life of the Thai population.

Materials and Methods

Study design

HIV-1-infected individuals receiving ART at Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand were enrolled in this study. LA was defined as the presence of fat loss at any site of the body (face, arms, legs, or buttocks). LD was defined as LA combined with fat accumulation diagnosed at the same time. Fat accumulation was defined as the presence of at least one alteration, such as buffalo hump, or fat accumulation in the breast, abdomen, neck, or lipomas. To qualify, the alteration had to be recognized by both the patient and the physician.³⁵ Most patients who developed LA or LD changed regimen for those without d4T. A 200 μ l sample of whole blood was collected from each patient and stored at –20°C until DNA extraction. The genotypes of *ApoC3* –455, *AR β 3* cod64, and *Fas* –670 were compared between patients who developed LA or LD and those who did not. Patients' age, gender, transmission route, CD4 counts at pre-ART and at 1 year post-ART (cells/ μ l), drug regimens, and duration of therapy were obtained from their medical records. Lipid profile data were obtained from patients' records of serum biochemical tests. Blood for this test was collected when patients were in the fasting state; however, not all patients had serum biochemical test data as the test incurs extra costs for patients. All participants signed an informed consent form. The study was approved by the institutional ethical committees at the Bamrasnaradura Infectious Diseases Institute and Department of Disease Control, Ministry of Public Health, Thailand.

DNA extraction

Genomic DNA was extracted from 200 μ l of each whole blood sample using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.³⁶

Genotyping

Genotyping of *Fas* gene promoter polymorphism, A–670G (rs1800682). Polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis for *Fas* A–670G, modified from a previously published technique,³⁷ was conducted with the primers 5'-CTACCTAAGAGCTATCTACCGTTC-3' and 5'-GGCTGTCCATGTTGTGGCTGC-3'. The PCR product was digested with the restriction enzyme *Mva*I (Roche Biochemicals, Mannheim, Germany) at 37°C overnight. Two polymorphic alleles could be distinguished on the basis of restriction length polymorphisms (188 bp for G and 232 bp for A).

Genotyping of *ApoC3* gene promoter polymorphism, T–455C (rs2854116). TaqMan SNP assay was performed using the StepOne real-time PCR system (Applied Biosystems, Foster City, CA) with primers (5'-GAGCTCAGCCCCCTGTAACCAG-3' and 5'-ACACAGCCTGGAGTAGAGGG-3') and TaqMan MGB probes (5'-VIC-CTCCAAACACCCC-MGB-3' for the C allele and 5'-FAM-TTTACTCCAAA CATCC-MGB-3' for the T allele).

Genotyping of *ARβ3* gene coding polymorphism, Tcod64C (rs4994). TaqMan SNP assay was performed with a TaqMan SNP assay kit (C_2215549_20; Applied Biosystems).

Statistical analysis

Person-year analysis was conducted to investigate a single endpoint: the incidence of LA or LD. If the patient was diagnosed as both LA and LD, the earlier date of the first diagnosis was used as the endpoint. We focused on the time to the occurrence of the first event. Person-years at risk were calculated from the start date of ART until the last available follow-up or development of the event, whichever occurred first. The Poisson regression univariate model was used to determine whether the genetic polymorphisms as well as patients' age, gender, transmission route, CD4 counts at pre-ART and at 1 year post-ART, and starting drug regimes were predictors of LA or LD. Associations were expressed as incidence rate ratio (IRR) and corresponding 95% confidence interval (CI). Factors showing significant association with LA or LD were then included as covariates in the Poisson regression multivariate model to adjust for their possible confounding effects. Starting drug regimens were grouped into either d4T-containing regimens or other backbone regimens. Continuous variables were compared using the Wilcoxon signed rank test for paired samples and Kruskal–Wallis test for unpaired samples. A variable was considered significant at $p < 0.05$.

Results

Incidences of LA and/or LD in HIV-1-infected Thai patients undergoing ART

A total of 829 HIV-1-infected individuals who had started ART were enrolled in this study at the Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand, from April 2008 to August 2009. Patient demographic data are shown in Table 1. The study population consisted of 478 males (57.7%) and 351 females (42.3%). Most of the patients contracted infection through sexual contact. Almost half of the patients started ART immediately after being diagnosed with HIV infection. Median CD4 count per microliter pre-ART was 45

and that post-ART (after 1 year) was 210.5. The mean follow-up period was 4.85 years (SD=2.42).

We first combined patients with LA and those with LD for analysis, as fat loss was commonly observed in both LA and LD cases. Among the 829 patients, 270 (32.6%) were diagnosed with LA or LD within the observation period. We kept on monitoring the other 559 (67.4%) until the end of the observation period and found that they developed neither LA nor LD (non-LA/LD cases). The median duration of therapy until diagnosis was 2.63 years for the LA or LD cases and 5.53 years for the non-LA/LD cases. In a follow-up of 4019.8 person-years, the incidence rate of LA or LD was 6.70 per 100 person-years. LA or LD cases were found more frequently in females (40.7%) than in males (26.6%), and the incidence rate per 100 person-years for females (9.11) was significantly higher than that for males (5.18) (IRR=1.76, 95% CI=1.37–2.25, $p < 0.001$) (Table 2). Patients using d4T/3TC backbone regimens as starting drugs showed a significantly higher incidence rate per 100 person-years (7.71) than those with other non-d4T-containing (mostly AZT/3TC) regimens (3.49) (IRR=2.21, 95% CI=1.53–3.28, $p < 0.001$) (Table 2). These findings were consistent with those of previous studies in other ethnic groups.^{9,20,29,38,39} In contrast, age, transmission route, and CD4 counts pre-ART and at 1 year post-ART did not significantly affect the incidence of LA or LD (Table 2).

Fas – 670AA, but not *ApoC3* – 455 or *ARβ3* cod64 genotype, affects incidence of LA or LD in HIV-1-infected Thai patients

To elucidate the possible effects of genetic polymorphisms on the development of LA or LD, we determined the genotypes of *Fas* – 670, *ApoC3* – 455, and *ARβ3* cod64 of 829 HIV-1-infected Thai patients. LA or LD cases were found significantly more frequently among patients with the *Fas* – 670AA genotype than in those with other *Fas* – 670 genotypes (IRR=1.41, 95% CI=1.06–1.86, $p = 0.015$, Table 2). The *Fas* – 670AA genotype was found in 25.9% of the LA or LD cases and in 20.2% of the non-LA/LD cases during the observation period. Increased frequency of the *Fas* – 670AA genotype in LA or LD cases was observed in both males and females (data not shown). In cases of *ApoC3* – 455 and *ARβ3* cod64 genotypes, however, there were no statistically significant differences in the frequency of LA or LD between groups (Table 2). Adjustment for gender and starting drug regimens in multivariate Poisson regression analysis resulted in a slightly higher IRR for *Fas* – 670AA (1.47, 95% CI=1.12–1.94, $p = 0.005$, Table 2). This difference was statistically significant after correction for multiple testing of three SNPs ($p = 0.015$).

Fas–670AA genotype affects incidence of LA but not that of LD in HIV-1-infected Thai patients

Among 270 LA and/or LD cases, 148 were diagnosed as LA singly and 105 were diagnosed as LD singly. There were 17 cases diagnosed as LA first who then subsequently developed fat accumulation. These 17 cases were grouped as LD cases. The median durations of therapy until diagnosis were 2.58 years for LA and 2.83 years for LD cases. When we analyzed LA and LD separately, females showed a higher IRR in both LA (1.60, 95% CI=1.14–2.24, $p = 0.005$) and LD (2.31, 95% CI=1.59–3.38, $p < 0.001$) (Tables 3 and 4). Similarly, d4T-containing regimens showed significantly higher IRR in both

TABLE 1. DEMOGRAPHIC DATA OF ALL THE PATIENTS

Demographic data	n=829
Mean age (SD) years	41.12 (7.77)
Male (%)	478 (57.66)
Transmission	
Sexual (%)	806 (97.23)
Intravenous drug user (%)	8 (0.97)
Unknown (%)	15 (1.81)
Median baseline CD4 (cells/μl) (IQR)	45 (15, 127.5)
Median CD4 (cells/μl) after treatment (IQR)	210.5 (142, 305)
Median duration of therapy before onset of lipoatrophy or lipodystrophy (years, IQR) (n=270)	2.63 (1.67, 4.00)
Median duration of therapy of nonlipoatrophy or nonlipodystrophy (years, IQR) (n=559)	5.53 (4.10, 7.18)

SD, standard deviation; IQR, interquartile range.

TABLE 2. UNIVARIATE AND MULTIVARIATE ANALYSIS OF ALL PATIENTS (N=829)

Factors	Events ¹	Person-years	Events/100 person-years	Crude		Adjusted ² (n=829)	
				IRR (95% CI)	p-value	IRR (95% CI)	p-value
Gender							
Male	127	2449.9	5.18	1			
Female	143	1569.9	9.11	1.76 (1.37–2.25)	<0.001	1.70 (1.33–2.16)	<0.001
Transmission							
Unknown ³	7	95.9	7.3	1			
Sexual	263	3924.0	6.7	0.92 (0.44–2.31)	0.782		
NRTIs							
No-d4T	33 ⁴	945.8	3.49	1			
d4T	237 ⁵	3074.0	7.71	2.21 (1.53–3.28)	<0.001	2.05 (1.42–2.96)	<0.001
Age×10 (years)				0.87 (0.74–1.02)	0.090		
CD4 at baseline ⁵				0.9988 (0.9973–1.0003)	0.107		
CD4 after therapy				0.9497 (0.9987–1.0005)	0.458		
<i>Fas</i> –670							
AG/GG	200	3221.6	6.21	1			
AA	70	798.2	8.77	1.41 (1.06–1.86)	0.015	1.47 (1.12–1.94)	0.005
<i>ApoC3</i> –455							
CT/TT	206	3139.0	6.56	1			
CC	64	880.9	7.27	1.11 (0.82–1.47)	0.474		
<i>ARβ3</i> cod64							
CC/CT	58	815.8	7.11	1			
TT	212	3204.0	6.62	0.93 (0.69–1.27)	0.621		

¹Diagnosis of lipodystrophy or lipodystrophy.²Gender, NRTIs, and *Fas* –670 were included as covariates.³Including eight intravenous drug users.⁴No-d4T group mainly used AZT. One case had received ddI instead of AZT as an initial regimen.⁵CD4 cells/ μ L.

IRR, incidence rate ratios; NRTIs, nucleoside reverse transcriptase inhibitors; d4T, stavudine.

LA (2.46, 95% CI=1.48–4.36, $p<0.001$) and LD (2.10, 95%CI=1.23–3.80, $p=0.003$) (Tables 3 and 4). In the case of the *Fas* –670AA genotype, however, significantly higher IRR was observed only in LA (1.68, 95% CI=1.15–2.41, $p=0.006$) but not in LD (1.19, 95%CI=0.74–1.83, $p=0.433$) (Tables 3 and 4). The statistical significance of higher IRR of LA for *Fas* –670AA did not change after adjustment for gender and starting drug regimens in multivariate Poisson regression analysis (1.72, 95% CI=1.20–2.45, $p=0.003$, Table 3). The difference was statistically significant even after correction for multiple testing of three SNPs in two groups (LA or LD) ($p=0.018$). These results suggested that the *Fas* –670 genotype affects LA without fat accumulation.

Lipid profiles of HIV-1-infected Thai patients

We analyzed the serum lipid profiles of the patients. From the records of 829 patients, serum cholesterol levels were obtained in 610 and 628 cases at the first and last measurement of the observation period, respectively. Serum triglyceride (TG) levels were obtained in 646 and 716 cases at the first and last measurement, respectively. Serum HDL levels were obtained in 393 and 367 cases at the first and last measurement, respectively. Serum low-density lipoprotein (LDL) levels were obtained in 382 and 438 cases at the first and last measurement, respectively. Serum cholesterol levels showed a normal distribution, while serum TG, HDL, and LDL levels did not. Therefore, we used a nonparametric method to evaluate the statistical significance of differences in patient lipid profiles. Slight increases in serum cholesterol levels were

observed in the LA (from median 194.5 to 206 mg/dL, $p=0.016$), LD (from median 194 to 205 mg/dL, $p=0.001$), and non-LA/LD cases (from median 195 to 200 mg/dL, $p=0.033$) during ART (Table 5). Similarly, significant increases in TG were observed in the LA (from median 130 to 183.5 mg/dL, $p=0.019$) and LD (from median 122 to 165.5 mg/dL, $p<0.001$), but not in non-LA/LD cases (from median 138 to 148.5 mg/dL, $p=0.359$; Table 5). Accordingly, the LA and LD cases showed significantly higher TG levels than the non-LA/LD cases at the last measurement ($p=0.007$; Table 5). These results indicated that lipid metabolism in LA and LD cases was more severely damaged by ART than that in non-LA/LD cases. It should also be noted here that the LA and LD cases showed weak but clear trends toward lower TG levels than non-LA/LD cases at the first measurement ($p=0.091$; Table 5). In fact, when combined, the LA and LD cases showed significantly lower TG levels (median 122 mg/dL) than the non-LA/LD cases (median 138 mg/dL) at the first measurement ($p=0.049$). On the other hand, HDL and LDL levels remained almost unchanged during the treatment period in the LA and LD, but non-LA/LD cases showed a slight decrease in LDL (from median 137 to 132.5 mg/dL, $p=0.026$; Table 5).

As the LA and LD cases showed similar lipid profiles, we combined the LA and LD cases again and analyzed the effects of patients' genotypes on the lipid profile together. The results indicated that the *ApoC3* –455CC genotype was associated with decreased serum cholesterol levels in non-LA/LD cases at the last measurement ($p=0.035$; Table 6). However, this association was not observed in LA and LD cases ($p=0.393$;

TABLE 3. LIPOATROPHY: RATE OF INCIDENCE AND MULTIVARIATE ANALYSIS (N=707)

Factors	Events ¹	Person-years	Events/100 person-years	Crude		Adjusted ² (n=707)	
				IRR (95% CI)	p-value	IRR (95% CI)	p-value
Gender							
Male	76	2282.1	3.33	1			
Female	72	1350.9	5.33	1.60 (1.14–2.24)	0.005	1.56 (1.13–2.16)	0.007
Transmission							
Unknown	3	85.2	3.52	1			
Sexual	145	3547.8	4.09	1.16 (0.39–5.69)	0.865		
NRTIs							
No-d4T	17	880.5	1.93	1			
d4T	131	2752.5	4.76	2.46 (1.48–4.36)	<0.001	2.32 (1.40–3.85)	0.001
Age × 10 (years)				0.88 (0.71–1.08)	0.228		
CD4 at baseline ³				0.9985 (0.9964–1.0006)	0.151		
CD4 after therapy ³				0.9991 (0.9979–1.0003)	0.151		
Fas–670							
AG/GG	105	2920.4	3.59	1			
AA	43	712.6	6.03	1.68 (1.15–2.41)	0.006	1.72 (1.20–2.45)	0.003
ApoC3–455							
CT/TT	118	2866.1	4.12	1			
CC	30	766.9	3.91	0.95 (0.61–1.43)	0.818		
ARβ3 cod64							
CC/CT	38	748.0	5.08	1			
TT	110	2885.0	3.81	0.75 (0.51–1.12)	0.134		

¹Diagnosis of lipodystrophy.²Gender, NRTIs, and Fas–670 were included as covariates.³CD4 cells/μl.

IRR, incidence rate ratios; NRTIs, nucleoside reverse transcriptase inhibitors; d4T, stavudine.

TABLE 4. LIPODYSTROPHY: RATE OF INCIDENCE AND MULTIVARIATE ANALYSIS (N=681)

Factors	Events ¹	Person-years	Events/100 person-years	Crude		Adjusted ² (n=681)	
				IRR (95% CI)	p-value	IRR (95% CI)	p-value
Gender							
Male	51	2234.3	2.28	1			
Female	71	1346.3	5.27	2.31 (1.59–3.38)	<0.001	2.19 (1.53–3.15)	<0.001
Transmission							
Unknown	4	86.0	4.65	1			
Sexual	118	3494.5	3.38	0.73 (0.28–2.71)	0.508		
NRTIs							
No-d4T	16	860.3	1.86	1			
d4T	106	2720.2	3.90	2.10 (1.23–3.80)	0.003	1.82 (1.07–3.09)	0.027
Age × 10 (years)				0.85 (0.67–1.07)	0.171		
CD4 at baseline ³				0.9988 (0.9966–1.0010)	0.278		
CD4 after therapy ³				1.0000 (0.9990–1.0014)	0.696		
Fas–670							
AG/GG	95	2888.0	3.29	1			
AA	27	692.6	3.90	1.19 (0.74–1.83)	0.433	1.27 (0.83–1.95)	0.278
ApoC3–455							
CT/TT	88	2781.9	3.16	1			
CC	34	798.7	4.26	1.34 (0.88–2.02)	0.148		
ARβ3 cod64							
CC/CT	20	714.5	2.80	1			
TT	102	2866.1	3.56	1.27 (0.78–2.17)	0.328		

¹Diagnosis of lipodystrophy.²Gender, NRTIs, and Fas–670 were included as covariates.³CD4 cells/μl.

Lipodystrophy cases included 17 cases diagnosed with both lipodystrophy and lipodystrophy.

IRR, incidence rate ratios; NRTIs, nucleoside reverse transcriptase inhibitors; d4T, stavudine.

TABLE 5. LIPID PROFILE OF PATIENTS

Lipid profile	LA cases ¹			LD cases ²			Non-LA/LD cases ³			p-value ⁴
	n	Median	IQR	n	Median	IQR	n	Median	IQR	
Cholesterol (mg/dl)										
First test ⁵	96	194.5	165.25–231.25	77	194	159.00–216.50	437	195	160.00–224.00	0.507
Last test ⁶	121	206	177.50–236.00	95	205	180.00–239.00	412	200	176.500–227.75	0.106
p-value ⁷		0.016			0.001			0.033		
TG (mg/dl)										
First test ⁵	98	130	92.25–190.25	89	122	88.00–164.50	459	138.0	95.00–201.00	0.091
Last test ⁶	140	183.5	109.50–285.00	116	165.5	103.75–256.25	460	148.5	97.00–224.75	0.007
p-value ⁷		0.019			<0.001			0.359		
HDL (mg/dl)										
First test ⁵	43	50	40.00–67.00	45	51	44.00–70.00	305	50	40.00–62.00	0.648
Last test ⁶	63	52	42.00–66.00	51	52	42.00–63.00	253	50	40.00–62.00	0.419
p-value ⁷		0.933			0.885			0.392		
LDL (mg/dl)										
First test ⁵	41	136	117.00–174.00	51	133	116.00–168.00	290	137	108.00–167.00	0.731
Last test ⁶	70	135.5	107.75–166.25	88	136.5	114.25–165.75	280	132.5	111.00–157.00	0.295
p-value ⁷		0.777			0.735			0.026		

¹Lipoatrophy cases.²Lipodystrophy cases.³Patients without lipoatrophy or lipodystrophy.⁴Kruskal–Wallis test.⁵The first measurement after patients started antiretroviral therapy.⁶The last measurement of the observation period. For LA and LD cases, this corresponded to the diagnosis of LA and LD, respectively.⁷Wilcoxon signed rank test.

LA, lipoatrophy; LD, lipodystrophy; IQR, interquartile range; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 6) or at the first measurement in both LA and LD cases and non-LA/LD cases ($p=0.254$ and $p=0.583$, respectively). The *ApoC3* –455CC genotype was also found to be associated with elevated serum TG levels in the LA and LD cases at the time of LA or LD diagnosis ($p=0.021$; Table 6). In the non-LA/LD cases, the *ApoC3* –455CC genotype had virtually no effect on serum TG levels. These results suggested that the *ApoC3* –455CC genotype promotes dysregulation of lipid metabolism in LA or LD cases, even though this allele had no deleterious effect on the onset of LA or LD (Table 2). On the other hand, the *ApoC3* –455CC genotype affected neither HDL nor LDL serum levels (data not shown). The *ARβ3* cod64 genotype had no effect on the serum lipid data of any patient group (Table 6). Similarly, *Fas* –670AA, which was found to be significantly associated with LA, did not show any effects on serum lipid levels (Table 6).

Discussion

In the present study, we evaluated the influence of SNPs involved in apoptosis and lipid metabolism on developing LA or LD in Thai HIV-1 patients. Specifically, we analyzed the SNPs of the *Fas* A–670G, *ApoC3* T–455C, and *ARβ3* Tcod64C in patients undergoing ART.

Of the SNPs investigated, our findings indicated that the AA genotype of *Fas* –670 was significantly associated with the risk of LA but not that of LD. This result is consistent with that of a previous study in Italian HIV patients by Zanone Poma *et al.*²⁹ It was suggested that adipocyte apoptosis induced by mitochondrial toxicity of NRTIs was the primary mechanism involved in LA.¹⁵ Consistent with this suggestion, increased *Fas* gene expression levels were observed in adipose tissue of HIV-1

patients with NRTI-associated LA.⁴⁰ The *Fas* A–670G polymorphism is located in the γ -interferon activation site, to which transcription factors such as signal transducer and activator of transcription (STAT) bind.⁴¹ Therefore, this SNP may affect the level of *Fas* gene transcription and may induce apoptosis, consequently increasing the risk of LA. The *Fas* A–670G polymorphism has been reported to be associated with several autoimmune diseases and inflammatory disorders,^{41–43} which may involve a mechanism similar to LA. The frequency of the AA genotype of *Fas* –670 in 829 HIV-1-infected Thais was 22.1%, which is similar to the percentages in Europeans and Japanese but is markedly higher than that in Africans. As *Fas* –670AA caused a 70% increase in risk of LA, this genotype is a candidate for future pretherapy genetic screening, although it is still necessary to find other SNPs that increase the risk of LA for accurate screening. It is also necessary to confirm our findings on *Fas* –670AA in a larger patient cohort.

On the other hand, the frequency distribution of the *ARβ3* cod64 and *ApoC3* –455 genotypes was not significantly different between LA or LD cases and non-LA/LD cases. The *ARβ3* Tcod64C polymorphism failed to show any effect on lipid metabolism among Thai patients in this study. The results for *ARβ3* cod64 were consistent with a previous report that showed no association of this polymorphism with lipid metabolism during weight loss in obese subjects.⁴⁴ However, in the Italian cohort, this genotype was associated with the risk of developing LA.²⁹ Several studies have reported that people with this SNP showed an increased risk of developing obesity and glucose intolerance,^{32,33} whereas other studies failed to find any correlation between this mutation and body mass index.^{45,46}

The results for *ApoC3* –455 were also not consistent with those of the Italian cohort, which showed an association of the

TABLE 6. PATIENT GENOTYPE AND LIPID PROFILE

Genotype	Cholesterol (mg/dl)											
	LA and LD cases ¹					Non-LA/LD cases ²						
	First test		Last test		n	First test		Last test		n		
Median	IQR	Median	IQR	Median		IQR	Median	IQR				
ApoC3 – 455 CC	42	202	172.50–225.00	183.00–239.00	51	214	183.00–239.00	196	154.75–220.25	86	193.5	164.50–220.50
ApoC3 – 455 CT/TT	131	191	159.00–218.00	179.50–240.00	165	204	179.50–240.00	195	161.00–224.00	326	202.5	177.75–229.00
<i>p</i> -value ³		0.254		0.393				0.583			0.035	
ARβ3 cod64 TT	138	194	159.75–224.25	177.50–216.50	169	206	177.50–216.50	198	162.00–224.00	332	202	175.25–228.00
ARβ3 cod64 CT/CC	35	194	177.00–207.00	178.00–246.00	47	202	178.00–246.00	189	156.00–223.50	80	194	174.25–221.00
<i>p</i> -value ³		0.806		0.658				0.360			0.328	
Fas – 670 AA	39	192	158.00–217.00	182.50–240.00	53	211	182.50–240.00	197	158.00–223.00	85	201	176.50–232.50
Fas – 670 AG/GG	134	195	165.75–223.50	173.50–236.75	163	205	173.50–236.75	195	161.00–224.00	327	199	175.50–226.00
<i>p</i> -value ³		0.259		0.602				0.820			0.493	
TG (mg/dL)												
Genotype	LA and LD cases ¹					Non-LA/LD cases ²						
	First test		Last test		n	First test		Last test		n		
	Median	IQR	Median	IQR		Median	IQR	Median	IQR			
ApoC3 – 455 CC	44	132	102.25–209.75	129.00–327.50	61	222	129.00–327.50	145	99.00–204.00	94	158	99.25–210.25
ApoC3 – 455 CT/TT	143	122	87.00–179.00	101.00–248.25	195	166	101.00–248.25	136	93.25–201.00	366	144	97.00–226.00
<i>p</i> -value ³		0.277		0.021				0.771			0.945	
ARβ3 cod64 TT	150	126	93.00–185.75	77.50–281.00	198	175	77.50–281.00	140	97.00–199.00	360	144.5	93.25–218.75
ARβ3 cod64 CT/CC	37	108	82.50–160.00	109.00–274.50	58	167.50	109.00–274.50	124.5	77.75–206.75	100	154	107.00–232.50
<i>p</i> -value ³		0.174		0.973				0.368			0.500	
Fas – 670 AA	42	120	89.50–162.75	99.25–270.75	64	167	99.25–270.75	128	94.00–202.50	96	123.5	96.50–223.75
Fas – 670 AG/GG	145	123	89.00–184.00	103.00–260.00	192	175	103.00–260.00	140	95.00–201.00	364	152	97.00–224.75
<i>p</i> -value ³		0.580		0.530				0.655			0.498	

¹Lipoatrophy and lipodystrophy cases.²Patients without lipoatrophy or lipodystrophy.³Kruskal–Wallis test.

LA, lipoatrophy; LD, lipodystrophy; IQR, interquartile range.

ApoC3 –455T allele with LA. However, it was also reported that the *ApoC3* –455C but not the *ApoC3* –455T allele was associated with metabolic syndrome.⁴⁷ In fact, a recent study in French HIV patients showed that the *ApoC3* –455C but not the *ApoC3* –455T allele is associated with the severity of ART-induced dyslipidemia and occurrence of LD.^{48,49} Therefore, the precise role of the *ApoC3* T–455C polymorphism in LA or LD during ART should be carefully reevaluated in a larger cohort of HIV patients.

Nevertheless, our study also showed that the *ApoC3* –455CC genotype was associated with elevated serum levels of TG, which was consistent with the findings of a previous report showing that the *ApoC3* –455C allele was associated with metabolic syndrome.⁴⁷ These data suggest that the mechanism underlying the promotion of LA and LD in HIV-infected individuals receiving ART may be different from that promoting metabolic syndrome. However, the differences in results may be due to ethnic differences in the cohorts. It was reported that genetic polymorphisms in hepatic lipase showed different effects on plasma lipid levels in different ethnic groups.⁵⁰ Foulkes *et al.* found race- and ethnic-specific differences in plasma lipid levels under ART, as well as differences in the influence of *ApoC3* T–455C and the other SNP in *ApoC3* gene on the development of protease inhibitor-related hypertriglyceridemia.⁵¹ Further studies including reevaluation of these SNPs in larger patient cohorts are therefore necessary to elucidate the precise mechanisms underlying LA and LD in HIV-infected individuals receiving ART.

In conclusion, the *Fas* –670AA genotype, but not the *ApoC3* –455 or *ARβ3* cod64 genotypes, affected the incidence of LA in HIV-1-infected Thai patients. None of these alleles affected the incidence of LD. On the other hand, the *ApoC3* –455CC, but not the *ARβ3* cod64 genotype, affected the serum levels of TG.

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Author Disclosure Statement

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Address correspondence to:

Sirirat Likanonsakul
Bamrasnaradura Infectious Diseases Institute
Department of Disease Control
Ministry of Public Health
126 Tiwanon Road
Muang, Nonthaburi 11000
Thailand

E-mail: siratlik@gmail.com