AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 31, Number 8, 2015
Mary Ann Liebert, Inc.
DOI: 10.1089/aid.2014.0266

Genome-Wide Association Study of HIV-Related Lipoatrophy in Thai Patients: Association of a DLGAP1 Polymorphism with Fat Loss

Sumonmal Uttayamakul,[1,*] Tiphaine Oudot-Mellakh,[2,*] Emi E. Nakayama,[3] Pimrapat Tengtrakulcharoen,[4] Julien Guergnon,[2] Jean-Francois Delfraissy,[5,6] Srisin Khusmith,[7] Chariya Sangsajja,[1] Sirirat Likanonsakul,[1] Ioannis Theodorou,[2,8] and Tatsuo Shioda[3]

Abstract

HIV-related lipoatrophy (LA) is a major adverse drug effect among HIV patients receiving the antiretroviral drug stavudine (d4T) in Southeast Asia. Although the development of LA could be observed in almost all HIV patients administered d4T for extended periods, there is considerable variation in the duration required to develop LA within this patient population. This study aimed to identify host genetic polymorphisms affecting the rate of LA onset in Thai HIV patients. We performed a genome-wide association study of HIV-related LA among patients at the Bamrasnaradura Infectious Diseases Institute, Thailand. Genotypes of HIV patients who developed LA within 2 years of treatment were compared with those of patients who did not develop LA after at least 4 years of treatment (non-LA patients). Genotypes of 49 LA and 92 non-LA patients at 578,525 single nucleotide polymorphisms (SNPs) were determined by Illumina bead arrays. The TaqMan real-time PCR method was used in a replication study. Five SNPs in the bead arrays, which showed the lowest *p* values in a comparison of LA with non-LA patients, were further tested in independent and sex-matched subpopulations consisting of 95 LA and 95 non-LA patients. This replication study revealed a significant association of LA with an SNP (rs12964965) in the gene encoding the Disks Large Homolog-Associated Protein 1 (DLGAP1), even after the correction for five multiple comparisons. These results strongly suggested involvement of the *DLGAP1* gene product in the development of LA in Thai HIV patients.

Introduction

Lipoatrophy (LA) is localized fat loss observed in HIV patients receiving antiretroviral therapy (ART).[1] The underlying mechanism of ART-related LA has not been fully elucidated. Several mechanisms, including mitochondrial toxicity by nucleoside reverse transcriptase inhibitors (NRTIs), inhibition of adipocyte differentiation by protease inhibitors (PIs), increased levels of inflammatory cytokines, and HIV particles themselves, have been suggested.[2–4] Apparently, mitochondrial toxicity plays a pivotal role; in particular, thymidine analogs are strong inhibitors of DNA polymerase-γ and lead to mitochondrial depletion and dysfunction, with the use of thymidine analogs stavudine (d4T), zidovudine (AZT), and didanosine (ddI) strongly correlated with the occurrence of LA.[5–7] Other nucleoside and

[1]Bamrasnaradura Infectious Diseases Institute, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand.
[2]INSERM, U1135, CIMI, Plateforme génomique ANRS, Paris, France.
[3]Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan.
[4]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
[5]AP-HP, Hôpital Kremlin-Bicêtre, Internal Medicine Department, Le Kremlin-Bicêtre, France.
[6]Faculty of Medicine Paris-Sud, Le Kremlin-Bicêtre, France.
[7]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.
[8]AP-HP, Hôpital Pitié-Salpêtrière, Département d'Immunologie, Paris, France.
*These authors contributed equally to this work.

nucleotide agents, such as lamivudine (3TC), emtricitabine, abacavir, and tenofovir, appear to be much weaker inhibitors of mitochondrial function and are associated with a lower risk of LA.[8]

LA, however, is observed less frequently during short treatment periods, and there is a large degree of variability in the timing of emergence and severity of symptoms among patients. Therefore, host genetic polymorphisms may have a significant effect on the response to ART in terms of metabolism and fat tissue distribution. Previous studies have suggested that single nucleotide polymorphisms (SNPs) in *Apolipoprotein C3* (ApoC3),[9] *Beta3 adrenergic receptor* (ARb3),[9] and *Fas* genes[9,10] affect the timing of LA emergence in HIV patients under ART. However, effect sizes of these genetic variants on LA emergence are relatively small, making it necessary to search further for other genetic variants involved in interindividual differences in LA emergence. In the present study, we performed a genome-wide association study (GWAS) of HIV-related LA in Thailand.

Materials and Methods

Patients

HIV-1-infected individuals receiving ART at Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand were recruited in this study.[10] All participants signed an informed consent form. Most of these HIV patients received a combination of d4T, 3TC, and nevirapine. LA was defined as the presence of fat loss at any site of the body (face, arms, legs, or buttocks). To qualify, the alteration had to be recognized by both the patient and the physician.[11] Aliquots of whole blood were collected from each patient and stored at −20°C until DNA extraction. Serum cholesterol and triglyceride (TG) data were obtained from the patients' records of serum biochemical tests. 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.

GWAS

The study enrolled 49 HIV patients who developed LA within 2 years of treatment, along with 92 patients who did not develop fat loss at any site of the body (face, arms, legs, or buttocks) (non-LA) even after at least 4 years of treatment (Table 1). The DNA samples from these patients were genotyped with Illumina Human660W-Quad v1 Beadarrays testing for 578,525 raw autosomal SNPs. All genotypes were determined using BeadStudio software (version 3.2.23) following the manufacturer's standard recommendations. From these SNPs, 458,071 passed the quality control (QC) criteria of genotyping rate >95%, with minor allele frequency >0.01, and with a Hardy–Weinberg p-value $> 10^{-5}$.

Individuals with genotyping success rates below 80%, with gender discrepancy (based on the genotyped sex chromosomes using PLINK[12]) and genetic outliers [using a principal components approach (PCA) calculated by EIGENSTRAT[13]] were excluded. As a result, 2 out of 49 LA and 3 out of 92 non-LA patients were excluded from the subsequent analysis. After applying these filters, the overall genotyping rate was 99.5%.

The association between SNPs and disease status was assessed by the use of logistic regression in adjusting for the

Table 1. Demographic Data of All Patients

	GWAS[a]		*Replication study*		*GWAS*[b] *+ replication study*	
	LA (n = *49*)	*Control* (n = *92*)	*LA* (n = *95*)	*Control* (n = *95*)	*LA* (n = *142*)	*Control* (n = *184*)
Age (years)—mean (SD)	41.0 (7.6)	41.5 (7.3)	41.1 (6.7)	40.8 (6.5)	41.0 (6.8)	41.2 (6.9)
Gender (%)						
Male	27 (55.1%)	46 (50%)	49 (51.6%)	49 (51.6%)	76 (53.5%)	94 (51.1%)
Female	22 (44.9%)	46 (50%)	46 (48.4%)	46 (48.4%)	66 (46.5%)	90 (48.9%)
Transmission (%)						
Unknown	1 (2.0%)	2 (2.2%)	2 (2.1%)	0 (0.0%)	2 (1.4%)	2 (1.1%)
Sexual	48 (98.0%)	90 (97.8%)	93 (97.9%)	95 (100.0%)	140 (98.6%)	182 (98.9%)
NRTI (%)						
No d4T	3 (6.1%)	3 (3.3%)	14 (14.7%)	0 (0%)	17 (12.0%)	3 (1.6%)
d4T	46 (93.0%)	89 (96.7%)	81 (85.3%)	95 (100%)	125 (88.0%)	181 (98.4%)
Median baseline CD4 (cells/μl) (IQR)	45 (16, 114)	61 (17, 150)	28 (10.5, 88)	39 (13, 72)	35 (12, 103)	42 (14, 119)
Median CD4 (cells/μl) after treatment (IQR)	199 (146, 286)	247 (138, 306)	199 (152, 269)	201 (152, 279)	199 (149, 282)	221 (143, 297)
Median duration of therapy (years, IQR)[c]	1.5 (1.3, 1.8)	7 (6.3, 7.5)	3.3 (2.6, 4.3)	4.8 (4.0, 6.3)	2.6 (1.7, 4.0)	6.2 (4.8, 7.3)

[a]All the patients in GWAS.
[b]Two LA and three control patients were excluded based on the filters and results of principal component analysis.
[c]Median duration of therapy before onset of LA in LA patients and median duration of therapy in control patients.
GWAS, genome-wide association study; LA, lipoatrophy; SD, standard deviation; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; d4T, stavudine.

first four principal components using PLINK version 1.07.[12] The genomic inflation factor was 1.000, indicating no population substructure in this patient group. Statistical significance was inferred when $p \leq 1.09 \times 10^{-7}$, which corresponds to the Bonferroni threshold corrected for the number of studied SNPs (=0.05/458,071).

A replication study

Ninety-five HIV patients who developed LA and 95 patients who did not develop fat loss at any site of the body (face, arms, legs, or buttocks) were enrolled (Table 1). These 190 patients were independent from the 141 patients described above. The DNA samples from these patients were genotyped for rs817027, rs12964965, rs2515037, rs2350983, and rs10748965 using TaqMan real-time PCR probes (Applied Biosystems, C__9552190__10, C__31422313__20, C_26371334_10, C__15985880_10, and C__1798792_10, respectively). Differences in the allele frequencies between LA and non-LA cases were evaluated by a single-sided *z*-test with Bonferroni correction.

Statistical analysis of all patients

Logistic regression univariate and multivariate models were used to determine whether the genetic polymorphisms were predictors of LA. Associations were expressed as an odds ratio (OR) and corresponding 95% confidence interval (CI). The Wilcoxon signed rank test and Mann–Whitney test were used to evaluate the differences in serum cholesterol and TG levels in patients.

Results

GWAS and replication studies on LA

We performed a GWAS of 49 LA and 92 non-LA HIV patients. Demographic data on these 141 patients are summarized in Table 1. In our previous study, treatment with d4T was significantly associated with earlier onset of LA.[10] Therefore, we intentionally enrolled more non-LA patients treated with d4T-containing regimens in order to exclude the possibility that levels of potential LA risk were lower in control patients than in LA patients. We also selected non-LA patients treated for longer periods. Except for the duration of the LA-free period and the drug regimen, there was no difference in demographic data between LA and non-LA patients (Table 1).

When we compared allele frequencies of SNPs between validated 47 LA and 89 non-LA patients, no SNP was significantly associated with the disease ($p < 10^{-7}$). Nevertheless, five SNPs showed suggestive evidence of association ($p < 10^{-4}$) with disease. The smallest p value was 1.87×10^{-5} at rs817027, the OR of which was 6.775. The four other SNPs were rs12964965 ($p = 2.21 \times 10^{-5}$, OR = 4.987), rs2515037 ($p = 2.46 \times 10^{-5}$, OR = 4.348), rs2350983 ($p = 2.66 \times 10^{-5}$, OR = 4.69), and rs10748965 ($p = 3.56 \times 10^{-5}$, OR = 12.06). To validate these results, we performed a replication study of these SNP associations using sex-matched 95 LA and 95 non-LA HIV patients who were totally independent from the 141 patients described above. Again, we intentionally recruited more non-LA patients with d4T-containing regimens and longer treatment periods, but the median duration of treatment in non-LA patients in this replication study was shorter than that in the initial GWAS study.

As shown in Table 1, no difference in demographic data of these 190 patients was observed between LA and non-LA patients, except for the duration of the LA-free period and the drug regimen. Genotyping results of the replication study confirmed that rs12964965, but not the other four SNPs, showed a statistically significant difference ($p = 0.0091$) in the minor allele frequency between LA (0.305) and non-LA (0.2) patients. This difference was still significant ($p = 0.046$) even after the Bonferroni correction.

Table 2 shows the results of univariate and multivariate logistic regression analysis for the 326 total patients analyzed in the present study. Age and CD4 counts did not exhibit a correlation with the onset of LA (Table 2). As we reported previously, AA homozygotes of *Fas*-670 developed LA significantly earlier than AG heterozygotes and GG homozygotes.[10] The OR for carriers of rs12964965 minor allele (CC homozygotes and CT heterozygotes) was 2.43 (95% CI: 1.55–3.80, $p = 0.000106$), a value that was slightly higher than that (2.23; 95% CI: 1.31–3.79, $p = 0.003037$) for AA homozygotes of *Fas*-670 (Table 2). These ORs did not change after adjustment for the effect of each other in multivariate logistic regression analysis (Table 2), indicating that *Fas*-670 and rs12964965 independently affect early onset of LA.

Since we recruited an excess of non-LA patients treated with d4T-containing regimens, we attempted to normalize for

Table 2. Univariate and Multivariate Logistic Regression Analysis of Patients ($n = 326$)

Factors	*LA* (n = *142*)	*Control* (n = *184*)	*Crude*			*Adjusted*		
			OR	*(95% CI)*	p *value*	*OR*	*(95% CI)*	p *value*
Age × 10 (years)			0.96	(0.70–1.32)	0.801			
CD4 × 100 at baseline[a]			0.94	(0.71–1.25)	0.677			
CD4 × 100 after therapy[a]			0.93	(0.77–1.13)	0.485			
Fas-670								
AG/GG	99 (69.7%)	154 (83.7%)	1					
AA	43 (30.3%)	30 (16.3%)	2.23	(1.31–3.79)	0.003037	2.27	(1.32–3.92)	0.003087
rs12964965								
TT	57 (40.1%)	114 (62.0%)	1					
CC/CT	85 (59.9%)	70 (38.0%)	2.43	(1.55–3.80)	0.000106	2.46	(1.56–3.88)	0.000108

[a]CD4 cells/μl.
LA, lipoatrophy; CI, confidence interval.

drug regimen by excluding from the univariate logistic regression analysis all 20 of the patients treated with non-d4T-containing regimens. The ORs of CC homozygotes and CT heterozygotes of rs12964965 and that for the AA homozygotes of *Fas*-670 were 2.37 (95% CI: 1.48–3.81, $p=0.00035$) and 2.31 (95% CI: 1.32–4.06, $p=0.003562$), respectively. These results confirmed the association of rs12964965 with early onset of LA in patients treated with d4T-containing regimens, which are still the most prevalent HIV drug regimens in Thailand.

Serum cholesterol and TG levels of HIV patients

Previously we reported significant increases of serum cholesterol in LA and non-LA cases during ART.[10] In the present study, we were able to confirm this trend in the LA cases, since significant increases of cholesterol were observed in the 142 LA cases (from median 194 to 206 mg/dl, $p=0.0109$). We also previously reported significant increases of serum TG in LA but not in non-LA cases during ART.[10] We confirmed these trends in the present study, since the significant increases of TG were observed in the 142 LA cases (from median 130 to 181 mg/dl, $p=0.0054$) but not in the 184 non-LA cases (from 145 to 152 mg/dl, $p=0.4268$). Accordingly, these LA cases showed significantly higher TG levels than the non-LA cases ($p=0.0133$) at the end of the observation period.

These results indicated that lipid metabolism in the LA cases was more severely damaged than in the non-LA cases. However, further stratification of the LA cases by the *DLGAP1* genotypes did not result in a significant association of higher TG levels with particular genotypes (data not shown), suggesting that *DLGAP1* genotypes have little to no effect on serum TG levels.

Discussion

The present study employed a GWAS followed by a replication study. Our work revealed a statistically significant association of rs12964965 with early onset of LA in HIV patients in Thailand. Although the number of patients analyzed in this study is relatively small, this is (to our knowledge) the first GWAS report on HIV-related LA; our results suggest a novel candidate gene involved in LA development. It will be necessary to analyze this SNP in larger patient cohorts to confirm our observations.

rs12964965 is located in the intron of the extended *disks large homolog-associated protein 1* gene (*DLGAP1*), a locus that spans 959.31 kb in chromosome 18p11.31. The *DLGAP1* gene product is also known as synapse-associated protein 90/postsynaptic density 95 (PSD95)-associated protein 1 (SAPAP1),[13] DLG and PSD95-associated protein (DAP-1),[14] or guanylate-kinase-associated protein (GKAP).[15] *DLGAP1* is involved in the development and maintenance of normal brain function[16] and interacts with the motor protein dynein.[17] At present, it is unclear how the SNP in the *DLGAP1* intron could affect the rate of HIV-related LA, which is apparently a nonneurological disease.

However, it was recently reported that certain genetic variants in the *DLGAP1* gene were underrepresented in patients with type 2 diabetes in the Netherlands and Korea.[18,19] Since both LA and type 2 diabetes are characterized by the loss of accumulated substances from the human body, it is possible that *DLGAP1* plays an unidentified common role in the pathogenesis of LA and type 2 diabetes. It is also possible that functional variants in the *DLGAP1* gene or genetic variants in neighboring genes, which are in strong linkage disequilibrium with the SNP in the *DLGAP1* gene, could be responsible for the observed early onset of HIV-related LA. Further studies will be needed to elucidate a mechanistic role for this SNP in the occurrence of LA in HIV-infected individuals.

We previously reported the association of *Fas*-670 AA homozygotes with the early onset of HIV-related LA.[10] Since the *Fas* gene is located on chromosome 19, it is not surprising that the *Fas*-670 and *DLGAP1* SNPs independently affect LA (Table 2). It is, however, noteworthy that the *DLGAP1* SNP effect may be larger in AA homozygotes of *Fas*-670 than in GG homozygotes and AG heterozygotes, since the OR of the *DLGAP1* SNP in AA homozygotes of *Fas*-670 was 6.81 (95% CI: 2.36–19.64, $p=0.000208$), while that in GG homozygotes and AG heterozygotes was 1.88 (95% CI: 1.13–3.14, $p=0.015$). These results suggest that the *DLGAP1* SNP might promote cell death of adipocytes, a process that is already augmented in AA homozygotes of *Fas*-670. Further studies will be required to test this hypothesis.

Acknowledgments

We thank the HIV patients for their kind participation in the study. We thank Tippawan Rattanatham and Siriluk Feangvad for their excellent assistance. We also thank Drs. Wisit Prasithsirikul, Agostino Riva, Massimo Galli, and Patrice Debre for critical discussions. This study was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology, the Ministry of Health, Labour, and Welfare, Japan, and those from ANRS, France. S.U. was a recipient of the Royal Golden Jubilee Ph.D. Program (grant PHD/0094/2551) of the Thailand Research Fund. We dedicate this research communication to Siriluk Feangvad, who passed away on November 30, 2013.

Author Disclosure Statement

No competing financial interests exist.

References

1. Carr A: HIV lipodystrophy: Risk factors, pathogenesis, diagnosis and management. AIDS 2003;17(Suppl 1):S141–148.
2. Birkus G, Hitchcock MJ, and Cihlar T: Assessment of mitochondrial toxicity in human cells treated with tenofovir: Comparison with other nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother 2002;46:716–723.
3. Maggiolo F, Roat E, Pinti M, *et al.:* Mitochondrial changes during D-drug-containing once-daily therapy in HIV-positive treatment-naive patients. Antivir Ther 2010;15:51–59.
4. Giralt M, Domingo P, Guallar JP, *et al.:* HIV-1 infection alters gene expression in adipose tissue, which contributes to HIV- 1/HAART-associated lipodystrophy. Antivir Ther 2006;11:729–740.
5. Collins ML, Sondel N, Cesar D, and Hellerstein MK: Effect of nucleoside reverse transcriptase inhibitors on mitochondrial DNA synthesis in rats and humans. J Acquir Immune Defic Syndr 2004;37:1132–1139.
6. Mallal SA, John M, Moore CB, *et al.:* Contribution of nucleoside analogue reverse transcriptase inhibitors to

subcutaneous fat wasting in patients with HIV infection. AIDS 2000;14:1309–1316.
7. van Vonderen MG, van Agtmael MA, Hassink EA, *et al.:* Zidovudine/lamivudine for HIV-1 infection contributes to limb fat loss. PLoS One 2009;4:e5647.
8. Moyle G: Mechanisms of HIV and nucleoside reverse transcriptase inhibitor injury to mitochondria. Antivir Ther 2005;10(Suppl 2):M47–52.
9. Zanone Poma B, Riva A, Nasi M, *et al.:* Genetic polymorphisms differently influencing the emergence of atrophy and fat accumulation in HIV-related lipodystrophy. AIDS 2008;22:1769–1778.
10. Likanonsakul S, Rattanatham T, Feangvad S, *et al.:* Polymorphisms in Fas gene is associated with HIV-related lipoatrophy in Thai patients. AIDS Res Hum Retroviruses 2013;29:142–150.
11. Blanco F and Carr A: Lipodystrophy syndrome: Diagnostic, clinic and therapeutic aspects. AIDS Rev 2001;3: 98–105.
12. Purcell S, Neale B, Todd-Brown K, *et al.:* PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575.
13. Takeuchi M, Hata Y, Hirao K, *et al.:* SAPAPs. A family of PSD-95/SAP90-associated proteins localized at postsynaptic density. J Biol Chem 1997;272:11943–11951.
14. Satoh K, Yanai H, Senda T, *et al.:* DAP-1, a novel protein that interacts with the guanylate kinase-like domains of hDLG and PSD-95. Genes Cells 1997;2:415–424.
15. Kim E, Naisbitt S, Hsueh YP, *et al.:* GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules. J. Cell Biol 1997;136:669–678.
16. Kawashima N, Takamiya K, Sun J, *et al.:* Differential expression of isoforms of PSD-95 binding protein (GKAP/SAPAP1) during rat brain development. FEBS Lett 1997; 418:301–304.
17. Naisbitt S, Valtschanoff J, Allison DW, *et al.:* Interaction of the postsynaptic density-95/guanylate kinase domain-associated protein complex with a light chain of myosin-V and dynein. J. Neurosci 2000;20:4524–4534.
18. Aulchenko YS, Pullen J, Kloosterman WP, *et al.:* LPIN2 is associated with type 2 diabetes, glucose metabolism, and body composition. Diabetes 2007;56:3020–3026.
19. Bae JS, Cheong HS, Kim JH, *et al.:* The genetic effect of copy number variations on the risk of type 2 diabetes in a Korean population. PLoS One 2011;6:e19091.

Address correspondence to:
Tatsuo Shioda
Research Institute for Microbial Diseases
Osaka University
3-1 Yamada-oka, Suita
Osaka 565-0871
Japan

E-mail: shioda@biken.osaka-u.ac.jp