

Effects of PPAR γ and RBP4 Gene Variants on Metabolic Syndrome in HIV-Infected Patients with Anti-Retroviral Therapy

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Abstract

Background: PPAR γ and RBP4 are known to regulate lipid and glucose metabolism and insulin resistance. The influences of PPAR γ (C1431T and Pro12Ala) and RBP4 (–803GA) polymorphisms on metabolic syndrome in HIV-infected patients receiving anti-retroviral therapy were examined in this study.

Materials and Methods: A cross-sectional study of HIV-1 infected adults with antiretroviral therapy for more than one year in the National Cheng Kung University Hospital was conducted. The gene polymorphisms were determined by quantitative PCR.

Results: Ninety-one patients were included in the study. Eighty-two (90.1%) patients were males with a mean age of 44.4 years. For the C1431T polymorphism in PPAR γ , while patients with the T allele (48.4%) had trends toward lower rate of hypertriglyceridemia, the borderline significance together with insignificant power did not support the protective effect of the T allele against development of hypertriglyceridemia. For the Pro12Ala polymorphism in PPAR γ , although patients with the *Pro/Ala* genotype (8.8%) had a higher level of serum LDL (138.0 vs. 111.5 mg/dl, $P=0.04$) and trends toward higher rates of hypercholesterolemia and serum LDL>110 mg/dl, these variables were found to be independent of the *Pro/Ala* genotype in the multivariate analysis. For the –803GA polymorphism in RBP4, patients with the A allele (23.1%) more often had insulin resistance (HOMA>3.8; 33.3 vs. 8.7%, $P=0.01$) and more often received anti-hypoglycemic drugs (14.3 vs. 1.4%, $P=0.04$). The detrimental effect of the A allele in RBP4 –803GA polymorphism on development of insulin resistance was supported by the multivariate analysis adjusting for covariates.

Conclusion: The impacts of PPAR γ C1431T and Pro12Ala polymorphisms on metabolism in HIV-infected patients are not significant. RBP4 –803GA polymorphism has increased risk of insulin resistance in HIV-infected patients with anti-retroviral therapy.

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Introduction

The annual, newly reported cases with human immunodeficiency virus (HIV) infection in Taiwan has been increasing since 2003, with an 11% increase in 2003, a 77% increase in 2004 and 123% increase in 2005 [1]. The introduction of highly active antiretroviral therapy (HAART) has dramatically decreased the morbidity and mortality in HIV-infected patients

[2,3]. However, prolonged use of HAART is associated with many metabolic complications, such as lipodystrophy, dyslipidemia and glucose metabolism disorder [4,5]. The prevalence of metabolic syndrome among HIV-infected persons is 26% in the United States, and 81% met at least one of the criteria for the risk of metabolic syndrome regardless of the use of HAART or the type of HAART [6,7]. A similar clinical issue was noted

among HIV-infected patients receiving HAART in Taiwan. A study of 242 Taiwanese receiving HAART reported that 79 (32.6%) had hypertriglyceridemia (>250 mg/dL) [8]. In another study consisting of 877 HIV-infected Taiwanese, 210 (26.2%) had metabolic syndrome, especially in those with HAART [9]. Thus, metabolic complications are an important issue in HIV-infected patients under anti-retroviral therapy. However, not all HIV-infected patients receiving HAART develop metabolic syndrome. Many factors have been shown to contribute to these metabolic alternations, including genetics, cytokines, diet, drinking, gender and age [10].

Peroxisome proliferator-activated receptor γ (PPAR γ), a nuclear receptor, stimulates lipid uptake and adipogenesis by fat cells and is a key regulator of lipid metabolism, adipogenesis and insulin resistance [11]. The actions of PPAR γ are mediated by two protein isoforms, the widely expressed PPAR γ 1 and adipose tissue-restricted PPAR γ 2. Yen *et al.* performed a molecular scanning of human PPAR γ in diabetic Caucasians and identified two variants in the coding region of the gene: Pro12Ala missense (rs1801282) and C1431T silent mutations (rs3856806; also known as His477His or C161T) [12]. Further studies identified that these variants are associated with insulin sensitivity and glucose metabolism in non-HIV population [13,14]. An alanine substitution at position 12, located within the extra N-terminal residues of adipose tissue-restricted PPAR γ 2, leads to a lower DNA-binding affinity and decreased transactivation in *in vitro* studies [13,15]. The C1431T polymorphism is a silent (C>T) substitution at a nucleotide 1431 in exon 6. While the mechanism by which the C1431T mutation in PPAR γ affects its activity remains unclear, it has been suggested that it is in linkage disequilibrium with mutations in other regions of the gene that regulate the activity of PPAR γ [16]. The estimated frequencies of PPAR γ Pro12Ala and C1431T polymorphisms in Chinese are about 7.2% and 44.3% [17].

Retinol-binding protein 4 (RBP4) is an adipocytokine, secreted from adipocytes and released into circulation [18]. Serum RBP4 levels are elevated in insulin-resistant mice and humans with obesity and type 2 diabetes, and are normalized by rosiglitazone, a PPAR γ agonist [19]. Experiments in mice indicated that elevated RBP4 levels cause insulin resistance, suggesting that the improvement of glucose metabolism by PPAR γ activation may be through the reduction of circulating RBP4 levels [20]. Furthermore, RBP4 -803GA polymorphism (rs3758539), located at 5' upstream of the translational start site within a putative enhancer region, is associated with an increased risk of type 2 diabetes in non HIV-infected patients [21]. *In vitro* studies showed that -803 A allele induces greater transcriptional activity and higher binding affinity with the transcription factor hepatocyte nuclear factor 1 alpha (HNF1 α) than the G allele, which may cause increased serum RBP4 levels in diabetic patients [22,23]. Antiretroviral therapy in HIV-infected patients has been reported to induce a pronounced increase of plasma RBP4 [18], which is associated with obesity, insulin resistance and dyslipidemia [24]. However, the relationship between PPAR γ or RBP4 polymorphism and insulin resistance or dyslipidemia in HIV-infected patients receiving HAART remains unclear. The objective of the present study is to investigate the effect of PPAR γ and RBP4 polymorphisms on body mass, insulin resistance and dyslipidemia among HIV-infected patients with anti-retroviral therapy after adjusting other risk factors, including influence of anti-retroviral regimen, diet and drinking.

Materials and Methods

A cross-sectional study of HIV-1 infected patients in the National Cheng Kong University Hospital was conducted. HIV-infected patients aged 18 years or older with regular outpatient follow-ups and antiretroviral therapy (efavirenz or lopinavir/ritonavir plus two nucleoside reverse transcriptase inhibitors [NRTIs]) for at least one year were included. Patients, who had diabetes mellitus (DM), dyslipidemia or lipodystrophy before the clinical diagnosis of HIV infection, or had active opportunistic infections, were excluded.

Demographic data, duration of HIV disease, regimen and duration of HAART were collected. Body height, body weight, waist and hip circumference, and blood pressure were measured at outpatient visits. The waist circumference was measured at the midpoint of the rib margin and the iliac crest at the end of expiration, and the hip circumference was measured at the greatest protuberance of the buttocks [25]. Central obesity was defined as waist >90 cm for men and >80 cm for women according to the Bureau of Health Promotion, Department of Health, Taiwan. The diet condition and habitual of the patients were assessed by a modified questionnaire [26,27]. The nutrition content of the diet was analyzed. Daily metabolic rate and total energy consumption in calories (including resting energy expenditure and total energy expenditure [TEE]) were calculated from age, gender, weight and height of the patients [26]. Patients who ate more than TEE were regarded as intake over-TEE, otherwise under-TEE.

Overnight fasting blood samples were taken for the measurement of serum concentrations of glucose, insulin, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol. Dyslipidemia was defined as either of serum cholesterol >200 mg/dl, triglyceride >150 mg/dl, LDL >110 mg/dl, or HDL <40 mg/dl for men and <50 mg/dl for women [28,29]. Insulin resistance was estimated by the model of homeostasis model assessment (HOMA) [5,17], which was derived from fasting insulin (μ U/ml) \times fasting glucose (mmol/l)/22.5. A HOMA score higher than 3.8 was regarded to have insulin resistance [17,30]. For patients starting current anti-retroviral therapy regimen after January 2005, serum lipid profiles at less than 3 months, 6~9 months, 12~15 months, 18~21 months, 24~27 months, and 30~33 months were recorded.

Patients were solicited about their average amount of alcohol consumption per week. A drink was defined as one can, bottle, or glass of beer, a glass of wine, a shot of liquor, a mixed drink with that amount of liquor, or any other kind of alcoholic beverage [31]. A patient who consumed more than seven drinks per week was considered to be a hazardous alcohol drinker [31,32].

Human DNA was extracted using a kit (Geneaid Genomic DNA Mini Kit) according to the manufacturer's instructions. Pro12Ala and C1431T polymorphisms of PPAR γ and -803GA polymorphism of RBP4 were examined by real-time quantitative PCR (Applied Biosystems) using TaqMan Pre-Designed SNP Genotyping Assays.

Statistical analysis was performed with statistical software (SPSS, version 13.0). Continuous data were expressed as means \pm standard deviations. The χ^2 test with Yates' correction or Fisher's exact test was used for comparing categorical variables and the independent *t*-test was used for comparing continuous variables between two groups. A two-tailed *P* value less than 0.05 was considered to be statistically significant. Multivariate analysis was performed by binary logistic regression model. Variables of multivariate analysis include gender, age, C1430T polymorphism, P12A polymorphism, RBP4 polymorphism, hazardous drinking,

HCV co-infection, calorie over-TEE, and efavirenz use, which have been shown to influence metabolic syndrome in HIV-infected patients in previous studies [25,32,33,34]. The deviation from Hardy-Weinberg equilibrium for genotypes was tested by the χ^2 test. The mixed effect model was used to examine the difference in serum triglyceride and cholesterol between patients with different gene variants over time. Bonferroni correction for multiple testing was applied. We performed post hoc analysis and statistical power test to exam the differences between pairs of groups after the global analysis.

Results

There were 312 patients who had received two NRTIs plus efavirenz (a non-nucleoside reverse transcriptase inhibitor (NNRTI)) or lopinavir/ritonavir (a combination of protease inhibitor (PI)) at the National Cheng Kung University Hospital between Oct. 2000 and Jul. 2008. Among these patients, 114 patients fulfilling the inclusion criteria were evaluated. However, 23 patients declined to participate in the study resulting in 91 patients joining the study and their blood samples were collected for genetic polymorphism analyses. Of these 91 patients, 82 (90.1%) were males with a mean age of 44.4 years (Table 1). Mean duration of HIV infection was 70.2 months. Main risk factors for HIV infection were for men having sex with men in 6 (6.6%) patients and intravenous drug use in 33 (36.3%). The others were heterosexual or unknown. Mean duration of HAART was 40.6 months. Mean CD4⁺ cell count was 520.7 cells/mm³. As for HAART, 43 (47.3%) patients were treated by efavirenz plus two NRTIs and 48 (52.7%) by lopinavir/ritonavir plus two NRTIs. Patients with efavirenz therapy, than those with lopinavir/ritonavir-based regimens, had higher serum levels of fasting glucose (106.1 vs. 90.7 mg/dl, $P=0.01$) and LDL (124.6 vs. 104.1 mg/dl, $P<0.01$), HOMA index (2.6 vs. 1.7, $P=0.02$), but lower serum levels of uric acid (5.6 vs. 6.2 mg/dl, $P=0.03$), and more often had hypercholesterolemia (cholesterol >200 mg/dl; 67.4 vs. 37.5%, $P=0.01$) and serum LDL>110 mg/dl (72.1 vs. 41.7%, $P=0.01$) (Table 2).

For the C1431T polymorphism in PPAR γ , 47 (51.6%) patients were the CC genotype, 41 (45.1%) CT genotype, and 3 (3.3%) TT genotype. Allele frequency for the C allele was 0.74 and T allele 0.26. The P value of χ^2 test was 0.09 and such a result was consistent with the Hardy-Weinberg equilibrium. There was no discernible difference in the prevalence of smoking, hazardous drinking, presumed routes of HIV infection, HCV co-infection, duration of HIV infection, CD4⁺ cell counts, or HAART regimen (NNRTI or PI use) between patients with the T allele (CT+TT genotype) and without the T allele (CC genotype)(data not shown). No difference in BMI, waist circumference, systolic and diastolic blood pressure, fasting glucose and insulin, HOMA index, serum cholesterol, LDL, HDL and anti-dyslipidemic therapy was detectable between patients with and without the T allele (Table 3 and data not shown). Patients with the T allele had a trend toward lower rate of hypertriglyceridemia (triglyceride >150 mg/dl; 65.9 vs. 85.1%, $P=0.06$; $\alpha=0.05$; statistical power = 0.57 in post hoc analysis) and had lower levels of serum uric acid (5.5 vs. 6.3 mg/dl, $P=0.01$) than those without the T allele. While the multivariate analysis supported the protective effect of the T allele against development of hypertriglyceridemia (odds ratio [OR] 0.282, 95% confidence interval [CI] 0.087~0.921, $P=0.04$) (Table 4), there was no statistical significance under Bonferroni correction for multiple testing. For 46 patients with current anti-retroviral therapy after January 2005, their longitudinal lipid profiles were recorded. Serum triglyceride

levels in patients with the T allele were significantly lower than those without the T allele at several time points after antiretroviral therapy (Figure 1). The effect is of statistical significance in serum triglyceride in patients with the T allele over time using the mixed effect model ($P=0.006$, statistical power = 0.79). Although the differences of fasting insulin and HOMA index between patients with and without the T allele did not reach statistical significance, there were trends toward a lower fasting insulin level (7.5 vs. 10.3 mg/dl; $P=0.07$) and less insulin resistance (HOMA index >3.8; 6.8 vs. 21.7%; $P=0.09$) in those with the T allele.

For the Pro12Ala polymorphism in PPAR γ , 83 (91.2%) patients belong to the *Pro/Pro* genotype, and 8 (8.8%) the *Pro/Ala* genotype. No patient with the *Ala/Ala* genotype was identified. Allele frequency for the *Pro* allele was 0.96 and *Ala* allele 0.04. The P value of χ^2 test for the Hardy-Weinberg equilibrium was 0.66. There was no difference in the prevalence of smoking, hazardous drinking, risk factors of HIV infection, HCV co-infection, duration of HIV infection, CD4⁺ cell counts, or HAART regimen between patients with the *Pro/Pro* genotype and with the *Pro/Ala* genotype (data not shown). For the metabolic profiles, such as BMI, waist circumference, blood pressure, fasting glucose and insulin, serum triglyceride, uric acid, and HDL, no difference was noted between two genotypes (Table 3 and data not shown). However, patients with the *Pro/Ala* genotype had a higher level of serum LDL (138.0 vs. 111.5 mg/dl, $P=0.04$), and trends toward higher rates of hypercholesterolemia (87.5 vs. 48.2%, $P=0.06$) and serum LDL>110 mg/dl (87.5 vs. 53.0%, $P=0.07$) than those with the *Pro/Pro* genotype. The variables of hypercholesterolemia (OR: 7.373, 95% CI: 0.667~81.524, $P=0.10$) and serum LDL>110 mg/dl (OR: 6.946, 95% CI: 0.623~77.481, $P=0.12$) were found to be independent of the *Pro/Ala* genotype in the multivariate analysis (Table 4 and data not shown). Longitudinal profiles of serum cholesterol in 46 patients with antiretroviral therapy showed significant higher serum cholesterol levels in patients with the *Pro/Ala* genotype than those with the *Pro/Pro* genotype at 12~15 months, and trends toward higher serum cholesterol levels at 6~9 and 24~27 months (Figure 2). There was marginal statistical significance in serum cholesterol in patients with the *Pro/Ala* genotype over time using the mixed effect model ($P=0.04$, statistical power = 0.54).

For the -803GA polymorphism in RBP4, 70 (76.9%) patients belong to the GG genotype, 19 (20.9%) GA genotype, and 2 (2.2%) AA genotype. Allele frequency for the G allele was 0.87 and A allele 0.13. The P value of χ^2 test for the Hardy-Weinberg equilibrium was 0.60. Patients with the A allele (GA+AA genotype) more often had insulin resistance (33.3 vs. 8.7%, $P=0.01$; $\alpha=0.05$; statistical power = 0.72 in post hoc analysis) and more often had treated for DM (14.3 vs. 1.4%, $P=0.04$) than those without the A allele (GG genotype) (Table 3). Furthermore, the multivariate analysis supported the A allele as a predictor of insulin resistance with an odds ratio of 8.8 (95% CI, 1.6~49.1, $P=0.01$) (Table 4). It is of statistical significance under Bonferroni correction for multiple testing ($P<0.017$).

Discussion

In this study, we investigated the effect of PPAR γ and RBP4 polymorphisms on body mass, insulin resistance and dyslipidemia among HIV-infected patients with anti-retroviral therapy (HAART). Our results showed that the T allele of PPAR γ C1431T polymorphism was marginally associated with a lower rate of hypertriglyceridemia in HIV-infected patients receiving anti-retroviral therapy. However, the borderline significance together with insignificant power did not support the protective

Table 1. Baseline clinical characteristics of 91 HIV-infected patients receiving lopinavir/ritonavir - or efavirenz-based antiretroviral therapy.

Clinical characters	Total N = 91	Lopinavir/ritonavir N = 48	Efavirenz N = 43	P value
Male gender	82 (90.1)	42 (87.5)	40 (93.0)	0.49
Age, years	44.4 \pm 13.1	43.1 \pm 13.0	45.9 \pm 13.3	0.31
Hazardous drinking	8 (8.8)	4 (8.3)	4 (9.3)	1.00
Smoking	38 (41.8)	22 (45.8)	16 (37.2)	0.54
Family history of DM	34 (37.4)	16 (33.3)	18 (41.9)	0.53
Risk factor of HIV infection	6 (6.6)	4 (8.4)	2 (4.7)	0.36
Intravenous drug abuse	33 (36.3)	20 (41.7)	13 (30.2)	
Men having sex with men				
Recognized duration of HIV infection, months	70.2 \pm 49.1	79.3 \pm 55.3	59.7 \pm 38.6	0.06
Duration of antiretroviral therapy, months	40.6 \pm 25.9	35.2 \pm 23.3	46.7 \pm 27.6	0.03
CD4 ⁺ count at enrollment, cells/mm ³	520.7 \pm 226.1	516.8 \pm 220.5	525.1 \pm 235.0	0.86
Daily calorie intake, kcal (88 [*])	1625.8 \pm 412.9	1645.7 \pm 396.7	1755.5 \pm 462.0	0.24
Intake over-TEE, %	30 (33.0)	15 (31.9)	15 (36.6)	0.76
Fat composition, %	36.1 \pm 9.7	36.3 \pm 9.3	36.0 \pm 10.2	0.94
Body mass index (BMI), kg/m ²	22.8 \pm 3.4	22.8 \pm 3.5	22.9 \pm 3.4	0.88
BMI >24 kg/m ²	31 (34.1)	17 (35.4)	14 (32.6)	0.95
Waist circumference, cm	78.9 \pm 9.7	79.1 \pm 7.5	78.8 \pm 11.8	0.90
Waist >90 (men) or >80 cm (women)	11 (12.1)	8 (16.7)	3 (7.0)	0.27
Ratio of waist/hip circumference	0.90 \pm 0.04	0.90 \pm 0.04	0.90 \pm 0.04	0.89
Uric acid, mg/dl	5.9 \pm 1.3	6.2 \pm 1.3	5.6 \pm 1.3	0.03
ALT, IU/ml	32.9 \pm 29.8	25.2 \pm 16.0	41.3 \pm 38.2	0.01

Data are expressed as numbers (percentages) or mean values \pm standard deviations.

TEE = total energy expenditure; ALT = alanine transaminase.

*The number of patients with indicated information.

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Table 2. Genetic and metabolic characteristics of 91 HIV-infected patients receiving lopinavir/ritonavir- or efavirenz-based antiretroviral therapy.

Characters	Total N = 91	Lopinavir/ritonavir N = 48	Efavirenz N = 43	P value
C1431T polymorphism, TC+TT genotype	44 (48.4)	23 (47.9)	21 (48.8)	1.00
P12A polymorphism, Pro/Ala genotype	8 (8.8)	4 (8.3)	4 (9.3)	1.00
RBP4 polymorphism, GA+AA genotype	21 (23.1)	11 (22.9)	10 (23.3)	1.00
Receipt of anti-hypoglycemic drugs	4 (4.4)	1 (2.1)	3 (7.0)	0.34
Fasting glucose, mg/dl	98.0 \pm 24.5	90.7\pm9.0	106.1\pm32.7	0.01
Fasting insulin, IU/ml	8.9 \pm 7.4	8.0 \pm 6.4	9.9 \pm 8.4	0.21
HOMA index	2.2 \pm 1.9	1.7\pm1.3	2.6\pm2.2	0.02
HOMA index >3.8	13 (14.3)	4 (8.5)	9 (20.9)	0.17
Receipt of anti-dyslipidemic drugs	35 (38.5)	18 (37.5)	17 (39.5)	1.00
Cholesterol, mg/dl	205.9 \pm 42.6	198.3 \pm 49.3	214.2 \pm 41.5	0.10
Cholesterol >200 mg/dl	47 (51.6)	18 (37.5)	29 (67.4)	0.01
LDL cholesterol, mg/dl	113.8 \pm 34.1	104.1\pm36.5	124.6\pm27.7	<0.01
LDL cholesterol >110 mg/dl	51 (56.0)	20 (41.7)	31 (72.1)	0.01
HDL cholesterol, mg/dl	45.4 \pm 11.7	44.7 \pm 12.2	46.1 \pm 11.1	0.57
HDL <40 (men) or <50 mg/dl (women)	27 (29.7)	17 (35.4)	10 (23.3)	0.30
Cholesterol/HDL ratio	4.9 \pm 1.9	4.8 \pm 2.3	4.9 \pm 1.5	0.94
Triglyceride, mg/dl	327.1 \pm 359.4	379.4 \pm 454.2	268.7 \pm 198.5	0.13
Triglyceride >150 mg/dl	69 (75.8)	38 (79.2)	31 (72.1)	0.59

LDL = low-density lipoprotein; HDL = high-density lipoprotein.

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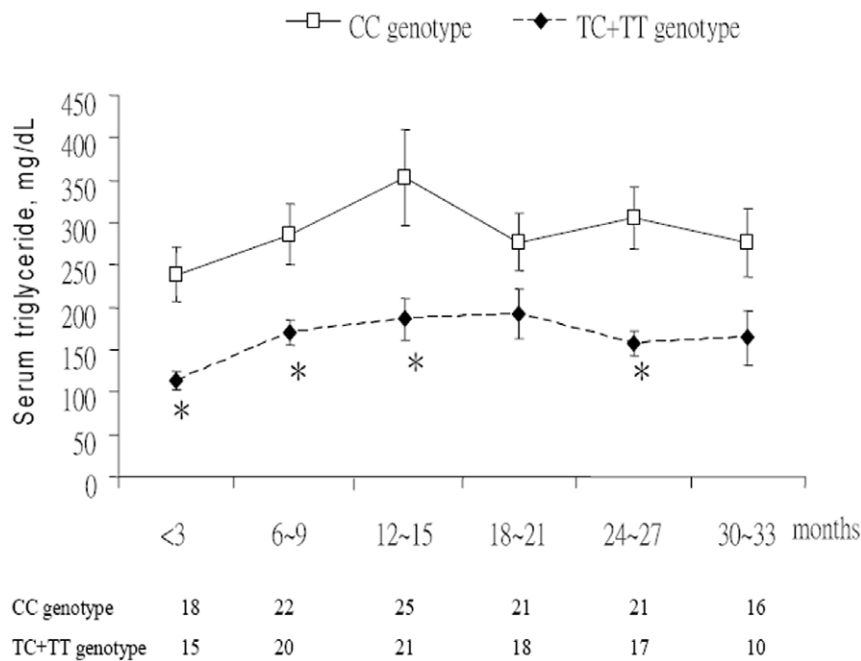


Figure 1. The influence of PPAR γ C1431T polymorphism on serum triglyceride levels in HIV-infected patients after anti-retroviral therapy. The numbers below the months are the patient numbers. * $P < 0.05$ for comparing serum triglyceride levels between patients with and without the T allele. $P = 0.006$ for difference in serum triglyceride levels over time in the mixed effect model. doi:10.1371/journal.pone.0049102.g001

Table 3. PPAR γ (C1431T and Pro12Ala) and RBP4 (–803GA) polymorphisms and metabolic syndrome among 91 HIV-infected patients.

Characters	PPAR γ						RBP4		
	C1431T (rs3856806)			Pro12Ala (rs1801282)			–803GA (rs3758539)		
	CC N = 47 (51.6%)	TC+TT N = 44 (48.4%)	<i>P</i> value	Pro/Pro N = 83 (91.2%)	Pro/Ala N = 8 (8.8%)	<i>P</i> value	GG N = 70 (76.9%)	GA+AA N = 21 (23.1%)	<i>P</i> value
Fasting glucose, mg/dl	95.0 \pm 16.5	101.2 \pm 30.7	0.23	98.1 \pm 25.3	96.4 \pm 15.1	0.85	95.0 \pm 11.2	107.8 \pm 46.2	0.22
Fasting insulin, IU/ml	10.3 \pm 9.3	7.5 \pm 4.6	0.07	8.9 \pm 7.8	8.6 \pm 2.1	0.90	8.5 \pm 7.2	10.3 \pm 8.1	0.31
HOMA index	2.4 \pm 2.1	2.0 \pm 1.6	0.29	2.2 \pm 1.9	2.1 \pm 0.8	0.89	2.0 \pm 1.6	2.8 \pm 2.5	0.15
HOMA index >3.8	10 (21.7)	3 (6.8)	0.09	13 (15.9)	0 (0)	0.60	6 (8.7)	7 (33.3)	0.01
Receipt of anti-hyperglycemic drugs	1 (2.1)	3 (6.8)	0.35	4 (4.8)	0 (0)	1.00	1 (1.4)	3 (14.3)	0.04
Cholesterol, mg/dl	206.7 \pm 44.8	204.9 \pm 48.3	0.85	204.2 \pm 47.3	223.1 \pm 30.3	0.27	206.3 \pm 46.6	204.3 \pm 46.0	0.87
Cholesterol >200 mg/dl	23 (48.9)	24 (54.5)	0.75	40 (48.2)	7 (87.5)	0.06	38 (54.3)	9 (42.9)	0.50
LDL cholesterol, mg/dl	113.9 \pm 34.4	113.7 \pm 34.1	0.98	111.5\pm34.4	138.0\pm18.5	0.04	111.9 \pm 34.7	120.2 \pm 31.7	0.33
LDL cholesterol >110 mg/dl	27 (57.4)	24 (54.5)	0.95	44 (53.0)	7 (87.5)	0.07	39 (55.7)	12 (57.1)	1.00
HDL cholesterol, mg/dl	44.7 \pm 11.4	46.1 \pm 12.1	0.57	45.3 \pm 11.7	46.3 \pm 11.8	0.83	46.0 \pm 12.1	43.4 \pm 10.0	0.39
HDL <40 (men) or <50 mg/dl (women)	13 (27.7)	14 (31.8)	0.84	25 (30.1)	2 (25.0)	1.00	21 (30.0)	6 (28.6)	1.00
Triglyceride, mg/dl	355.9 \pm 421.1	296.3 \pm 280.7	0.43	335.2 \pm 374.1	243.3 \pm 113.4	0.49	335.8 \pm 400.0	298.1 \pm 168.3	0.68
Triglyceride >150 mg/dl	40 (85.1)	29 (65.9)	0.06	63 (75.9)	6 (75.0)	1.00	51 (72.9)	18 (85.7)	0.36
Receipt of anti-dyslipidemic drugs	16 (34.0)	19 (43.2)	0.50	31 (37.3)	4 (50.0)	0.48	26 (37.1)	9 (42.9)	0.83
Uric acid, mg/dl	6.3\pm1.3	5.5\pm1.3	0.01	5.9 \pm 1.4	6.3 \pm 1.0	0.46	6.0 \pm 1.4	5.6 \pm 1.2	0.19

Anti-hyperglycemic or anti-dyslipidemic medications use is expressed as number (percentage within each group); others are expressed as means \pm standard deviations. doi:10.1371/journal.pone.0049102.t003

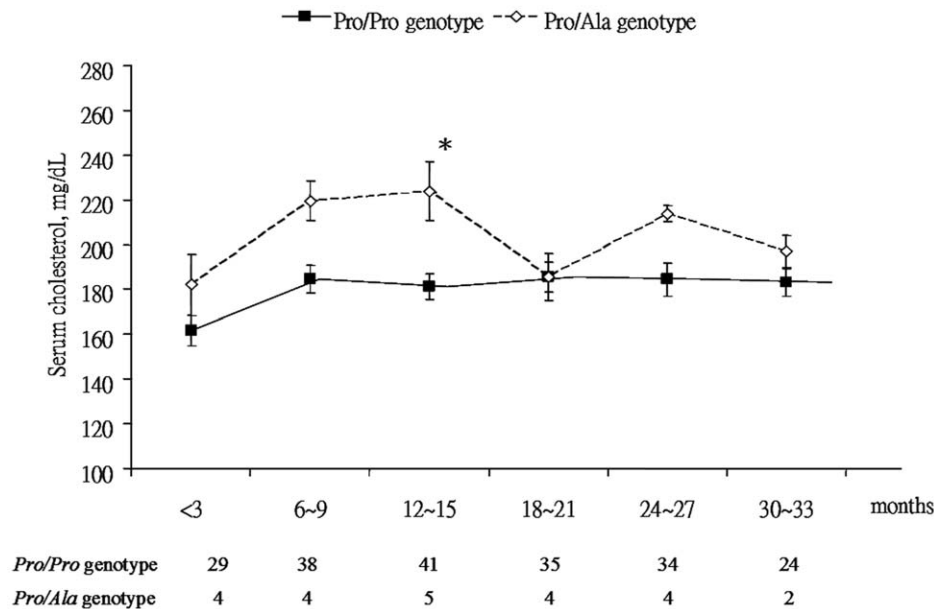


Figure 2. The influence of PPAR γ Pro12Ala polymorphism on serum cholesterol levels in HIV-infected patients after anti-retroviral therapy. The numbers below the months are the patient numbers. * $P < 0.05$ for comparing serum cholesterol levels between patients with and without the *Ala* allele. $P = 0.04$ for difference in serum cholesterol levels over time in the mixed effect model. doi:10.1371/journal.pone.0049102.g002

effect of the T allele against development of hypertriglyceridemia. No dramatic impact of the *Ala* allele of PPAR γ Pro12Ala polymorphism on the metabolic parameters we examined was identified. Finally, the A allele of RBP4 -803GA polymorphism was associated with a higher rate of insulin resistance in HIV-infected patients receiving anti-retroviral therapy.

HIV-infected patients with lipodystrophy had been found to have a lower PPAR γ level in the subcutaneous adipose tissue, and a higher waist-to-hip ratio, higher serum insulin and triglyceride levels [17]. Although genetic variants of PPAR γ have been associated with insulin sensitivity and glucose metabolism in non HIV-infected people, the influence of PPAR γ polymorphism on the metabolism in HIV-infected patients receiving HAART was not conclusive in previous studies [13,14,35]. The inconsistency of PPAR γ genetic variation on the metabolism can stem from the influences of diet, drinking, or antiretroviral therapy in each HIV-infected individual. For example, increased fat intake is associated with a higher serum triglyceride level in HIV-infected patients [25,36]. Similarly, hazardous alcohol consumption is associated with several metabolic consequences in HIV-infected patients, such as lipodystrophy and the increases in cholesterol, triglyceride

and LDL [32,33]. To our knowledge, our study is the first one to assess the influence of genetic factor of PPAR γ on metabolism after multivariate analyses adjusting for different factors, such as diet, drinking, and antiretroviral regimens, on BMI, waist circumference, insulin resistance and dyslipidemia of HIV-infected patients.

Although C1431T is a silent polymorphism, it was found to be a better predictor of fasting insulin levels and insulin sensitivity than Pro12Ala, suggesting that the C1431T polymorphism may be in tight linkage disequilibrium with a functional variant in PPAR γ or nearby gene [37,38]. While, in non HIV-infected patients, the effects of PPAR γ C1431T polymorphism on metabolic syndrome varied in different studies, the majority of them indicated the association of C1431T polymorphism with obesity and hyperglycemia. For example, in a Finnish study, obese women with the TT genotype had an increased BMI and waist circumference [39]. Similarly, in Chinese population, the CT and TT genotypes in C1431T polymorphism have been associated with a higher fasting blood sugar in patients with metabolic syndrome [35]. However, in an Asian population, the CT and TT genotypes had a lower risk of diabetes, but a higher BMI than those with the CC genotype

Table 4. Multivariate analysis of factors associated with serum triglyceride >150 mg/dl, serum cholesterol >200 mg/dl, or HOMA index >3.8.

Genetic polymorphisms	triglyceride >150 mg/dl			cholesterol >200 mg/dl			HOMA index >3.8		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
PPAR γ C1430T (TC+TT)	0.282	0.087~0.921	0.04	1.360	0.472~3.920	0.57	0.272	0.049~1.517	0.14
PPAR γ Pro12Ala (<i>Pro/Ala</i>)	0.539	0.081~3.583	0.52	7.373	0.667~81.524	0.10	0.751	0.112~5.063	0.77
RBP4 -803GA (GA+AA)	1.934	0.373~10.027	0.43	0.943	0.255~3.491	0.93	8.788	1.573~49.087	0.01

Variables of multivariate analysis include gender, age, C1430T polymorphism, P12A polymorphism, RBP4 polymorphism, hazardous drinking, HCV co-infection, calorie over-TEE, and efavirenz use. CI denotes confidence interval.

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[14]. In HIV-infected patients, the association of C1431T polymorphism with metabolic syndrome is not well documented. For example, Nazih *et al.* found there was no convincing association between the His449His (equal to C1431T) polymorphism and individual components of the metabolic syndrome [40]. In the study by Zanone *et al.*, the C161T (equal to C1431T) polymorphism had no influence on the presence of atrophy and fat accumulation in individuals with HIV-related lipodystrophy [41]. In our study, we found that the T allele of PPAR γ C1431T polymorphism was marginally associated with a lower rate of hypertriglyceridemia in HIV-infected patients receiving antiretroviral therapy in univariate and multivariate analyses. However, it did not reach a desirable power in the post hoc analysis and statistical significance after the correction for multiple testing. Finally, the statistical significance (0.006) with a nearly acceptable power (0.79) indicates that the T allele carriers have lower serum triglyceride levels at several time points of the longitudinal follow-up. There are many reasons to reduce the power of mixed models. For example: smaller sample size, lower number of repeated measures, higher between-subject variance, higher intraclass correlation, and smaller difference between two groups, and so on. Thus, the effect of PPAR γ C1431T polymorphism on serum triglyceride in HIV-infected patients is not significant and requires further large scale study.

The Pro12Ala polymorphism in PPAR γ represents the first genetic variant with a broad impact on the risk and complications of type 2 diabetes. *In vitro* studies showed that Ala variant exhibited moderate reduction of target gene transactivation due to decreased DNA binding capacity [13]. The effect of the Ala carriage is not conclusive in the literature both in non HIV-infected and in HIV-infected patients. While most studies in non HIV-infected patients found that the Ala carrier exerted a protective effect from development of type 2 diabetes, insulin resistance or obesity, some studies found it to have a deleterious effect [14,42,43,44]. In Chinese population, the Ala carrier was associated with a higher fasting level of blood sugar in patients with the metabolic syndrome [35]. Similarly, the influence of the Ala carriage on metabolism in HIV-infected patients is not evident. For example, in the study by Saumoy *et al.*, the Pro12Ala polymorphism had no effect on the risk of developing lipodystrophy in HIV-1-infected patients treated with HAART [45]. In the study by Nazih *et al.*, there was no convincing association between the Pro12Ala polymorphism and individual components of the metabolic syndrome, except for the association of the Pro12Ala polymorphism with diabetes in HIV-infected patients [40]. Thus, these suggest that Pro12Ala polymorphism is unlikely to have a significant impact on the metabolic syndrome in HIV-infected patients. Consistently, our study showed that the Ala carriers of Pro12Ala polymorphism in PPAR γ were not associated with serum levels of cholesterol and LDL in HIV-infected patients. While the Ala carriers had higher serum cholesterol levels at several time points of the longitudinal follow-up, the borderline significance (0.04) together with insignificant power (0.54) makes the results inapplicable at a population level. We noticed relatively few Ala carriers in our study, which may be related to the insignificant impact of Ala carriage on lipid metabolism in our findings. Thus further studies with more patients are warranted to reveal the impact of Pro12Ala polymorphism on metabolism in HIV-infected patients.

Because the $-803GA$ polymorphism is located in the enhancer region, the $G>A$ substitution alters transcriptional efficiency and affinity of the enhancer sequence for the transcription factor

HNF1 α [46]. The A allele of RBP4 $-803GA$ polymorphism was linked to an increased risk of type 2 diabetes in non HIV-infected population [21]. In HIV-infected patients receiving HAART, the serum RBP4 level has been positively correlated with obesity, insulin resistance and dyslipidemia [24]. However, the role of the A allele of RBP4 $-803GA$ polymorphism in HIV-infected patients is still unknown. Our study revealed that the A allele of RBP4 $-803GA$ polymorphism was associated with insulin resistance in HIV-infected patients receiving HAART. This provides us another potential surrogate marker of insulin resistance in HIV-infected patients receiving antiretroviral therapy.

It has long been noted that PI treatment was associated with dyslipidemia, particularly hypertriglyceridemia [47]. The class of NNRTI, in contrast, induces dyslipidemia to a lesser degree than PI. However, NNRTI-related dyslipidemia can still be found in several studies [48,49,50]. The effect of NNRTI or PI therapy on the lipid profile of HIV-infected patients in Asia is not clear. In our study, patients with efavirenz therapy had higher serum levels of fasting glucose, LDL, and HOMA index, lower serum levels of uric acid, and more often had hypercholesterolemia and LDL >110 mg/dl, than those with lopinavir/ritonavir-based regimens. These results suggest that efavirenz may be associated with more metabolic side effects than lopinavir/ritonavir in Asian population. Thus, further randomized, double-blind trials will be required to clarify the effect of efavirenz or lopinavir/ritonavir on the metabolic profile in an Asian population.

There are several limitations in our study. First, the case number is relatively small in our study. Many effects of exposure may not exhibit a logistic difference and dose-response effect in our study. Second, exercise habits, which may influence BMI, insulin resistance or lipid profiles of our patients, were not recorded. Third, our study is primarily a cross-sectional study, and some longitudinal effects may not be observed in this setting. Fourth, we did not measure serum levels of RBP4 in these patients due to no permission from the Institutional Review Board and no available stored serum samples. However, it is the first study revealing the different effects of PPAR γ and RBP4 polymorphisms on the metabolic syndrome after multivariate analysis adjusting for anti-retroviral drug, diet and drinking in HIV-infected patients receiving anti-retroviral therapy.

In conclusion, the A allele of $-803GA$ polymorphism in RBP4 is associated with a higher rate of insulin resistance. These results suggest that certain genetic factors can affect the metabolic syndrome in HIV-infected patients receiving anti-retroviral therapy. Identification of the individuals with unfavorable genotypes may be helpful to select more appropriate drugs to minimize the risk of metabolic syndrome. Moreover, the knowledge of function alterations of the vulnerable genes could be used as the surrogates of therapeutic targets in the future.

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Author Contributions

Conceived and designed the experiments: YPH YST WCK. Performed the experiments: YPH NYL HCC CJW CMC PLC. Analyzed the data: HJL YHW PJT SHL. Contributed reagents/materials/analysis tools: HJL YHW PJT. Wrote the paper: YPH YST WCK.

References

- Chen YM, Kuo SH (2007) HIV-1 in Taiwan. *Lancet* 369: 623–625.
- Michaels SH, Clark R, Kissinger P (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N Engl J Med* 339: 405–406.
- Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, et al. (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 338: 853–860.
- Behrens GM, Stoll M, Schmidt RE (2000) Lipodystrophy syndrome in HIV infection: what is it, what causes it and how can it be managed? *Drug Saf* 23: 57–76.
- Palacios R, Merchante N, Macias J, Gonzalez M, Castillo J, et al. (2006) Incidence of and risk factors for insulin resistance in treatment-naïve HIV-infected patients 48 weeks after starting highly active antiretroviral therapy. *Antivir Ther* 11: 529–535.
- Mondy K, Overton ET, Grubb J, Tong S, Seyfried W, et al. (2007) Metabolic syndrome in HIV-infected patients from an urban, midwestern US outpatient population. *Clin Infect Dis* 44: 726–734.
- Jacobson DL, Tang AM, Spiegelman D, Thomas AM, Skinner S, et al. (2006) Incidence of metabolic syndrome in a cohort of HIV-infected adults and prevalence relative to the US population (National Health and Nutrition Examination Survey). *J Acquir Immune Defic Syndr* 43: 458–466.
- Chang SY, Ko WS, Kao JT, Chang LY, Sun HY, et al. (2009) Association of single-nucleotide polymorphism 3 and c.553G>T of APOA5 with hypertriglyceridemia after treatment with highly active antiretroviral therapy containing protease inhibitors in hiv-infected individuals in Taiwan. *Clin Infect Dis* 48: 832–835.
- Wu PY, Hung CC, Liu WC, Hsieh CY, Sun HY, et al. (2012) Metabolic syndrome among HIV-infected Taiwanese patients in the era of highly active antiretroviral therapy: prevalence and associated factors. *J Antimicrob Chemother* 67: 1001–1009.
- Rosenkranz SL, Yarasheski KE, Para MF, Reichman RC, Morse GD (2007) Antiretroviral Drug Levels and Interactions Affect Lipid, Lipoprotein, and Glucose Metabolism in HIV-1 Seronegative Subjects: A Pharmacokinetic-Pharmacodynamic Analysis. *Metab Syndr Relat Disord* 5: 163–173.
- Tsai YS, Maeda N (2005) PPARgamma: a critical determinant of body fat distribution in humans and mice. *Trends Cardiovasc Med* 15: 81–85.
- Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, et al. (1997) Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 241: 270–274.
- Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, et al. (1998) A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20: 284–287.
- Tai ES, Corella D, Deurenberg-Yap M, Adiconis X, Chew SK, et al. (2004) Differential effects of the C1431T and Pro12Ala PPARgamma gene variants on plasma lipids and diabetes risk in an Asian population. *J Lipid Res* 45: 674–685.
- Masugi J, Tamori Y, Mori H, Koike T, Kasuga M (2000) Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-gamma 2 on thiazolidinedione-induced adipogenesis. *Biochem Biophys Res Commun* 268: 178–182.
- Knouff C, Auwerx J (2004) Peroxisome proliferator-activated receptor-gamma calls for activation in moderation: lessons from genetics and pharmacology. *Endocr Rev* 25: 899–918.
- Kannisto K, Sutinen J, Korshennikova E, Fisher RM, Ehrenborg E, et al. (2003) Expression of adipogenic transcription factors, peroxisome proliferator-activated receptor gamma co-activator 1, IL-6 and CD45 in subcutaneous adipose tissue in lipodystrophy associated with highly active antiretroviral therapy. *AIDS* 17: 1753–1762.
- Schindler K, Haider D, Wolz M, Rieger A, Gmeinhardt B, et al. (2006) Impact of antiretroviral therapy on visfatin and retinol-binding protein 4 in HIV-infected subjects. *Eur J Clin Invest* 36: 640–646.
- Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, et al. (2005) Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436: 356–362.
- Yang B, Lin P, Carrick KM, McNulty JA, Clifton LG, et al. (2005) PPARgamma agonists diminish serum VEGF elevation in diet-induced insulin resistant SD rats and ZDF rats. *Biochem Biophys Res Commun* 334: 176–182.
- van Hoek M, Dehghan A, Zillikens MC, Hofman A, Witteman JC, et al. (2008) An RBP4 promoter polymorphism increases risk of type 2 diabetes. *Diabetologia* 51: 1423–1428.
- Munkhtulga L, Nakayama K, Utsumi N, Yanagisawa Y, Gotoh T, et al. (2007) Identification of a regulatory SNP in the retinol binding protein 4 gene associated with type 2 diabetes in Mongolia. *Hum Genet* 120: 879–888.
- Kovacs P, Geyer M, Berndt J, Klötting N, Graham TE, et al. (2007) Effects of genetic variation in the human retinol binding protein-4 gene (RBP4) on insulin resistance and fat depot-specific mRNA expression. *Diabetes* 56: 3095–3100.
- Han SH, Chin BS, Lee HS, Jeong SJ, Choi HK, et al. (2009) Serum retinol-binding protein 4 correlates with obesity, insulin resistance, and dyslipidemia in HIV-infected subjects receiving highly active antiretroviral therapy. *Metabolism* 58: 1523–1529.
- Shah M, Tierney K, Adams-Huet B, Boonyavarakul A, Jacob K, et al. (2005) The role of diet, exercise and smoking in dyslipidaemia in HIV-infected patients with lipodystrophy. *HIV Med* 6: 291–298.
- Mahan LK, Escott-Stump S (2000) Krause's food, nutrition, & diet therapy. Philadelphia: W.B. Saunders. xxxiv, 1194 p. p.
- Gibson RS (2005) Principles of nutritional assessment. New York: Oxford University Press. xx, 908 p. p.
- Miller M, Zhan M, Havas S (2005) High attributable risk of elevated C-reactive protein level to conventional coronary heart disease risk factors: the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 165: 2063–2068.
- del Rincon Jarero JP, Aguilar-Salinas CA, Guillen-Pineda LE, Gomez-Perez FJ, Rull JA (2002) Lack of agreement between the plasma lipid-based criteria and apolipoprotein B for the diagnosis of familial combined hyperlipidemia in members of familial combined hyperlipidemia kindreds. *Metabolism* 51: 218–224.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412–419.
- Cook RL, Zhu F, Belnap BH, Weber K, Cook JA, et al. (2009) Longitudinal trends in hazardous alcohol consumption among women with human immunodeficiency virus infection, 1995–2006. *Am J Epidemiol* 169: 1025–1032.
- Miguez-Burbano MJ, Lewis JE, Malow R (2009) Alcohol and race/ethnicity elicit different changes in lipid profiles in HIV-infected individuals receiving highly active antiretroviral therapy. *J Assoc Nurses AIDS Care* 20: 176–183.
- Cheng DM, Libman H, Bridden C, Saitz R, Samet JH (2009) Alcohol consumption and lipodystrophy in HIV-infected adults with alcohol problems. *Alcohol* 43: 65–71.
- Peit JM, Carrat F, Duong M, Halfon P, Duvillard L, et al. (2010) Response to anti-HCV therapy in HIV-HCV-coinfected patients: does the lipid profile really have an effect? *Antivir Ther* 15: 797–800.
- Yang LL, Hua Q, Liu RK, Yang Z (2009) Association between two common polymorphisms of PPARgamma gene and metabolic syndrome families in a Chinese population. *Arch Med Res* 40: 89–96.
- Joy T, Keogh HM, Hadigan C, Lee H, Dolan SE, et al. (2007) Dietary fat intake and relationship to serum lipid levels in HIV-infected patients with metabolic abnormalities in the HAART era. *AIDS* 21: 1591–1600.
- Lagou V, Scott RA, Manios Y, Chen TL, Wang G, et al. (2008) Impact of peroxisome proliferator-activated receptors gamma and delta on adiposity in toddlers and preschoolers in the GENESIS Study. *Obesity (Silver Spring)* 16: 913–918.
- Moffett SP, Feingold E, Barmada MM, Damcott CM, Marshall JA, et al. (2005) The C161->T polymorphism in peroxisome proliferator-activated receptor gamma, but not P12A, is associated with insulin resistance in Hispanic and non-Hispanic white women: evidence for another functional variant in peroxisome proliferator-activated receptor gamma. *Metabolism* 54: 1552–1556.
- Valve R, Sivenius K, Miettinen R, Pihlajamaki J, Rissanen A, et al. (1999) Two polymorphisms in the peroxisome proliferator-activated receptor-gamma gene are associated with severe overweight among obese women. *J Clin Endocrinol Metab* 84: 3708–3712.
- Nazih H, Raffi F, Taieb A, Reynes J, Choutet P, et al. (2011) Peroxisome Proliferator Activating Receptor (PPAR) Alpha and Gamma Polymorphisms and Metabolic Abnormalities in HIV-Infected Patients Receiving Highly Active Antiretroviral Therapy: The ANRS CO8 APROCO-COPILOTE Study. *AIDS Res Hum Retroviruses*.
- Zanone Poma B, Riva A, Nasi M, Cicconi P, Brogini V, et al. (2008) Genetic polymorphisms differently influencing the emergence of atrophy and fat accumulation in HIV-related lipodystrophy. *AIDS* 22: 1769–1778.
- Meirhaeghe A, Amouyel P (2004) Impact of genetic variation of PPARgamma in humans. *Mol Genet Metab* 83: 93–102.
- Mohamed MB, Mtraoui N, Ezzidi I, Chaieb M, Mahjoub T, et al. (2007) Association of the peroxisome proliferator-activated receptor-gamma2 Pro12Ala but not the C1431T gene variants with lower body mass index in Type 2 diabetes. *J Endocrinol Invest* 30: 937–943.
- Doney AS, Fischer B, Cecil JE, Boylan K, McGuigan FE, et al. (2004) Association of the Pro12Ala and C1431T variants of PPARG and their haplotypes with susceptibility to Type 2 diabetes. *Diabetologia* 47: 555–558.
- Saumoy M, Veloso S, Alonso-Villaverde C, Domingo P, Chacon MR, et al. (2009) PPARgamma Pro12Ala polymorphism in HIV-1-infected patients with HAART-related lipodystrophy. *Curr HIV Res* 7: 533–540.
- Munkhtulga L, Nagashima S, Nakayama K, Utsumi N, Yanagisawa Y, et al. (2010) Regulatory SNP in the RBP4 gene modified the expression in adipocytes and associated with BMI. *Obesity (Silver Spring)* 18: 1006–1014.
- Oh J, Hegele RA (2007) HIV-associated dyslipidaemia: pathogenesis and treatment. *Lancet Infect Dis* 7: 787–796.
- van Leth F, Phanuphak P, Stroes E, Gazzard B, Cahn P, et al. (2004) Nevirapine and efavirenz elicit different changes in lipid profiles in antiretroviral-therapy-naïve patients infected with HIV-1. *PLoS Med* 1: e19.

49. Parienti JJ, Massari V, Rey D, Poubeau P, Verdon R (2007) Efavirenz to nevirapine switch in HIV-1-infected patients with dyslipidemia: a randomized, controlled study. *Clin Infect Dis* 45: 263–266.
50. Young J, Weber R, Rickenbach M, Furrer H, Bernasconi E, et al. (2005) Lipid profiles for antiretroviral-naive patients starting PI- and NNRTI-based therapy in the Swiss HIV cohort study. *Antivir Ther* 10: 585–591.