ELAINE REGINA DELICATO DE ALMEIDA

POLIMORFISMO *Pvu*II NO INTRON 15 DO GENE DO RECEPTOR DE LIPOPROTEÍNA DE BAIXA DENSIDADE (LDLR) EM PACIENTES INFECTADOS PELO VÍRUS DA IMUNODEFICIÊNCIA HUMANA TIPO 1 (HIV-1)

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Patologia Experimental da Universidade Estadual de Londrina como requisito para obtenção do título de doutor.

Orientadora: Prof[a]. Dr[a]. Maria Angelica Ehara Watanabe.
Co-orientadora: Prof[a]. Dr[a]. Edna Maria Vissoci Reiche.

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BANCA EXAMINADORA

_____
Orientadora: Prof[a]. Dr[a]. Maria Angelica Ehara Watanabe
Universidade Estadual de Londrina - UEL

_____
Prof. Dr. Dennis Armando Bertolini
Universidade Estadual de Maringá - UEM

_____
Prof[a]. Dr[a]. Tânia Longo Mazzuco
Universidade Estadual de Londrina - UEL

_____
Prof[a]. Dr[a]. Roberta Losi Guembarovski
Universidade Estadual de Londrina - UEL

_____
Prof[a]. Dr[a]. Karen Brajão de Oliveira
Universidade Estadual de Londrina - UEL

Londrina, 26 de setembro de 2013.

O Senhor é meu pastor, nada me faltará.

Restaura as forças de minha alma.

Pelos caminhos retos ele me leva, por amor do seu nome.

A vossa bondade e misericórdia hão de seguir-me por todos os dias de minha vida.

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ALMEIDA, E.R.D. **Polimorfismo *Pvu*II no intron 15 do gene do receptor de lipoproteína de baixa densidade (LDLR) em pacientes infectados pelo vírus da imunodeficiência humana tipo 1 (HIV-1)**. 2013. Departamento de Ciências Patológicas (CCB) - Universidade Estadual de Londrina, Londrina, 2013.

RESUMO

Com a introdução da terapia antirretroviral de alta potência (HAART), em 1996, tornou-se cada vez mais evidente que os pacientes infectados pelo HIV-1 têm um risco aumentado de desenvolver dislipidemia. A proposta deste estudo foi avaliar a associação entre o polimorfismo *Pvu*II localizado no intron 15 do gene do receptor de lipoproteína de baixa densidade (LDLR) e as alterações no perfil lipídico dos pacientes infectados pelo HIV-1, submetidos ou não a HAART, pois não se tem conhecimento da avaliação deste polimorfismo nestes indivíduos. Foram avaliados 355 pacientes infectados pelo HIV-1. Destes, 100 (28,2%) eram virgens de tratamento e 255 (71,8%) foram tratados com HAART. O grupo controle consistiu de 116 indivíduos saudáveis. Os genótipos *Pvu*II do *LDLR* foram identificados pelo método da PCR-RFLP. O alelo P2 inclui o sítio de restrição para a *Pvu*II o que resulta em dois fragmentos: um de 200 pb e outro de 600 pb após a digestão, já o alelo P1 é identificado por um fragmento de 800 pb. As frequências dos genótipos P1P1, P1P2 e P2P2 do polimorfismo *Pvu*II no intron 15 do *LDLR* obtidas nos pacientes e nos controles foram 52,4%, 39,1% e 8,5%; e 55,2%, 42,2% e 2.6%; respectivamente. Os pacientes (submetidos ou não ao HAART) apresentaram maior concentração sérica de triglicerídeos (TG) e menor colesterol de lipoproteína de alta densidade (HDL-C) do que os controles ($p<0,0001$). Os pacientes em uso de HAART apresentaram níveis mais elevados de TG ($p<0,0001$), colesterol total ($p<0,0001$) e colesterol de lipoproteína de baixa densidade (LDL-C, $p=0,0003$) do que aqueles virgens de HAART. A frequência dos pacientes com níveis de colesterol total ≥ 200 mg/dL, LDL-C ≥ 100 mg/dL e TG ≥ 150 mg/dL foi maior entre aqueles que usavam HAART ($p<0,0001$, $p=0,0248$ e $p=0,0269$, respectivamente). Quando os pacientes com HIV-1 foram categorizados de acordo com os níveis de HDL-C, a frequência de indivíduos com baixos níveis de HDL-C não diferiram entre os que estavam virgens de tratamento ou em tratamento com HAART ($p=0,7375$). No entanto, quando os valores de HDL-C foram avaliados de acordo com o polimorfismo *Pvu*II no intron 15 do *LDLR*, a frequência de HDL-C ≥ 40 mg/dL para homens e ≥ 50 mg/dL para mulheres foi maior entre os portadores do genótipo P2P2 ($p = 0,0415$). Os resultados obtidos mostraram que a frequência de indivíduos com níveis aumentados de colesterol total, LDL-C e TG foi maior entre os pacientes infectados pelo HIV-1 em uso de HAART. Quando os valores de HDL-C foram avaliados de acordo com o polimorfismo *Pvu*II no intron 15 do *LDLR*, a frequência de valores elevados de HDL-C foi maior entre os portadores do genótipo P2P2. Os resultados sugerem que a infecção pelo HIV-1 *per se* e o uso de HAART alteram o colesterol total, LDL-C e TG em pacientes infectados pelo HIV-1 independentemente do polimorfismo *Pvu*II no intron 15 do *LDLR*; entretanto, os efeitos desses fatores no HDL-C podem ser atenuados em parte, pelo genótipo P2P2 deste polimorfismo.

Palavras chaves: dislipidemia, HIV-1, HAART, polimorfismo genético, receptor de lipoproteína de baixa densidade.

ALMEIDA, E.R.D**. *Pvu*II intron 15 polymorphism of the *low density lipoprotein receptor* (*LDLR*) gene in human immunodeficiency virus type 1(HIV-1)- infected patients**. 2013. Department of Pathological Sciences – State University of Londrina, 2013.

ABSTRACT

The introduction of highly active antiretroviral therapy (HAART) in 1996 has become increasingly clear that HIV-1-infected patients exhibit an increased risk for developing dyslipidemia. The proposal of this study was to evaluate the association between the *Pvu*II intron 15 polymorphism at the *low-density lipoprotein receptor (LDLR)* gene and the changes in lipid profile among the patients with HIV-1 infection submitted or not to HAART, since there is no knowledge of the evaluation of this polymorphism in these individuals. A total of 355 HIV-1-infected patients were analyzed. Of them, 100 (28.2%) were antiretroviral naïve and 255 (71.8%) were treat with HAART. The control group consisted of 116 healthy individuals. The *Pvu*II *LDLR* genotypes were determined from the genomic DNA using PCR-RFLP methods. The P2 allele includes a restriction site for *Pvu*II which results in two fragments: one of 200 bp and one of 600 bp after digestion, whereas the P1 allele is identified by one fragment with 800 bp. The frequencies of P1P1, P1P2, and P2P2 *Pvu*II intron 15 LDLR genotypes obtained among the patients and healthy controls were 52.4%, 39.1%, and 8.5%; and 55.2%, 42.2%, and 2.6%; respectively. The patients (submitted or not to HAART) presented higher serum triglycerides (TG) and lower serum high-density lipoprotein cholesterol (HDL-C) concentration than controls ($p<0.0001$). The patients treated with HAART showed higher TG ($p<0.0001$), total cholesterol ($p<0.0001$), and low-density lipoprotein cholesterol (LDL-C, $p=0.0003$) than those without HAART. The frequency of patients with total cholesterol levels ≥ 200mg/dL, LDL-C levels ≥ 100mg/dL, and TG ≥ 150mg/dL was higher among those using HAART ($p<0.0001$, $p=0.0248$, and $p=0.0269$, respectively). When HIV-1 patients were categorized according to HDL-C values, the frequency of individuals with low HDL-C levels did not differ among the HIV-1 infected patients antiretroviral naïve or on HAART ($p=0.7375$). However, when the HDL-C values were evaluated according to *LDLR Pvu*II intron 15 polymorphism, the frequency of HDL-C ≥ 40 mg/dL for men and ≥ 50 mg/dL for women was higher among those carrying the P2P2 genotype ($p=0.0415$). The results obtained showed that the frequency of individuals with increased total cholesterol, LDL-C, and TG was higher among the HIV-1 patients using HAART. When the HDL-C values were evaluated according to *LDLR Pvu*II intron 15 polymorphism, the frequency of high HDL-C values was higher among those carrying the P2P2 genotype. The results underscore that the HIV-1 infection *per se* and the HAART change the total cholesterol, LDL-C and TG in HIV-1-infected patients independent of the *LDLR Pvu*II intron 15 polymorphism; however, the effects of these factors on the HDL-C can be mitigated, in part, by the P2P2 genotype of this polymorphism.

Keywords: dyslipidemia, HIV-1, HAART, genetic polymorphism, low-density lipoprotein receptor.

LISTA DE ABREVIATURAS E SIGLAS

ADH	Hipercolesterolemia autossômica dominante
APO	Apolipoproteína
CCR5	*CC-Chemokine receptor 5*; receptor de CC-quimiocina 5
DAC	Doença arterial coronariana
DCV	Doença cardiovascular
DNA	Ácido desoxirribonucleico
FDB	Defeito familiar de apolipoproteína B-100
HAART	*Highly active antirretroviral therapy;* terapia antirretroviral de alta potência
HDL-C	*High-density lipoprotein choleterol;* colesterol de lipoproteína de alta densidade
HF	Hipercolesterolemia familiar
HMG-CoA	*3-hydroxi-3-methylglutaryl-coenzyme A;* 3-hidroxi-3-metil-glutaril-coenzima A
HIV	Vírus da imunodeficiência humana
IC	Intervalo de confiança
IFN-γ	Interferon gama
IMC	Índice de massa corpórea
IPs	Inibidores de protease
INS/DEL	Inserções/deleções
ITRNs	Inibidores de transcriptase reversa análogos de nucleosídeos
ITRNNs	Inibidores da transcriptase reversa não análogos de nucleosídeos
LDL-C	*Low-density lipoprotein cholesterol;* Colesterol de lipoproteína de baixa densidade

LDLR	*Low-density lipoprotein receptor;* receptor de lipoproteína de baixa densidade
PCR	*Polymerase chain reaction;* reação em cadeia da polimerase
PCSK9	*Proprotein convertase subtilisin/kexin type* 9; pró-proteína convertase subtilisina/kexina tipo 9
RNA	Ácido ribonucleico
SLHIV	Síndrome lipodistrófica do vírus da imunodeficiência humana
SM	Síndrome metabólica
SNP	*Single nucleotide polymorphism*; polimorfismo de nucleotídeo simples
TG	Triglicerídeo
TH1	Linfócito T *helper* 1
3' UTR	3' *untranslated region*; região 3' não transcrita
VLDL-C	very l*ow-density lipoprotein cholesterol;* Colesterol de lipoproteína de densidade muito baixa

SUMÁRIO

1 INTRODUÇÃO

1.1 VÍRUS DA IMUNODEFICIÊNCIA HUMANA TIPO 1 (HIV-1) E TERAPIA ANTIRRETROVIRAL DE ALTA POTÊNCIA (HAART)

A epidemia da infecção pelo vírus da imunodeficiência humana tipo 1 (HIV-1) e, como consequência, a síndrome da imunodeficiência adquirida (aids), remontam dos anos oitenta, quando os primeiros casos foram divulgados em 1981. Nos primeiros anos, mais precisamente a partir de 1987, as novas drogas empregadas em monoterapia, ou mesmo em terapia dupla, não produziram os efeitos necessários para a sobrevida dos que as utilizavam. Com a introdução da terapia antirretroviral de alta potência (*highly active antiretroviral therapy* – HAART) em 1996, observou-se um profundo impacto na história natural desta infecção (DETELS et al., 1998; HOGG et al., 1999; PALELLA et al., 1998).

No entanto, dados recentes mostram que, aproximadamente, 34 milhões de pessoas são portadoras do HIV-1 no mundo e apenas 50% delas sabem que estão infectadas. Além disto, 14,8 milhões de pessoas seriam elegíveis para o tratamento, mas apenas 8 milhões de pessoas estão em tratamento para este vírus no mundo (UNAIDS, 2012). Passados dois anos pós-HAART, o acesso ao tratamento cresceu cerca de 63% em todo o mundo, permitindo que milhares de pessoas que vivem com HIV-1 recebessem o tratamento pela primeira vez. Na região do sub-Saara africano, aproximadamente 2,3 milhões de pessoas entraram nos programas de tratamento nos últimos anos, refletindo um aumento de 59%. O número de pessoas que está morrendo de causas relacionadas à aids começou a declinar em meados dos anos 2000, devido ao aumento do uso de HAART e ao declínio estável na incidência da infecção desde o pico da epidemia em 1997 (UNAIDS, 2012). Com a introdução da HAART, verificou-se um aumento considerável na expectativa e qualidade de vida dos portadores da infecção pelo HIV-1 (GUIMARÃES, 2007).

A HAART é baseada em esquemas terapêuticos combinados contendo, pelo menos, três drogas antirretrovirais, de forma a ser extremamente efetiva na redução da carga viral plasmática do ácido ribonucleico (RNA) do HIV-1 para níveis indetectáveis (DETELS et al., 1998; HOGG et al., 1999; PALELLA et al., 1998; WLODAWER; VONDRASEK, 1998). O tratamento antirretroviral consiste em

uma combinação de drogas capazes de inibir diferentes etapas da replicação viral, divididas em seis classes: 1) inibidores de transcriptase reversa análogos de nucleosídeos (ITRNs): abacavir, didanosina, estavudina, lamivudina, zidovudina e tenofovir; 2) inibidores da transcriptase reversa não análogos de nucleosídeos (ITRNNs): efavirenz e nevirapina; 3) inibidores de protease (IPs): amprenavir, atazanavir, darunavir, indinavir, nelfinavir, ritonavir, ritonavir + lopinavir e saquinavir; 4) inibidor de fusão: enfuvirtida; 5) inibidor do receptor de quimiocina 5 (CCR5): maraviroc; e 6) inibidor da integrase: raltegravir (DUBÉ; CADDEN, 2011; MENÉNDEZ-ARIAS, 2013; WLODAWER; VONDRASEK, 1998).

A adesão ao tratamento HAART e a boa resposta à terapia são fatores que têm contribuído para o aumento da expectativa de vida e melhor prognóstico destes pacientes. A maior sobrevida destes indivíduos os tem exposto aos efeitos da idade, a outros fatores relacionados ao indivíduo e ao ambiente que aumentam o risco de obesidade, diabetes e doença cardiovascular (DCV) na população em geral. A infecção pelo HIV-1 *per se* pode causar anormalidades nos lipídeos incluindo elevação dos triglicerídeos (TG) e diminuição da fração lipoprotéica do colesterol de alta densidade (HDL-C) (GRUNFELD et al., 1992) e estas alterações têm sido correlacionadas com o grau de imunossupressão causada pelo HIV-1 (DUCOBU; PAYEN, 2000; KHIANGTE et al., 2007). Muitos autores têm sugerido que a infecção não tratada é um exemplo de inflamação que pode ocasionar aterosclerose e alterações metabólicas que aumentam o risco de DCV (ADEYEMI et al., 2008; KOTLER, 2008).

O mecanismo das desordens lipídicas em pacientes com HIV-1 virgens de tratamento pode ser modulado pelas citocinas e uma associação entre os níveis plasmáticos de TG e interferon gama (IFN-γ) circulante tem sido observada em pessoas com aids. Acredita-se que o IFN-γ aumenta os níveis de TG pela diminuição do *clearance* de TG no período pós prandial assim como pelo aumento da lipogênese hepática *de novo* e da síntese de colesterol de lipoproteína de densidade muito baixa (VLDL-C) (GRUNFELD et al., 1992). No entanto, tanto na população em geral como em pacientes com HIV-1, a DCV é um processo multifatorial, que inclui fatores genéticos, ambientais, o próprio HIV-1 e a terapia antirretroviral (AMADO; RUIZ, 2007).

1.2 EFEITOS COLATERAIS DA HAART

Sabe-se que a terapia antirretroviral apresenta inúmeros efeitos adversos e que, em curto prazo, são bem tolerados (RACHID; SCHECHTER, 2008). Entretanto, o tratamento antirretroviral pode induzir complicações metabólicas graves, tais como resistência à insulina, síndrome metabólica (SM), lipodistrofia e DCV. Os efeitos metabólicos do tratamento antirretroviral no aumento do risco de aterosclerose precoce e acelerada em pacientes infectados por HIV-1 são bem reconhecidos. Essas condições clínicas inter-relacionadas têm prevalência significativamente maior entre pacientes infectados por HIV-1 em uso de terapia antirretroviral (GUIMARÃES et al., 2007).

A SM é um conjunto de anormalidades metabólicas relacionadas ao excesso de adiposidade visceral que confere um risco aumentado de DCV e diabetes *mellitus* tipo 2 (KASSI et al., 2011). A SM que aparece em indivíduos infectados pelo HIV-1 está associada ao aumento da incidência de DCV e da mortalidade associada à mesma. Estudos mostram que, aproximadamente, 36% dos pacientes infectados pelo HIV-1 apresentam SM (DIEHL et al., 2008), com valores que variam de 7 a 45% (WORM; LUNDGREN, 2011). Um estudo do tipo coorte de pacientes americanos infectados pelo HIV-1 que incluía indivíduos virgens de tratamento e em tratamento, apresentou uma incidência de 1,2 por 100 pessoas/mês (JACOBSON et al., 2006) e um estudo internacional de indivíduos iniciando a terapia demonstrou uma incidência de 12 por 100 pessoas/ano (WAND et al., 2007).

Um estudo recente (KRISHMAN et al., 2012) registrou que a prevalência de SM em pacientes infectados pelo HIV-1 em início de terapia antirretroviral era de 20% e que, após o início da terapia, a incidência de SM foi de 8,5 por 100 pessoas-ano. Na análise realizada após o ajuste das características demográficas e índice de massa corpórea (IMC), o risco de SM diminuiu [RR=0,62; IC 95%=0,43-0,90] quando os pacientes tinham mais que 500 linfócitos T $CD4^{+}/mm^{3}$; no entanto, o risco de SM aumentou quando a carga viral foi maior que 400 cópias/mL (RR=1,55; IC 95%=1,25-1,92) e com o uso de IPs (RR=1,25; IC 95%= 1,04-1,51). Outro estudo avaliou o perfil lipídico de indivíduos normotensos não diabéticos, não obesos e infectados pelo HIV-1 virgens de tratamento, e os resultados demonstraram que os níveis de TG foram mais elevados em indivíduos com HIV-1, e os níveis de colesterol total e HDL-C foram menores comparados com

os controles não infectados. Os resultados também demonstraram que valores diminuídos de HDL-C foram associados com contagem de linfócitos T $CD4^+$ menores que 200 células/mm^3 (DANIYAM, IROENZIDU, 2013).

Valente et al. (2005) demonstram que o uso prolongado das drogas antirretrovirais está associado com a síndrome lipodistrófica do HIV (SLHIV), que inclui dislipidemia, alteração glicêmica, DCV, resistência insulínica e lipodistrofia. A lipodistrofia pode ser lipoatrofia, com redução de gordura em regiões periféricas, proeminência vascular e venosa, devido ao fato da pele ficar mais adelgaçada, o que permite a visualização, quase anatômica, dos grupamentos musculares e vasos sanguíneos superficiais; lipohipertrofia, com acúmulo de gordura em região abdominal, gibosidade dorsal, ginecomastia e o aumento das mamas em mulheres; e mista, com associação da lipoatrofia e lipohipertrofia.

A elevada prevalência de alterações metabólicas observada em pacientes que vivem com HIV-1/aids foi atribuída, recentemente, a vários fatores, tais como história familiar de dislipidemia e tempo de uso e ao tipo de terapia antirretroviral utilizada, em especial os IPs (FARHI; LIMA; CUNHA, 2008). Inicialmente, a SLHIV foi atribuída ao uso dos IPs (MONTESSORI et al., 2004). No entanto, outros estudos mostraram que a infecção pelo HIV-1, *per se,* está associada com estas alterações metabólicas, especialmente com a diminuição dos níveis séricos do HDL-C (RACHID; SCHECHTER, 2008).

A SLHIV é uma preocupação em relação às crianças infectadas verticalmente pelo HIV-1, devido à exposição à HAART por um longo período de tempo. Estudos mostraram que 39% das crianças com SLHIV apresentaram quatro ou mais sinais de lipodistrofia após seis anos de tratamento, enquanto que 14% apresentaram quatro ou mais sinais de lipodistrofia com menos de três anos de tratamento. Crianças que receberam tratamento em doses pediátricas apresentaram menor probabilidade de desenvolver a SLHIV comparadas com crianças que receberam doses de adultos (ALVES et al., 2008). Sarni et al. (2009) verificaram que 60% de crianças e adolescentes com aids em uso regular de HAART apresentaram alteração no perfil lipídico.

Flint et al. (2009), analisando o papel dos IPs na patogênese da lipodistrofia associada ao HIV-1, sugeriram novos regimes HAART efetivos que minimizem ou eliminem as complicações metabólicas associadas a essas drogas. As associações com IPs e/ou estavudina causam mais efeitos metabólicos adversos e

estes deveriam ser evitados em pacientes infectados pelo HIV-1 com alto risco cardiovascular (DOMINGOS et al., 2009).

Resino et al. (2008) realizaram um estudo que analisou a recuperação imunológica de 55 crianças infectadas pelo HIV-1 e que fizeram o uso de HAART por longo período de tempo e verificaram que, aquelas que possuíam uma contagem de linfócitos T $CD4^+$ muito baixa, não conseguiram a recuperação imunológica após oito anos de tratamento; já as crianças com uma rápida recuperação imunológica tiveram uma maior prevalência de SLHIV.

1.3 Dislipidemia nos Pacientes Infectados pelo HIV-1

Dois padrões de dislipidemia são fatores de risco para a DCV: o primeiro está relacionado com um aumento da fração da lipoproteína de baixa densidade do colesterol (LDL-C), geralmente por predisposição genética, e o segundo está relacionado com aumento da concentração de TG e diminuição de HDL-C, que tem sido comumente encontrado em pacientes com outras alterações metabólicas como obesidade central, diabetes *mellitus* e hipertensão (KOTLER, 2008; SAMARAS et al., 2007).

A dislipidemia que está sendo frequentemente observada em pacientes infectados pelo HIV-1 em uso de HAART é caracterizada pelas mudanças no perfil lipídico com aumento das concentrações de colesterol total, LDL-C, TG e diminuição de HDL-C, o que constitui um perfil lipídico altamente aterogênico (CHACRA et al., 2006). A patogênese da dislipidemia na infecção pelo HIV-1 é complexa e envolve fatores relacionados ao vírus, ao hospedeiro e à terapia antirretroviral, uma vez que nem todos os pacientes em uso da HAART apresentam distúrbios metabólicos (OH; HEGELE, 2007).

A importância das desordens no perfil lipídico de pacientes infectados pelo HIV-1 se deve ao aumento do risco cardiovascular decorrente da terapia antirretroviral, principalmente associado ao uso contínuo de IPs (CARR et al., 1998; CHI et al., 2000; ESTRADA; PORTILLA, 2011). Segundo Carr et al. (1998), o tratamento desses pacientes com IPs está associado com a síndrome de lipodistrofia periférica, adiposidade central, hiperlipidemia e resistência à insulina. A melhora considerável na condição destes pacientes devido ao uso da HAART tem progressivamente transformado a aids em uma doença crônica (HOGG et al., 1999;

MARINS et al., 2003; PALELLA et al., 1998). Considerando o aumento da expectativa de vida destes pacientes, tem-se proposto uma avaliação sistemática e precoce dos riscos para eventos cardiovasculares nesta população (GUIMARÃES et al., 2007).

Friis-Moller et al. (2003) analisaram a hipercolesterolemia em 17.852 pacientes Europeus, Americanos e Australianos infectados pelo HIV-1 e verificaram níveis elevados de colesterol em 10% dos indivíduos que recebiam apenas ITRN, em 23% dos que recebiam ITRN e ITRNN, em 27% dos que recebiam IPs e ITRN e em 44% dos pacientes que estavam em tratamento com as três classes de antirretrovirais. A associação desses dados para hipertrigliceridemia foi de 23%, 32%, 40% e 54%, respectivamente, comparada com 15% observada dos pacientes virgens de tratamento.

Vários estudos também mostraram a presença de dislipidemia em pacientes infectados pelo HIV-1 em uso de HAART na população brasileira. Farhi et al. (2008) analisaram 235 pacientes atendidos no Hospital Universitário da Universidade do Estado do Rio de Janeiro e verificaram que 77,5% apresentavam alteração lipídica. Guimarães et al. (2007) avaliaram 176 pacientes infectados pelo HIV-1 atendidos no Hospital das Clínicas da Universidade Federal de Minas Gerais e verificaram que, dos 133 pacientes em uso de HAART, estes tiveram níveis mais elevados de TG e colesterol total.

O tipo e a gravidade da dislipidemia variam de acordo com o regime de tratamento com antirretrovirais. Hipertrigliceridemia ocorre frequentemente durante tratamento com ritonavir e o aumento de LDL-C é verificado com o uso de vários antirretrovirais, incluindo ITRNs (estavudina), PIs e NNRTI (principalmente efavirenz). Os NNRTIs, particularmente a nevirapina, estão relacionados com aumento do HDL-C (ARNEDO et al., 2007). Entretanto, os inibidores de fusão, inibidor da integrase e inibidor do CCR5 parecem ter um efeito neutro sobre os lipídios, mas o efeito desses medicamentos sobre os eventos cardiovasculares ainda é desconhecido (DUBÉ; CADDEN, 2011). Mesmo assim, a dislipidemia não ocorre em todos os pacientes em uso do mesmo regime de HAART e expostos às mesmas características demográficas, imunológicas e virológicas. Essas diferenças parecem estar relacionadas a fatores genéticos (EGAÑA-GORROÑO et al., 2013; TAR et al., 2010).

1.4 Fatores Genéticos Associados à Dislipidemia

Os níveis séricos de lipídios têm etiologia multifatorial determinada por um grande número de fatores genéticos e ambientais (ANDRADE; HUTZ, 2002). Fatores genéticos e da dieta influenciam a concentração sérica de colesterol total, mas mecanismos detalhados de sua interação não são bem conhecidos. O aumento da ingestão de colesterol na dieta aumenta a concentração sérica de colesterol total em alguns indivíduos, mas não em outros. Variações genéticas em apoproteínas (Apo), enzimas e receptores que atuam principalmente no metabolismo do LDL-C estão envolvidas, pelo menos em parte, na regulação da concentração sérica do colesterol total e LDL-C (SALAZAR et al., 2000a). Segundo Andrade e Hutz (2002), a identificação do componente genético na causa da dislipidemia tem sido intensamente investigada nos últimos anos. Estudos demonstram que o efeito de polimorfismos genéticos depende, em parte, da interação com fatores ambientais, tais como tabagismo, sobrepeso ou sedentarismo (FIEGENBAUM, 2001; FREEMAN et al., 1994; VOHL et al., 1999).

Variações em um grande número de genes envolvidos na síntese de proteínas estruturais e enzimas relacionadas com o metabolismo de lipídios podem responder por variações do perfil lipídico de cada indivíduo (ANDRADE; HUTZ, 2002). Tais variações genéticas, quando encontradas com frequência na população estudada (mais de 1% de frequência do alelo mais raro), são chamadas de polimorfismos genéticos. A base genética para estas variações pode ser uma troca, deleção ou inserção de um único nucleotídeo no ácido desoxirribonucleico (DNA), denominado polimorfismo de um único nucleotídeo ou *single nucleotide polymorphism* (SNP) ou duplicação ou deleção de vários pares de bases (LANDER et al., 2001). Desta maneira, os genes que codifiquem proteínas envolvidas no metabolismo dos lipídios poderiam ser genes candidatos para investigação de variações genéticas dos níveis lipídicos (ANDRADE; HUTZ, 2002).

A hipercolesterolemia é o principal fator de risco para aterosclerose e complicações cardiovasculares prematuras, podendo ser multifatorial ou menos frequentemente monogênica, levando à doença denominada hipercolesterolemia autossômica dominante (ADH), que se caracteriza pela elevação plasmática de LDL-C, xantoma, xantelasma e doença coronariana prematura. O diagnóstico de ADH é difícil devido à sobreposição de valores de colesterol entre as formas monogênicas e

multifatoriais. Testes de análise do DNA fornecem um diagnóstico inequívoco e permitem a identificação de indivíduos que são portadores de uma ou mais variantes genéticas associadas ao risco maior de DCV e assim podem, precocemente, se beneficiar de medidas preventivas para mudanças de hábitos de vida e de terapias hipolipeminantes (HUMPHRIES et al., 2008).

O primeiro gene identificado na ADH foi o que codifica o receptor de LDL (LDLR) (GOLDSTEIN et al 1973). Esta doença autossômica dominante foi denominada hipercolesterolemia familiar (HF) e a prevalência da sua heterozigose foi estimada em 1/500 indivíduos. O *LDLR* localiza-se no cromossomo 19, compreende 18 éxons e 17 introns e codifica uma proteína de 839 aminoácidos (SUDHOF et al., 1985). Já foram descritas mais de 1288 variantes diferentes para o *LDLR* em pacientes com HF: 55% destas variantes correspondem a substituições em regiões de éxons, 22% são pequenos rearranjos em éxons com menos de 100 pares de bases (pb), 11% são grandes rearranjos com mais de 100 pb, 2% ocorrem em regiões promotoras, 10% em introns e 1% são variações na região 3' não transcrita (3' UTR) (LEIGH et al., 2008; USIFO et al., 2012). Algumas dessas variações genéticas estão associadas com aumento dos níveis séricos de lipídios e, portanto, podem ser associadas com alto risco para doença arterial coronariana (DAC) (REGIS-BAILY et al., 1996; SAHA et al., 1992; SALAZAR et al., 1999 e 2000a; STEPANOV et al., 1998).

Subsequentemente ao *LDLR*, um segundo gene foi envolvido na ADH após a descoberta de pacientes hipercolesterolêmicos com atividade normal do LDLR (INNERARITY et al., 1987). Estes indivíduos carregavam uma mutação não sinônima (*missense)* no gene *Apo B*, que codifica a molécula Apo B, o principal ligante para o LDLR (SORIA et al., 1989), sendo denominada de defeito familiar de apolipoproteína B-100 (FDB) com uma frequência estimada de 1/250 indivíduos Suíços e 1/1250 indivíduos Norte-Europeus e Norte-Americanos (RABÉS et al., 2000). Em seguida, um terceiro gene causador de ADH foi identificado como o que codifica a pró-proteína convertase subtilisina/kexina tipo 9 (PCSK9) (ABIFADEL et al., 2003). Tem sido reportado que esta enzima degrada o LDLR independentemente de sua atividade catalítica, o que contribuiria para elevação dos níveis circulantes de LDL-C (MCNUTT et al., 2007).

Recentemente, um quarto *locus* para ADH foi mapeado no cromossomo 16q22.1 (MARQUES-PINHEIRO et al., 2010). No entanto, a proporção

de pacientes com ADH em quem a doença não é explicada por mutações nos genes previamente descritos como *LDLR*, *Apo B* e *PCSK9* foi estimada em 15,25% (VARRET et al., 2008). Em uma meta-análise de 46 estudos genômicos de associação, outros 95 *loci* foram identificados como fatores genéticos que contribuem com as variações normais dos lipídeos e com os fenótipos extremos de dislipidemias na população em geral (TESLOVICH et al., 2010).

Estudos de epidemiologia molecular realizados em diferentes populações têm mostrado a elevada frequência de variantes nos genes *LDLR, Apo* B e *PCSK9* em pacientes com HF e seus probandos. Marduel et al. (2010) avaliaram 1358 probandos de diferentes regiões da França e identificaram 1111 eventos moleculares: 1012 (91,1%) mutações no *LDLR* em 1003 (73,9%) probandos, 9 com duas variantes do *LDLR* cada; 89 (8,0%) mutações no *Apo B* em 89 (6,6%) probandos incluindo 2 probandos também heterozigotos para mutação no *LDLR*; 10 (0,9%) mutações no *PCSK9* em 10 (0,7%) probandos. Para os demais 258 (19,0%) probandos, os autores não identificaram mutações nos três principais genes associados à ADH, o que reforça a existência de outras mutações associadas à ADH em genes ainda não identificados. A comparação dos dados clínicos e bioquímicos mostrou um gradiente de severidade para as mutações na ADH, sendo HF = PCSK9 > FDB > outros genes.

1.5 POLIMORFISMOS GENÉTICOS NO *LDLR*

O LDLR desempenha um papel importante na remoção das partículas de LDL-C do sangue (BROWN; GOLDSTEIN, 1986), regulando a homeostase do colesterol. Muitas mutações no *LDLR* têm sido associadas com a disfunção deste gene, que reduz significativamente o catabolismo de LDL-C e leva à desordem metabólica observada na HF (DAY et al., 1997; HOBBS; BROWN; GOLDSTEIN, 1992; LEVY et al., 1997; PEREIRA et al., 1995). Pacientes com mutações no *LDLR* possuem níveis plasmáticos elevados de colesterol total, duas ou mais vezes que a concentração normal, aumentando o risco de desenvolvimento de aterosclerose e DAC (BROWN; GOLDSTEIN, 1986).

O LDLR modula os níveis plasmáticos de LDL-C regulando a absorção das partículas de LDL-C pelo fígado e a entrega de colesterol para as glândulas supra-renais e gônadas para a síntese de hormônios esteróides e para o

fígado para a síntese de ácidos biliares (BROWN; GOLDSTEIN, 1986). Considerando o papel crucial do LDLR na homeostase do colesterol, alterações genéticas no *LDLR* têm contribuído para a variação dos níveis plasmáticos do colesterol na população em geral (SALAZAR et al., 1999).

Estudos relataram a associação entre o polimorfismo *Pvu*II no intron 15 do *LDLR* com diferentes níveis de LDL-C, onde indivíduos com o genótipo homozigoto raro P2P2 (presença do alelo polimórfico em homozigose), apresentaram uma redução de 10-20% dos níveis de LDL-C comparados com os indivíduos com outros genótipos (PEDERSEN et al., 1988; PERERSEN et al., 1989; SCHUSTER et al., 1990).

Humphries et al. (1991) analisaram a influência do polimorfismo *Pvu*II (intron 15) do *LDLR* na variação dos níveis de colesterol total e LDL-C em 289 indivíduos Italianos. Estes autores verificaram associação entre a presença do alelo P2 com baixos níveis de colesterol total e LDL-C. A alta frequência do alelo P2 nos indivíduos acima de 65 anos de idade associada com uma elevada frequência de valores baixos de LDL-C, sugeriu que este alelo poderia estar associado com aumento da sobrevida nestes indivíduos.

Gudnason et al. (1998) padronizaram o método da reação em cadeia da polimerase - polimorfismo do comprimento dos fragmentos de restrição (PCR-RFLP) para detectar o polimorfismo *Pvu*II (intron 15) do *LDLR*, substituindo a técnica de S*outhern Blotting,* usada anteriormente. O sítio da enzima *Pvu*II (CAGCTG) é criado pela troca de C para T dentro da sequência CAGCCG no intron 15, na qual localiza-se na posição de 900 pares de bases (pb) a partir da extremidade 3’ no final do exón 16. O alelo P2 inclui o sítio de restrição para a *Pvu*II o que resulta em dois fragmentos: um de 200 pb e outro de 600 pb após a digestão; já o alelo P1 é identificado por um fragmento de 800 pb e o genótipo heterozigoto P1P2 é identificado por três fragmentos (800 pb, 600 pb e 200 pb), como indicado na figura 1.

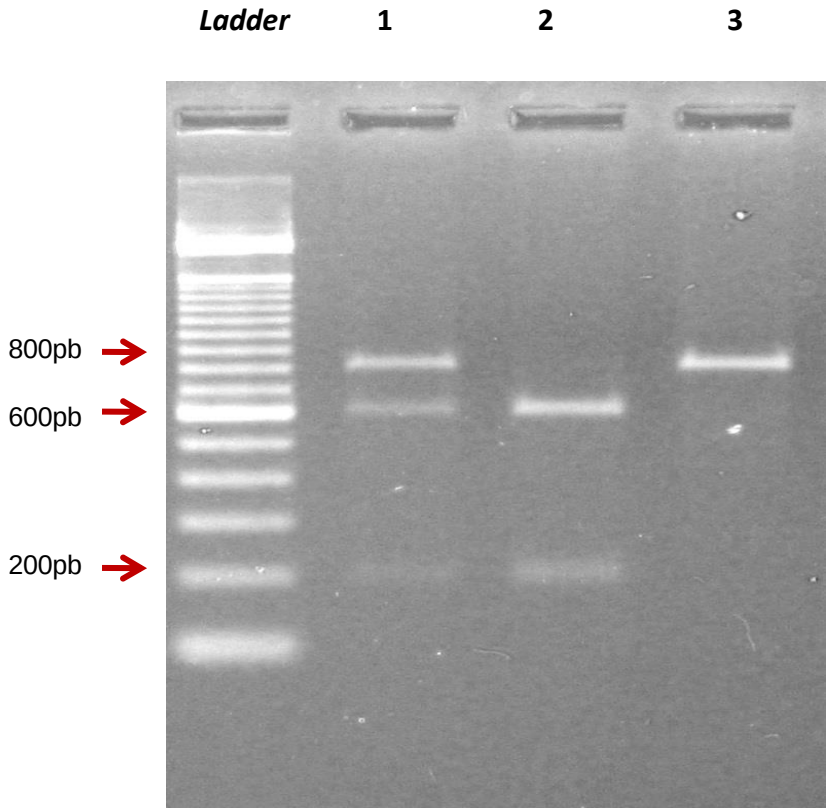

Figura 1 – Perfil de PCR-RFLP para o polimorfismo *Pvu*II (intron 15) do gene *receptor de lipoproteína de baixa densidade* (*LDLR)* em gel de agarose a 3%. *Ladder* (marcador molecular)*:* de 100pares de bases (pb); 1: genótipo P1P2, heterozigoto para o sítio de restrição, com fragmentos de 800, 600 e 200 pb; 2: genótipo P2P2, homozigoto para o sítio de restrição, com fragmentos de 600 e 200 pb; 3: genótipo P1P1, homozigoto para a ausência do sítio de restrição com fragmento de 800 pb.

Fonte: Elaine Regina Delicato de Almeida (2013).

O polimorfismo *Pvu*II localizado no intron 15 do *LDLR* foi considerado um marcador genético associado à variação do *LDLR* que pode alterar a estrutura, a atividade do receptor ou a regulação da expressão gênica (GUDNASON et al., 1998). Estudos têm demonstrado que o polimorfismo *Pvu*II no intron 15 do *LDLR* foi associado com diferenças na concentração do LDL-C em indivíduos normo e hipercolesterolêmicos de diferentes países (CHAKRAVARTI; HOBBS, 1989; BERTOLINE et al., 1992; CHAVES et al., 1996; HANSEN et al., 1997).

Salazar et al. (2000a) demonstraram a influência do polimorfismo *Pvu*II no intron 15 do *LDLR* nas concentrações séricas de lipídios em indivíduos com baixo e alto risco para DAC. Foram analisados 128 indivíduos brasileiros, caucasianos, com perfil lipídico sugestivo para DAC e 100 indivíduos

normolipêmicos e os resultados demonstraram que a frequência do alelo P1 em indivíduos com alto risco para DAC foi de 75%, similar à observada em indivíduos hipercolesterolêmicos de diferentes países como Israel, Itália, Espanha, Holanda e Dinamarca, Londres, América do Norte, Suíça e Alemanha (BERKMAN et al., 1992; BERTOLINI et al., 1992; CHAVES et al., 1996; HANSEN et al., 1997; HUMPHRIES et al., 1985; LEITERSDORF; CHAKRAVARTI; HOBBS, 1989; MISEREZ et al., 1993, respectivamente). O genótipo P1P1 deste polimorfismo foi mais frequente em indivíduos com alto risco para DAC quando comparado aos controles (57% *vs* 38%, p<0,05). Além disso, Salazar et al. (2000a) demonstraram uma forte associação entre a elevada concentração plasmática de colesterol total, TG, LDL-C, VLDL-C e baixa concentração plasmática de HDL-C em indivíduos com alto risco para DAC.

Em outro estudo, Salazar et al. (2000b) avaliaram três polimorfismos no gene *LDLR,* como o *Ava*II no exon 13 (T20001C, rs5925), *Hinc*II no exon 12 (C16730T, rs688) e *Pvu*II no intron 15 em 50 indivíduos brasileiros diagnosticados com HF e em 130 indivíduos normolipêmicos. Indivíduos com HF mostraram maior frequência dos genótipos homozigotos A+A+ (*Ava*II), H+H+ (*Hinc*II) *e* P1P1 (*Pvu*II) quando comparado ao grupo controle (p<0,05). Os indivíduos com HF apresentaram maior frequência dos alelos: A+ (58%), H+ (61%) e P1 (78%) do que os indivíduos normolipêmicos (45%, 45% e 64%, respectivamente). A forte associação observada entre esses alelos e HF sugere que os polimorfismos *Ava*II, *Hinc*II e *Pvu*II do *LDLR* podem ser utilizados para monitorar a susceptibilidade para HF em famílias brasileiras.

Em um estudo com mulheres brasileiras, caucasianas e com DAC, Salazar et al. (2000c) mostraram que a frequência dos genótipos homozigotos A+A+ e P1P1 para os polimorfismos *Ava*II e *Pvu*II respectivamente no *LDLR* foi significativamente maior naquelas com DAC do que no grupo controle (44% *vs* 16% e 64% *vs* 39%, p<0,05, respectivamente). Além disto, a frequência dos alelos A+ e P1 encontrados em mulheres com DAC também foi maior do que no grupo controle (62% *vs* 44%, p=0,005 e 78% *vs* 65%, p<0,05, respectivamente). No entanto, para o polimorfismo *Hinc*II do *LDLR* não foi demonstrada diferença significativa entre pacientes e controles.

Com relação à resposta terapêutica aos medicamentos inibidores da enzima 3-hidroxi-3-metilglutaril-coenzimaA (HMG-CoA) redutase utilizados no tratamento da hipercolesterolemia, Salazar et al. (2000d) demonstraram a

associação de polimorfismos no *LDLR* com a resposta terapêutica à fluvastatina em 55 pacientes brasileiros com hipercolesterolemia primária. Os resultados indicaram que os polimorfismos *Ava*II e *Pvu*II influenciam na resposta terapêutica à fluvastatina. Indivíduos com os genótipos homozigotos A+A+ (*Ava*II) e P1P1 (*Pvu*II) apresentaram menor redução dos níveis de colesterol total, LDL-C e Apo B após 16 semanas de tratamento com fluvastatina, quando comparados aos outros genótipos.

Salazar et al. (2002) realizaram um estudo demonstrando um largo espectro de mutações no *LDLR* em 35 pacientes brasileiros com HF em heterozigose, e destes, 22 pacientes apresentaram mutações. Das mutações no *LDLR* (8 previamente reportadas e 7 novas mutações), 11 foram não sinônimas *(missense*), 2 de códon de parada (*nonsense*) e 2 causaram alterações no quadro de leitura (*frameshift*).

Como descrito, a dislipidemia é frequente entre os pacientes infectados pelo HIV-1, principalmente naqueles que são tratados com regimes de HAART, o que eleva o risco de complicações metabólicas e cardiovasculares nesses pacientes. Essa alteração lipídica pode ser intensificada se o paciente apresentar polimorfismos genéticos relacionados ao metabolismo lipídico. Este fato justifica o atendimento multidisciplinar aos pacientes infectados pelo HIV-1 em que, além dos infectologistas, outros profissionais da área médica, como cardiologistas e endocrinologistas, devem ficar atentos e, sempre que possível, avaliar o perfil lipídico destes pacientes visando implementação imediata de medidas farmacológicas e não farmacológicas.

Além disto, devem ser propostas medidas preventivas sistemáticas a todos os pacientes infectados pelo HIV-1 para melhora da qualidade de vida, como evitar excesso ponderal e sedentarismo, estimular a interrupção do tabagismo e a manutenção de bons hábitos alimentares. Em pacientes com susceptibilidade genética para o desenvolvimento de alterações lipídicas, recomenda-se evitar o tratamento com os antirretrovirais que estão mais relacionados com dislipidemia. A monitorização cuidadosa e tratamento preventivo ajudam a otimizar a relação custo/benefício das terapias antirretrovirais (CALZA et al., 2004; GUIMARÃES et al., 2007; OH; HEGELE, 2007).

2 JUSTIFICATIVA

De acordo com os relatos da literatura, fica evidente a importância do estudo das alterações do perfil lipídico, da SM e do aumento na incidência de DCV em pacientes infectados pelo HIV-1, situações que têm como etiologia diferentes fatores genéticos e ambientais. Entre os fatores genéticos associados às alterações nos níveis circulantes de lipídeos, variações no *LDLR* têm sido extensamente avaliadas em populações de diferentes etnias e características clínicas, entre elas a população brasileira (Salazar et al., 2000a; 2000b; 2000c; 2000d). No entanto, não se tem conhecimento da avaliação do polimorfismo *Pvu*II no intron 15 do *LDLR* em indivíduos infectados pelo HIV-1.

O estudo da frequência da dislipidemia, das alterações metabólicas e do polimorfismo *Pvu*II no intron 15 do *LDLR* em indivíduos infectados pelo HIV-1 atendidos em serviços especializados de saúde de Londrina, Paraná, e a sua associação com o uso de HAART por estes pacientes poderá contribuir para o melhor entendimento dos mecanismos fisiopatológicos da infecção pelo HIV-1, além de proporcionar uma reavaliação das medidas de controle e manejo dos pacientes que vivem com HIV/aids em nossa população. Informações sobre o papel deste polimorfismo no *LDLR* poderão contribuir para a caracterização de um genótipo ou variante alélica que possa ser utilizado como marcador genético na identificação de indivíduos que teriam maior chance de apresentar a dislipidemia como um dos efeitos adversos da terapia antirretroviral.

Os resultados obtidos neste estudo poderão, também, indicar uma possível relevância da inclusão na rotina laboratorial de testes de genotipagem deste polimorfismo. Indivíduos infectados pelo HIV-1 que apresentem um determinado genótipo do *Pvu*II no intron 15 do *LDLR* associado à dislipidemia poderiam ser beneficiados com estratégias terapêuticas diferentes, tanto de HAART como de hipolipemiantes, ou submetidos a um monitoramento clínico e laboratorial em intervalos menores de tempo, ou ambos os procedimentos.

3 OBJETIVOS

3.1 OBJETIVO GERAL

- Avaliar as alterações metabólicas e o polimorfismo genético *Pvu*II no intron 15 do *LDLR* em pacientes infectados pelo HIV-1, atendidos no Centro Integrado de Doenças Infecciosas (CIDI) da 17ª Regional de Saúde do Paraná e Ambulatório do Hospital das Clínicas da Universidade Estadual de Londrina.

3.2 OBJETIVOS ESPECÍFICOS

- Descrever as características demográficas como sexo, etnia e idade; e antropométricas como índice de massa corpórea (IMC) e circunferência abdominal (ca) em pacientes infectados pelo HIV-1 (virgens de tratamento e em tratamento com HAART) e em indivíduos controles;
- Determinar os níveis séricos de TG, colesterol total e as frações HDL-C e LDL-C em pacientes infectados pelo HIV-1 (virgens de tratamento e em tratamento com HAART) e em indivíduos controles;
- Determinar a associação entre os genótipos do polimorfismo *Pvu*II do *LDLR* e a ocorrência de dislipidemia em pacientes infectados pelo HIV-1 virgens de tratamento e em tratamento com HAART.

THIS MANUSCRIPT HAS BEEN ACCEPTED FOR PUBLICATION IN BIOMED RESEARCH INTERNATIONAL.

THE ROLES OF GENETIC POLYMORPHISMS AND HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION IN LIPID METABOLISM

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Elaine Regina Delicato de Almeida[1,2], Edna Maria Vissoci Reiche[1], Ana Paula Kallaur[3], Tamires Flauzino[4], Maria Angelica Ehara Watanabe[5]

[1]Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil;

[2]Pathological Sciences Postgraduate Program, Biological Sciences Center, State University of Londrina, Londrina, Paraná, Brazil.

[3]Postgraduate Program of Health Sciences, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil;

[4]Clinical Immunology, Clinical Analysis Laboratory, University Hospital, State University of Londrina, Londrina, Paraná, Brazil;

[5]Department of Pathological Sciences, Biological Sciences Center, State University of Londrina, Londrina, Paraná, Brazil.

RUNNING TITLE: HIV infection and dyslipidemia

Corresponding author: Edna Maria Vissoci Reiche, Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, Londrina State University, Av. Robert Koch, 60, CEP 86.038-440, Londrina, Paraná, Brazil. Phone/FAX number: + 55-43-3371-2619. e-mail: reiche@sercomtel.com.br

ABSTRACT

Dyslipidemia has been frequently observed among individuals infected with human immunodeficiency virus type 1 (HIV-1), and factors related to HIV-1, the host, and antiretroviral therapy (ART) are involved in this phenomenon. This study reviews the roles of genetic polymorphisms, HIV-1 infection, and highly active antiretroviral therapy (HAART) in lipid metabolism. Lipid abnormalities can vary according to the HAART regimen, such as those with protease inhibitors (PIs). However, genetic factors may also be involved in dyslipidemia because not all patients receiving the same HAART regimen and with comparable demographic, virological, and immunological characteristics develop variations in the lipid profile. Polymorphisms in a large number of genes are involved in the synthesis of structural proteins, and enzymes related to lipid metabolism account for variations in the lipid profile of each individual. As some genetic polymorphisms may cause dyslipidemia, these allele variants should be investigated in HIV-1-infected patients to identify individuals with an increased risk of developing dyslipidemia during treatment with HAART, particularly during therapy with PIs. This knowledge may guide individualized treatment decisions and lead to the development of new therapeutic targets for the treatment of dyslipidemia in these patients.

Key words: Genetic polymorphism, dyslipidemia, HIV-1, cholesterol, HAART.

1 INTRODUCTION

Serum lipids have a multifactorial etiology that is determined by a large number of environmental and genetic factors [1]. Genetic and dietary factors influence serum cholesterol concentration, but detailed mechanisms of their interactions are not well known. An increase in dietary cholesterol intake raises serum cholesterol concentrations in some but not all subjects.

Human immunodeficiency virus type 1 (HIV-1) infected patients develop dyslipidemia, resulting in a highly atherogenic lipid profile with increased levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) and decreased levels of high-density lipoprotein cholesterol (HDL-C) [2]. The pathogenesis of dyslipidemia in HIV-1 infection is complex and involves factors related to the virus, the host, and to the antiretroviral therapy (ART). Moreover, HIV-1 infection and ART are associated with accelerated atherosclerosis and an increased number of cases of myocardial infarction [3].

Highly active antiretroviral therapy (HAART) consists of a combination of drugs that inhibit different stages of viral replication, and it is divided mechanistically into six classes [3] based on whether it targets the viral lifecycle or viral enzymes: nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitor (enfuvirtide or T-20), entry inhibitor chemokine receptor 5 (CCR5) antagonist maraviroc, and HIV-1 integrase strand transfer inhibitor [4, 5].

The introduction of HAART in 1996 dramatically reduced the mortality and morbidity in HIV-1-infected patients, leading to prolonged and improved quality of life and making HIV-1 infection a manageable chronic disease [6]. HAART uses combination formulations containing at least three antiretroviral drugs that are extremely effective in reducing the plasma viral load of HIV-1 RNA to undetectable levels [4, 7, 8].

However, it is increasingly clear that HIV-1-infected patients exhibit an increased risk of developing non-infectious consequences of HIV-1 infection over time. In the last few years, lipodystrophy (characterized by body fat redistribution), insulin resistance, central adiposity, and dyslipidemia have been reported in HIV-1-infected patients, and their relationships with antiretroviral drugs and HIV-1 infection are the subject of global debate and research [9]. Moreover, HAART can induce severe metabolic complications, such as insulin resistance, metabolic syndrome,

lipodystrophy, and cardiovascular diseases. The metabolic effects of HAART and the risk of premature and accelerated atherosclerosis in HIV-1-infected patients are well recognized. These clinical conditions have significantly high prevalence in patients infected with HIV-1 that are treated with these drugs [10].

The type and severity of lipid abnormalities vary according to the HAART regimen used. However, genetic factors may be involved in dyslipidemia because not all patients exposed to same HAART regimen and comparable demographic, virological, and immunological characteristics develop lipid profile variations [11-13].

Many polymorphic variants of the genes that regulate lipid metabolism are present in humans, and more than 400 genes are candidate regulators of lipid exchange. Carriers of abnormal alleles exhibit a high risk for obesity and its associated complications, and therefore there is the interest in the association between dyslipidemia, adiposity, and other diseases with different genotypes. The genes involved in the leptin-melanocortin system of regulation of energy metabolism, protein carriers of lipids and cholesterol in the blood, and enzyme-splitting lipids are of particular interest [14].

Genetic variations of enzymes, receptors, and apolipoproteins (apo), which are essential to LDL-C metabolism, are partially involved in the regulation of serum LDL-C and total cholesterol [15]. Recently, the genetic components of dyslipidemia have been intensively investigated. Variations in a large number of genes involved in the synthesis of structural proteins and enzymes associated with lipid metabolism account for variations in the lipid profile of each individual [1].

Genetic variations that occur at a frequency of more than 1% in a study population are called genetic polymorphisms. The genetic basis for these variations can be a single nucleotide change in the DNA sequence, known as single nucleotide polymorphisms (SNPs), insertions or deletions (indels) of one or more base pairs [16], repeats of a large number of nucleotides (variable number of tandem repeats (VNTR) or minisatellite), and repeats of a small number of nucleotides (short tandem repeat (STR) or microsatellite). SNPs are the most common type of sequence variation in the human genome. The 10 to 30 million SNPs in humans represent 90% of all sequence variations [17].

The effect of a polymorphism depends on its interactions with environmental factors that predispose patients to dyslipidemia, such as being overweight, physical inactivity or smoking [18-20].

There are several factors that can trigger the atherogenic process, including dyslipidemia, smoking, hypertension, diabetes mellitus, physical inactivity, obesity, and a history of premature atherosclerotic disease. However, dyslipidemia is a major risk factor for developing coronary artery disease (CAD) [21].

Among the genetic factors associated with CAD are variations in the genetic loci responsible for the lipoprotein structure and metabolism and the low-density lipoprotein receptor (LDLR), which may contribute to the development of CAD. Some of these genetic variations are associated with increased serum levels of lipids, and therefore, they may be associated with a high risk of CAD [15, 22, 23]. There is a direct relationship between the onset of CAD and high LDL-C because these particles contribute to atherosclerotic plaques [24]. The opposite effect is observed when HDL-C is high. This circulating lipoprotein has the protective effect of reversing cholesterol transport and promotes a set of anti-inflammatory, antioxidant, and anticoagulant actions that inhibit atherosclerosis [25].

CAD is the main cause of mortality in many parts of the industrialized world [26]. In Brazil, CAD is the major cause of mortality and morbidity in women over the age of 40 or 50 years [27]. Hence, the early identification of subjects at risk of developing CAD is an important public health issue. Salazar *et al*. [28] showed that Brazilian women with CAD had elevated total serum cholesterol, TG, and LDL-C concentrations. These results confirm the well-known association between CAD and high lipid concentration. According to Salazar *et al.* [23], common DNA polymorphisms in genes associated with lipid metabolism are potentially important genetic markers of variation in the plasma lipid profile and thus susceptibility or resistance to CAD.

Myocardial infarction, angina pectoris, and ischemic stroke resulting from atherosclerosis are the main causes of morbidity and mortality in adults in developed and developing countries [21]. A study showed that 38% of men and 42% of women in Brazil exhibit elevated serum cholesterol [29]. Lipid profile data and the study of polymorphisms in genes encoding structural proteins and enzymes regulating lipid metabolism reveal the prevalence of dyslipidemia in a population, allowing targeted intervention for the control and prevention of atherosclerotic diseases [1, 30].

The considerable improvement in the rates of morbidity and mortality among HIV-1-infected patients due to HAART has progressively transformed the infection into a chronic disease [6, 7, 31, 32]. Given the increased life expectancy of these

patients, a systematic evaluation of their risk for early cardiovascular events is important [10].

Considering the importance of determining the contribution of genetic polymorphisms to the multifactorial etiology of dyslipidemia, this study reviews the genetic polymorphisms associated with changes in serum lipids and assesses the role of these polymorphisms in lipid changes in patients with HIV-1.

2 DYSLIPIDEMIA IN HIV-1-INFECTED PATIENTS

Dyslipidemia is frequently observed in HIV-1-infected patients. Its pathogenesis is complex and includes factors related to the virus, the host, and the ART. Antiretroviral drugs are associated with a state of accelerated atherosclerosis and an increase in the number of cases of myocardial infarction [3]. Cardiovascular reactions are diverse, due to the HIV-1 infection itself, autoimmunity, immune responses against other viral infections, neoplasms, prolonged immunosuppression, malnutrition, drug cardiotoxicity [33, 34], and hormonal changes [35].

2.1 The role of HIV-1 Infection

HIV-1-associated dyslipidemia was recognized for years before the widespread use of PI-based HAART [36, 37]. Viremia-associated dyslipidemia is characterized by decreased plasma concentrations of total cholesterol, LDL-C, and HDL-C and elevated plasma TG [38-40]. Low HDL-C is correlated with immune activation early in the course of HIV-1 infection [41], the repercussions of which may extend beyond atherosclerosis because of the numerous functions of HDL-C, including antioxidant and anti-inflammatory activities [42-45]. HIV-1 is also associated with an increase in acute phase HDL that lacks the normal atheroprotective functions [46].

Cholesterol is critical for several steps in HIV-1 replication. HIV-1 decreases plasma HDL-C by impairing the cholesterol-dependent efflux transporter ATP-binding cassette protein A1 (ABCA1) in human macrophages, a condition that is highly atherogenic [47]. Additionally, the inflammation stimulates endothelial lipase and certain acute phase proteins, such as serum amyloid A. The plasma level of this enzyme in humans is inversely associated with HDL-C, and the acute phase proteins accelerate the removal of HDL-C by macrophages [45].

The dyslipidemia in HIV-1-infected patients resembles that observed in other chronic infections [48]. The chronic inflammatory processes are characterized by the

production of proinflammatory cytokines, such as tumor necrosis factor α (TNFα) and interferon α (IFNα), resulting in the impaired clearance of TG-rich lipoproteins and insulin resistance [49]. Moreover, the nutritional state of HIV-1-infected patients, who may undergo weight loss and protein depletion, might contribute to reduced total plasma cholesterol, HDL-C, and LDL-C levels [38, 50].

Figure 1 illustrates several effects of HIV-1 infection on lipid metabolism and regulation.

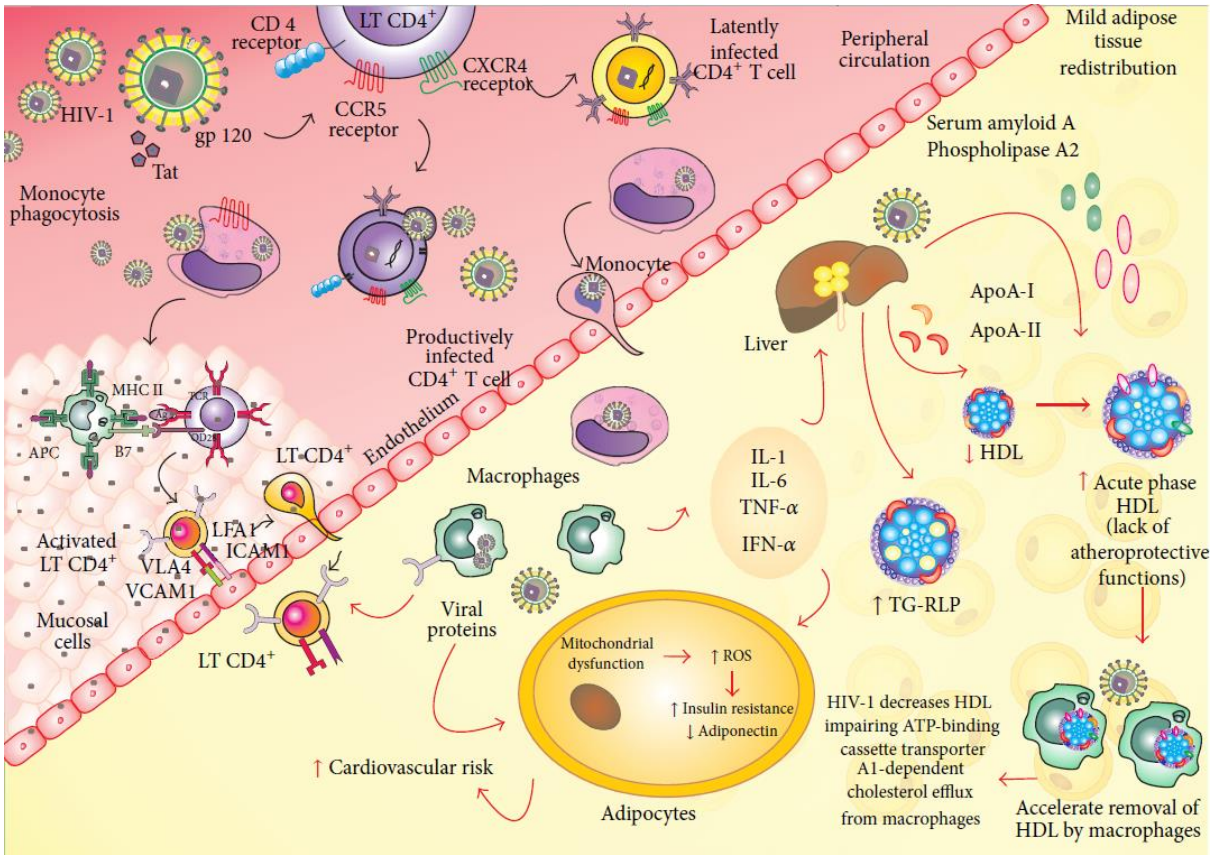

Figure 1 At tissues, human immunodeficiency virus type 1 (HIV-1) infects macrophages using the CD4 receptor and the CCR5 coreceptor and induces the local immune response. At peripheral circulation, HIV-1 infects the Th1 $CD4^+$ cells, particularly by the coreceptor CXCR4 that persist latently infected or become a productively infected cell. The viral proteins induce an proinflammatory response in peripheral circulation and in the tissues, decrease plasma HDL-C by impairing the cholesterol-dependent efflux transporter ATP-binding cassette protein A1 (ABCA1) in human macrophages, a condition that is highly atherogenic. Additionally, the viral proteins and the proinflammatory cytokines interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor α (TN-Fα), and interferon α (IFN-α) stimulate endothelial lipase and certain acute phase proteins, such as serum amyloid A. The viral proteins also

exert effects on the adipocytes resulting mitochondrial dysfunction, reactive oxygen species (ROS) production, insulin resistance, and decrease adiponectin. The chronic inflammatory processes increase the production of these proinflammatory cytokines, resulting in the impaired clearance of triglyceride-rich lipoproteins (TG-RLP) and insulin resistance. All these mechanisms increase the risk of cardiovascular diseases in the HIV-1-infected individuals.

2.2 The role of ART

HAART reduces the frequency of opportunistic infections and the number of AIDS-related deaths [6]. However, despite the improvements in quality of life and increased life expectancy gained with the continuous use of HAART, metabolic disorders characterized by hyperglycemia, dyslipidemia, and changes in the distribution of body fat (lipodystrophy) have been observed in HIV-1 seropositive patients [51].

The pathogenesis of HAART-related dyslipidemia is multifactorial and involves various drug-induced effects, chronic inflammatory status, hormonal influences, genetic predisposition, and HIV-1 infection itself [52].

The dyslipidemia associated with HAART is characterized by decreased plasma HDL-C and increased total cholesterol, TG, and LDL-C, which together constitute a highly atherogenic lipid profile [53].

HAART-related dyslipidemia appears mainly with the use of PIs. PIs may increase the hepatic synthesis of TG, VLDL-C, and to a lesser extent, cholesterol. Additionally, these drugs impair the hydrolysis of TG-rich lipoproteins by lipase, reduce free fatty acid trapping, and interfere with normal postprandial free fatty acid metabolism [54].

The treatment of HIV-1-infected patients is related to lipodystrophy, and dyslipidemia primarily affects those who use PIs. According to Carr *et al.* [55] and Chi *et al*. [56], over 60% of patients who are treated with PIs develop metabolic changes, such as hyperlipidemia, endothelial dysfunction, hyperglycemia, and central obesity. Persistent dyslipidemia in HIV-1-infected patients appears to be associated with increased cardiovascular risk, with a relative rate of myocardial infarction of 1.2 per year of PI exposure [57, 58].

One proposed mechanism of PI-induced dyslipidemia is based on the structural similarity between the catalytic region of HIV-1 protease and the LDL-receptor-

related protein (LRP). This receptor is a member of the LDLR superfamily and participates in lipid metabolism. LRP normally binds to lipoprotein lipase (LPL) on the capillary endothelium, which hydrolyzes fatty acids from TG to promote free fatty acid storage in adipocytes. PIs bind to LRP due to this structural similarity and interfere with LRP-LPL complex formation; as a result, they reduce the adipose storage capacity and increase plasma TG-rich lipoproteins [59].

PI-induced dyslipidemia is also based upon the structural similarity with the amino acid sequence of the C-terminal region of cytoplasmic retinoic acid-binding protein type 1 (CRABP-1). During normal lipid metabolism, CRABP-1 converts retinoic acid to cis-9-retinoic acid, which binds the retinoid X receptor-peroxisome proliferator-activated receptor γ (RXR-PPARγ) heterodimer found in adipocyte nuclei, inhibiting adipocyte apoptosis and stimulating adipocyte proliferation and differentiation. PIs likely bind to CRABP-1, increasing apoptosis and diminishing the proliferation of peripheral adipocytes [59, 60].

PIs also suppress the proteasome-mediated degradation of sterol regulatory element binding proteins (nSREBPs) in the liver and adipocytes. These transcription factors stimulate fatty acid and TG synthesis in the liver and adipose tissue and control several steps of cholesterol synthesis. The hepatic accumulation of nSREBPs increases TG and cholesterol biosynthesis, whereas accumulation in adipose tissue causes insulin resistance, reduced leptin expression and lipodystrophy [61].

In vitro, PIs and NRTIs increase the expression and secretion of pro-inflammatory cytokines, such as TNF-α, interleukin 6 (IL-6), and interleukin 1β (IL-1β), that are involved in altered adipocyte functions and decreased adiponectin. These alterations are also observed in fat and serum from HIV-1-patients with lipodystrophy that are treated with these drugs [62]. Upon entry into the cell, NRTIs are metabolized to the active triphosphorylated form and can be utilized as substrates by the mitochondrial DNA polymerase γ. Subsequently, they may inhibit mitochondrial DNA (mtDNA) replication and/or increase the number of mutations in mtDNA. This can lead to mtDNA depletion, the disruption of oxidative phosphorylation, decreases in ATP production, increases in reactive oxygen species and, ultimately, inappropriate mitochondrial and cellular toxicity.

HAART-related dyslipidemia may involve genetic predisposition, as not all patients taking HAART develop comparable metabolic disturbances [48]. In a study of 745 HIV-infected participants, Rotger *et a*l. [30] demonstrated that 42 SNPs of

genome-wide contribute to the development of dyslipidemia independent of other genetic variables, HAART, underlying conditions, sex, age, ethnicity, and HIV disease parameters. The genetic background alone explained up to 7.6% of lipid variation in HIV-infected patients (7.6% non-HDL cholesterol, 6.2% HDL-C and 6.8% TG), and HAART alone explained up to 6.2% of lipid variation (3.9% non-HDL cholesterol, 1.5% HDL-C and 6.2% TG). An individual with the most dyslipidemic antiretroviral and genetic background risk factors exhibits three- to fivefold increased risk of sustained dyslipidemia compared with an individual with the fewest dyslipidemic therapy and genetic background risk factors.

Figures 2 and 3 illustrate the main mechanisms involved in dyslipidemia associated with the PI and NRTI ART regimens, respectively.

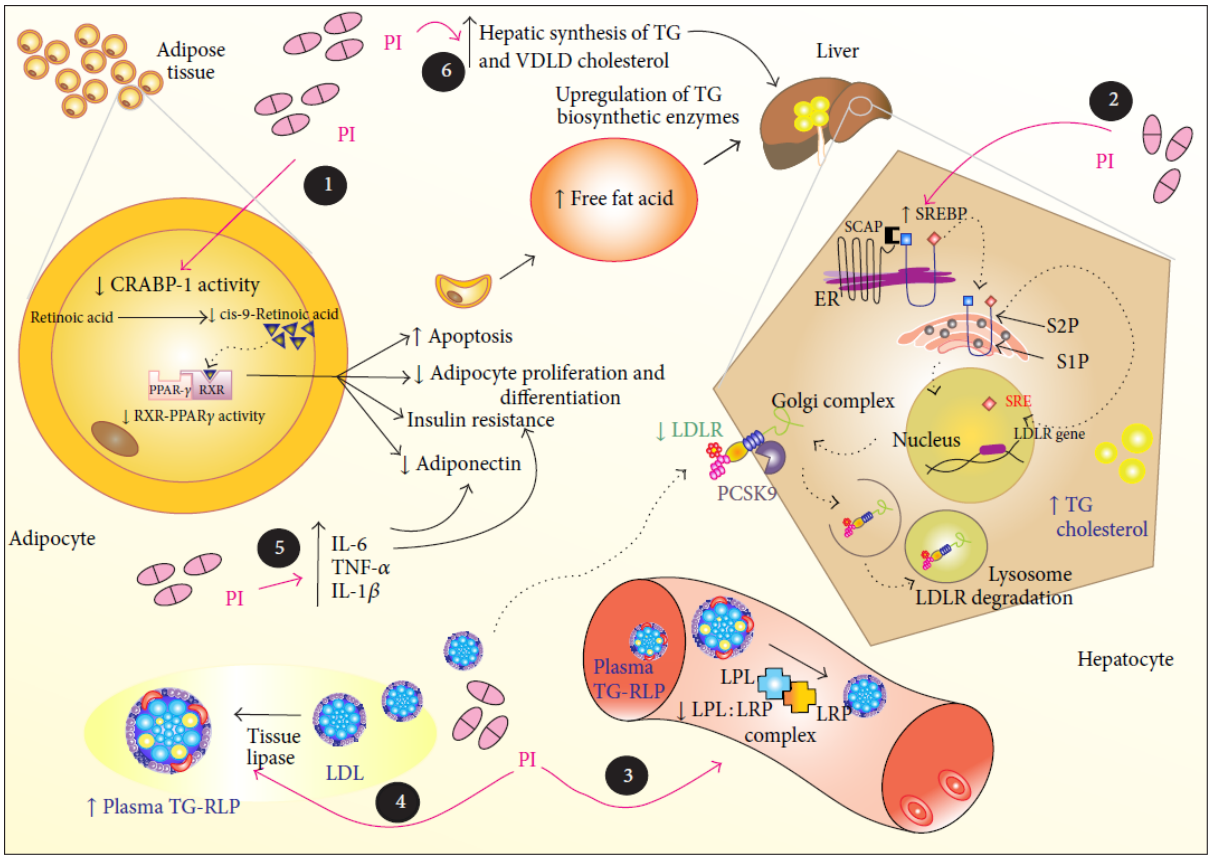

Figure 2 The dyslipidemia associated with protease inhibitor (PI) is characterized by decreased plasma high-density lipoprotein cholesterol (HDL-C) and increased total cholesterol, triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C), which together constitute a highly atherogenic lipid profile. Several mechamisms are proposed, such as: 1) The PI-induced dyslipidemia is based upon the structural similarity with the amino acid sequence of the C-terminal region of cytoplasmic retinoic acid-binding protein type 1 (CRABP-1). The PI likely binds to

CRABP-1, increasing apoptosis and diminishing the proliferation of peripheral adipocytes; 2) PI suppresses the proteasome-mediated degradation of sterol regulatory element binding proteins (nSREBP) in the liver and adipocytes. These transcription factors stimulate fatty acid and TG synthesis in the liver and adipose tissue and control several steps of cholesterol synthesis. The hepatic accumulation of nSREBP increases TG and cholesterol biosynthesis, whereas accumulation in adipose tissue causes insulin resistance, reduced leptin expression and lipodystrophy; 3 and 4) PI-induced dyslipidemia is also based on the structural similarity between the catalytic region of HIV-1 protease and the LDL-receptor-related protein (LRP). PI binds to LRP due to this the structural similarity and interferes with LRP-LPL complex formation, as a result, reduces the adipose storage capacity and increases plasma TG-rich lipoproteins; 5) PI also increases the expression and secretion of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin 1β (IL-1β), which are involved in altered adipocyte functions and decreased adiponectin; and 6) PI increases the hepatic synthesis of TG, very-low density lipoprotein cholesterol (VLDL-C), and to a lesser extent, cholesterol.

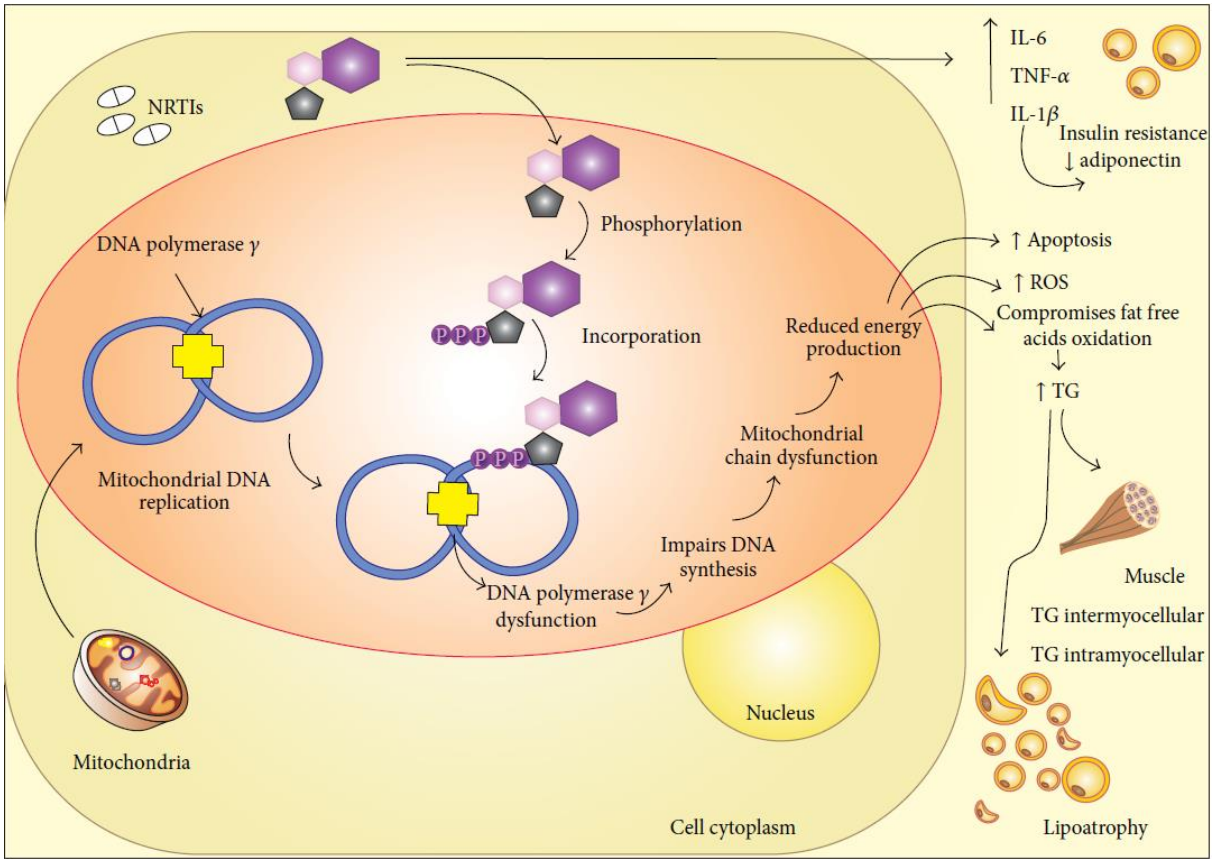

Figure 3 Some mechanisms are proposed to explain the effects of nucleoside reverse transcriptase inhibitors (NRTIs) in the lipid profile of human immunodeficiency virus type 1 (HIV-1)-infected individuals treated with this class of antiretroviral therapy. 1) NRTIs increase the expression and secretion of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin 1β (IL-1β), that are involved in altered adipocyte function, insulin resistance, and adiponectin expression; 2) Upon entry into the cell, NRTIs are metabolized to the active triphosphorylated form and can be utilized as substrates by the mitochondrial DNA polymerase γ. Subsequently, hey may inhibit mitochondrial DNA (mtDNA) replication and/or increase the number of mutations in mtDNA. This can lead to mtDNA depletion, the disruption of oxidative phosphorylation, decreases in ATP production, increases in reactive oxygen species (ROS) and, ultimately, inappropriate mitochondrial and cellular toxicity.

3 GENETIC POLYMORPHISMS ASSOCIATED WITH DYSLIPIDEMIA

Polymorphisms in genes associated with dyslipidemia in patients with HIV-1 infection, either treated with ART or untreated, are reviewed.

3.1 Polymorphisms in the *LDLR* gene

The LDLR plays a major role in the removal of LDL-C particles from the blood, which, in turn, regulates cholesterol homeostasis. The LDLR modulates plasma levels of LDL-C by regulating LDL-C particle uptake by the liver. It also delivers cholesterol to the adrenal gland and gonads for steroid hormone synthesis and to the liver for bile acid synthesis [63].

Many mutations in the *LDLR* gene have been identified in patients with familial hypercholesterolemia (FH) [64-66]. Individuals with these mutations exhibit plasma cholesterol concentrations that are elevated twofold or more above normal concentrations and have an increased risk of developing atherosclerosis and CAD [63]. Considering the crucial role of LDLR in cholesterol homeostasis, SNPs in the *LDLR* gene may also contribute to the variation in plasma cholesterol levels in the general population [23].

Located on chromosome 19p13.2, the *LDLR* gene comprises 18 exons and 17 introns and encodes a protein of 839 amino acids [67]. More than 1,288 different variants in the *LDLR* gene have been reported in FH patients, as follows: 55% exonic

substitutions, 22% exonic small rearrangements (<100 bp), 11% large rearrangements (>100 bp), 2% promoter variants, 10% intronic variants, and 1 variant in the 3' untranslated sequence [68].

The polymorphic nature of the *LDLR* gene has been demonstrated by its restriction fragment length polymorphisms (RFLPs) [35, 69]. The *Ava*II (T20001C, rs5925), *Hinc*II (C16730T, rs688) [23] and *Pvu*II (C>T, intron 15) polymorphisms in *LDLR* are associated with differences in serum lipid concentrations in Brazilian subjects with high risk for CAD [15].

Salazar *et al*. [23] investigated the effects of *LDLR* gene polymorphisms at the *Ava*II site in exon 13 (T20001C, rs5925) and the *Hinc*II site in exon 12 (C16730T, rs688) on circulating lipids of 170 unrelated white individuals presenting a lipid profile with high risk for coronary heart disease (HRG) and 130 controls. CHD subjects showed a higher frequency of the *Ava*II (A+) and *Hinc*II (H+) alleles compared with controls, and the frequency of the A+A+ (*Ava*II) and H+H+ (*Hinc*II) genotypes was greater in the HRG group than in the control group (32 *vs.* 16% and 32 *vs.* 18%, respectively). Moreover, in the HRG group, the A+A+ and H+H+ genotypes were associated with high concentrations of total serum cholesterol and LDL-C ($p = 0.0001$). Interestingly, neither the *Ava*II (rs5925) nor *Hinc*II (rs688) polymorphism was observed to affect serum lipid profiles in control individuals [23]. The strong association between A+A+ (*Ava*II) and H+H+ (*Hinc*II) genotypes with high total cholesterol and circulating LDL-C levels shows that *LDLR* genetic polymorphisms affect cholesterol levels in individuals with a high risk of CAD. Additionally, common polymorphisms in the *LDLR* gene are associated with inter-individual differences in plasma LDL-C levels in normal and hypercholesterolemic subjects [70-73].

The *Pvu*II intron 15 polymorphism is linked to other variations in *LDLR* that structurally alter the receptor activity or alter its function in a regulatory manner [73]. A *Pvu*II intron 15 polymorphism of the *LDLR* gene is associated with differences in LDL-C concentration in normal and hypercholesterolemic individuals from different countries [74, 75]. Salazar *et al.* [15] demonstrated the influence of *Pvu*II intron 15 polymorphisms of *LDLR* on serum lipid profiles in individuals with low or high risk for CAD (HRG). The authors analyzed 128 white subjects with lipid profiles suggesting HRG and 100 white normolipidemic individuals (controls). The P1P1 genotype frequency for the *Pvu*II intron 15 polymorphism (homozygous for the absence of a restriction site) was greater in HRG-affected individuals than in control subjects (57%

vs. 38%, $p<0.05$). Moreover, this genotype was strongly associated with high total cholesterol, TG, LDL-C, and VLDL-C and low HDL-C in HRG patients. Similarly, the control individuals with the P1P1 genotype presented higher concentrations of total cholesterol and LDL-C compared to those with other genotypes (P1P2 and P2P2) [15].

In a study of Brazilian Caucasian women with CAD, Salazar *et al*. [28] showed that the A+A+ and P1P1 homozygous genotypes (*Ava*II and *Pvu*II polymorphisms in the *LDLR* gene, respectively) were significantly higher in women with CAD than in the control group (44% *vs.* 16%, $p<0.001$ and 64% *vs*. 39%, $p<0.05$, respectively). Similarly, the frequency of the A+ and P1 alleles observed among women with CAD was higher than in controls (62% *vs*. 44%, $p<0.05$ and 78% *vs.* 65%, $p<0.05$, respectively). For the *Hinc*II polymorphism in *LDLR*, no significant difference in genotype distribution or in relative allele frequencies was observed between patients and controls.

Salazar *et al.* [76] also evaluated the *Ava*II (exon 13), *Hinc*II (exon 12), and *Pvu*II intron 15 polymorphisms in 50 unrelated Brazilian individuals clinically diagnosed as FH heterozygotes and in 130 normolipidemic controls. The FH subjects showed higher frequencies of A+A+ (*Ava*II), H+H+ (*Hinc*II), and P1P1 (*Pvu*II) homozygous genotypes compared with the control group ($p<0.05$). In addition, FH subjects presented higher frequencies of A+ (58%), H+ (61%), and P1 (78%) alleles compared with normolipidemic individuals (45%, 45%, and 64%, respectively). The strong association observed between these alleles and FH suggests that *Ava*II, *Hinc*II, and *Pvu*II polymorphisms could be useful for monitoring FH inheritance in Brazilian families.

3.2 *Apo E* gene polymorphism

The apo E protein is incorporated in the structure of HDLs-C, very low-density lipoproteins cholesterol (VLDLs-C), chylomicrons, and lipolytic degradation products (i.e., the remnants of chylomicrons and intermediate density lipoprotein cholesterol (IDL-C)). This plasma protein binds to cellular receptors. Furthermore, it is important for the transport of cholesterol and other lipids from peripheral tissues to the liver, where they are metabolized [77, 78].

Apo E is also important for the catabolism of TG-rich lipoproteins and reverse cholesterol transport in various tissues [79], which involves its binding to LDLR and

the apo E hepatic receptor, the activation of enzymes including hepatic lipase, and hepatic production of VLDL-C [80, 81]. The LDLR in the liver can clear both LDL- and apo E-containing lipoproteins, but the LRP-mediated clearance of remnants is absolutely dependent on apo E [82]. Moreover, apo E influences enteral cholesterol absorption, immunoregulation, and neurobiological events such as neuronal repair, remodeling, and protection [83, 84].

Apo E is synthesized primarily in the liver (>90%) and also in the gut, brain, lungs, kidneys, and macrophages, and it is secreted as a glycosylated protein [83] (SCHWANKE et al., 2002). In addition to its important effects on lipid metabolism, vascular disease, and cholesterol modulation, apo E also regulates the growth of smooth muscle cells in the arterial wall, which impacts the progression or regression of atherosclerotic lesions [85].

The *apo E* gene is located on the long arm of chromosome 19 and encodes a protein of 299 amino acids [79]. According to Andrade and Hutz (2002), the *apo E* gene exerts a strong influence on the serum levels of LDL-C.

The *apo E* gene has a common polymorphism, *Hha*I (T112C, rs429358 and C158T, rs7412), which is located in exon 4 and generates three alleles, ε2, ε3, and ε4; these alleles determine the six genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4) [79-84]. The allele frequencies differ significantly between ethnic groups [86, 87], but ε3 is the most common allele in several populations [88].

According to Schwanke et al. [83], the *apo E* polymorphisms modify the protein structure and function. Apo E isoforms interact differently with lipoprotein receptors, altering their metabolism and consequently the plasma level of the circulating lipids [89].

According to Davignon *et al*. [90], in industrialized societies, individuals carrying the ε4 allele exhibit high serum levels of total cholesterol and LDL-C, while individuals carrying the ε3 allele exhibit intermediate levels, and those carrying the ε2 allele present the lowest levels. Hallman *et al*. [86] reported that associations between the ε4 allele and increased total and LDL-C levels and between the ε2 allele and low levels of these lipids have been documented in many studies, independently of ethnic group.

The association between *apo E* polymorphisms and CAD has been studied with regard to cardiology, as apo E affects lipoprotein metabolism and cholesterol transport [80, 81, 91]. The *apo E* ε4 allele is consistently associated with an

increased risk of CAD, although its impact seems to vary according to other factors, such as gender, ethnic origin, and lifestyle [90, 92, 93].

Salazar *et al*. [28] demonstrated that the *Hha*I polymorphism in the *apo E* gene is strongly associated with CAD. Brazilian women with CAD present a higher frequency of the ε3/ε4 genotype compared with controls (40% *vs.* 14%, $p<0.001$). In addition, women with CAD present a higher frequency of the ε4 allele compared with controls (23% *vs.* 11%, $p<0.05$), suggesting that this allele promotes premature CAD. However, in a study of 184 Afro-Brazilian individuals, the *Hha*I polymorphism in *apo E* was not associated with hypertension or variations in serum lipid concentrations [94].

3.3 *Apo B* gene polymorphisms

Apo B is the major protein in human LDL-C and VLDL-C, and it is synthesized in the liver and intestine. This protein is essential for the assembly, secretion, and metabolism of lipoprotein particles and for the removal of LDL-C from the circulation by LDLR on cell surfaces [63, 95].

Structural and genetic alterations in *apo B* are associated with defective binding to LDLR and lead to hypercholesterolemia, an important risk factor for atherosclerosis and premature CAD [96-98].

The *apo B* gene is located on chromosome 2p23-p24, and several mutations and SNPs are associated with either variations in plasma lipid concentrations [79] or with CAD and myocardial infarction [99-101]. The SNPs in *apo B* include the *Xba*I at exon 26 (C7673T, rs693), *Eco*RI at exon 29 (G12669A, rs1042031), *Msp*I at exon 26 (rs676210), an indel at exon 1 within the signal peptide (rs17240441), and a hypervariable region at the 3' end (3'HVR) [102, 103].

Polymorphisms in the *apo B* gene, as evaluated by RFLP using the restriction enzymes *Xba*I (rs693), *Eco*RI (rs1042031), and *Msp*I (rs67210), are also associated with variability in serum cholesterol levels and coronary atherosclerosis [22, 104, 105, 106].

The indel, *Msp*I (rs676210), *Xba*I (rs693), and 3'HVR polymorphisms may be associated with variations in lipid levels, CAD, and myocardial infarction [104, 107, 108, 109, 111, 112], but these findings are controversial [113, 114].

The *Xba*I polymorphism in exon 26 of the *apo B* gene is associated with increased total cholesterol, altered postprandial lipoprotein metabolism, and

increased CAD [110, 115, 116, 117]. The *Eco*RI polymorphism in exon 29 is associated with variations in total cholesterol and TG levels, obesity, and CAD [22, 111, 118, 119]. Furthermore, the signal peptide indel polymorphism is associated with increased serum TG, total cholesterol, and LDL-C [120, 121].

Salazar *et al.* [28] demonstrated that women with CAD present a higher frequency of the X-X- genotype for the *Xba*I polymorphism compared with controls (42% *vs*. 12%, p<0.0001). The frequency of the X- allele is also higher in women with CAD compared with controls (0.66 vs. 0.39, p < 0.0001). The *Xba*I polymorphism is associated with increased total cholesterol, LDL-C, and CAD in Brazilian Caucasian women.

In a study of the genotypes at three polymorphic sites of *ApoB* (the indel at the signal peptide, *Xba*I at exon 26, and *Eco*RI at exon 29), Machado *et al*. [122] reported the simultaneous presence of the rare *X*+ and *Del* alleles (*X*+Del haplotype) in males with CHD was associated with significantly high serum levels of total cholesterol ($p < 0.01$), TG ($p < 0.05$), and LDL-cholesterol ($p < 0.05$) and with a high total cholesterol/HDL-C ratio ($p < 0.05$). These data indicate that a single haplotype, *X+Del,* within the *apo B* gene impacts lipid metabolism and may contribute to CHD susceptibility in Brazilian males.

Cavalli *et al*. [123] investigated four *apo B* gene polymorphisms, *Msp*I, (rs676210), *Xba*I (C7673T, rs693), the indel, and 3'HVR, in 177 white hypercholesterolemic Brazilian subjects and 100 control individuals. The genotype distribution and allele frequency of the *Msp*I, *Xba*I and indel polymorphisms were similar between hypercholesterolemic and control individuals, and the frequency of the alleles with ≤ 43 repeats in the 3'HVR was higher in the hypercholesterolemic group than in the control group (16.4 *vs.* 8.5%, p<0.05). Moreover, these alleles were associated with higher serum total cholesterol hypercholesterolemic individuals ($p< 0.05$). On the other hand, hypercholesterolemic individuals carrying at least one allele with ≤ 43 repeats presented higher total serum cholesterol compared with the individuals carrying both alleles with > 43 repeats. In addition, an association between the indel and 3'HVR polymorphisms was observed. The alleles with ≤ 43 repeats and the *Del* allele were more frequent in the hypercholesterolemic individuals ($p<0.05$). Taken together, these findings show that the *apo B* 3'HVR polymorphism may be an important genetic marker to evaluate the risk of atherosclerotic disease.

3.4 *Apo AI-CIII-AV* gene cluster polymorphisms

Apo A-I, apo C-III and apo A-V are mainly synthesized in the liver [124, 125]. Apo A-I is the major protein found in HDL cholesterol and is a cofactor for lecithin cholesterol acyltransferase (LCAT), the enzyme required for reverse cholesterol transport metabolism [126, 127]. The *Msp*I polymorphism in the promoter region of *apo AI* is associated with differences in the plasma levels of apo AI and HDL-C [128].

ApoC-III is the major apolipoprotein of hepatic VLDL-C and due to the role in the transport and metabolism of cholesterol, it is a candidate for determining genetic associations with serum lipid or lipoprotein levels and dyslipidemia. *In vitro* studies show that apo C-III is a non-competitive inhibitor of LPL activity, which suggests that it plays an important role in TG-rich lipoprotein catabolism [129]. There are several polymorphisms in the *apo C-III* gene, [130]. Genetic variations in the 3' untranslated region of *apo C-III* (*Sst*I polymorphism, rs10892152) are more frequent in hypertriglyceridemic individuals [108, 131].

Apo A-V is observed at lower concentrations than other apolipoproteins; however, studies have shown that it participates in TG metabolism. Apo A-V deficiency is associated with severe hypertriglyceridemia in humans because this apolipoprotein reduces plasma TG by reducing hepatic VLDL-TG production and by enhancing the lipolytic conversion of TG-rich lipoproteins [125, 132]. Three mutations in the *Apo A-V* gene have been described, at positions 148, 139, and 97 (Q148X, Q138X, and Q97X, respectively). These mutations produce three different glutamine nonsense mutations that result in Apo A-V deficiencies.

3.5 *PCSK9* gene polymorphisms

Another protein related to dyslipidemia is proprotein convertase subtilisin/kexin type 9 (PCSK9). The *PCSK9* gene is located on chromosome 1p32, has 12 exons and encodes a 692 amino acid protein. There are several mutations in *PCSK9*, including c.G1120T (p.Asp374Tyr), c.T381A (p.Ser127Arg), c.T646A (p.Phe216Leu), c.A654T (p.Arg218Ser), R46L (rs11591147), and rs11206510. Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia (ADH) [133]. The overexpression of PCSK9 in HepG2 cells accelerates the degradation of cell-surface LDLR through a non-proteasomal mechanism in a post-endoplasmic reticulum compartment and leads to increased total cholesterol and LDL-C [134, 135].

3.6 *Cholesteryl ester transfer protein* gene polymorphisms

Cholesteryl ester transfer protein (CETP) is an enzyme with a key role in HDL-C metabolism. CETP promotes the exchange of TG and cholesterol between lipoproteins, and it transfers cholesteryl esters from HDL-C to other lipoproteins for subsequent absorption of cholesterol by hepatocytes. Cholesteryl esters are transferred to LDL-Cs and VLDL-Cs in exchange for TG [136-138]. By increasing the amount of cholesteryl esters in LDL-Cs and VLDL-Cs, CETP increases the atherogenicity of these lipoproteins. A high plasma CETP concentration is associated with reduced HDL-C, a strong and independent risk factor for atherosclerosis [139, 140].

The *CETP* gene is located on chromosome 16 and contains 16 exons [141, 142]. The protein is expressed primarily in the liver, spleen, and adipose tissue, but low levels have been detected in the small intestine, adrenal glands, heart, kidney, and skeletal muscle [143]. CETP-deficient patients exhibit elevated plasma HDL-C levels and low plasma LDL-C levels [144].

The relationship between plasma CETP, HDL-C and atherosclerosis is complex, and *CETP* gene polymorphisms have been studied to better define this relationship [145]. Polymorphisms at the *CETP* gene locus are associated with the progression of coronary atherosclerosis independently of plasma lipase activity and HDL-C concentration.

The *Taq*IB (rs708272) polymorphism affects lipid transfer activity and HDL-C. *Taq*IB (rs708272) is one of the best studied polymorphisms in *CETP*; it consists of a silent guanine-to-adenine nucleotide substitution in intron 1. The less common allele, B2, is associated with decreased CETP activity, and in normolipemic individuals, this allele is associated with an increase in HDL-C due to decreased CETP activity [18, 146, 147, 148].

3.7 *Lipoprotein lipase* gene polymorphisms

Lipoprotein lipase (LPL) is linked to the vascular endothelium and plays a crucial role in plasma lipoprotein processing. LPL catalyzes TG hydrolysis, which is the limiting step in the removal of TG-rich lipoproteins such as chylomicrons, VLDL-C, and LDL-C from the circulation [149]. LPL acts as a ligand for LDLR-related protein and for the uptake of VLDL-C and LDL-C [150].

The *LPL* gene is located on chromosome 8 (8p22) and it is composed of 10

exons [151, 152]. Its known polymorphisms result in three functional variants: D9N (G28A, rs1801177), S291N (A1127G, rs268), and S447X or *Mnl*I (rs328), and two SNPs located on introns; *Hind*III at intron 8 (T381G, rs320) and *Pvu*II at intron 6 (rs285). Generally, these variants are associated with increased TG, but the S447X mutation, which truncates the last two amino acids of the polypeptide chain, decreases TG [153-155].

The *Hind*III (T381G, rs320) and *Pvu*II (rs285) polymorphisms, located on introns 8 and 6 of the *LPL* gene, respectively, are associated with angiographic CAD. However, Anderson *et al.* [156] demonstrated that *Hind*III (+) allele is moderately associated with CAD, and the *Pvu*II (-) allele is only modestly associated with CAD.

4 GENETIC POLYMORPHISMS ASSOCIATED WITH DYSLIPIDEMIA IN HIV-1 INFECTED PATIENTS

There have been few studies of the effects of the *LDLR* gene on plasma cholesterol in HIV-1-infected patients. Tran *et al.* [157] showed that HIV-1 patients receiving PIs such as nelfinavir have decreased LDLR and LRP mRNA and protein levels, resulting in the reduced functional activity of these two receptors, which are involved in cholesterol metabolism. Moreover, individuals receiving nelfinavir have reduced levels of active SREBP in the nucleus.

Plasma LDL-C levels may be influenced through the regulation of hepatic LDLR expression. The expression of LDLR is under metabolic and hormonal control. Insulin, dehydroepiandrosterone (DHEA), and growth hormone (GH) may stimulate LDLR expression and reduce plasma LDL cholesterol levels [158-160]. Petit *et al*. [35] evaluated the LDLR expression in HIV-patients with or without lipodystrophy. These authors found that HIV-lipodystrophy was associated with low expression of LDLR and that this decreased LDLR expression was independent of DHEA or insulin secretion.

A study of 60 HIV-1-infected patients receiving PI therapy showed an association between *apo C-III* polymorphisms and a genetic predisposition to develop high TG and low HDL-C levels [161]; these authors suggested that *apo C-III* polymorphism genotyping could identify patients who are at risk for both hypertriglyceridemia and lipoatrophy [162]. Foulkes *et al.* [163] showed that there are associations between ethnic differences, *apo C-III* variants and the development of hypertriglyceridemia in HIV-1- infected patients treated with PIs. These authors also

demonstrated that Hispanics carrying the variant alleles at *apo C-III* exhibited smaller TG increases after receiving PIs compared with those carrying the wild-type genotype. According to Aragones *et al*. [164], the *apo C-III* rs10892151 polymorphism predisposes HIV-1-infected patients, especially those treated with PIs, to an unfavorable lipid profile. *Apo A-V* polymorphisms also enhance PI-associated hyperlipidemia [52], and variations in this gene are risk factors for extreme hypertriglyceridemia [165].

Tarr *et al*. [166] evaluated the influence of *apo C-III*, *apo E,* and *TNF* polymorphisms on the risk of ART-associated lipid disorders. No association between TNF and lipoatrophy was observed, whereas apo C-III and apo E contributed to an unfavorable lipid profile in ART-treated HIV-1 infected patients. In another study, 20 SNPs of 13 genes involved in lipid transport and metabolism were evaluated in 438 HIV-infected individuals receiving ART, and the results showed that SNPs in the *ABCA1*, *apo A-V,* and *apo C-III* genes contributed to hypertriglyceridemia, whereas SNPs in the *apo A-V* and *CETP* genes contributed to low HDL-C [11]. In a recent report by Egaña-Gorroño *et al.* [13], 192 SNPs in 87 genes from the lipid metabolism pathway were assessed in 727 HIV-1-infected patients starting ART. The results of this study showed that one SNP in the *apo B* gene (rs10495712) was associated with high LDL-C levels.

5 CONCLUSION

Dyslipidemia leads to atherosclerosis and CAD; thus, understanding the etiology of changes in the lipid profile is extremely important. Dyslipidemia is a complex and multifactorial condition caused by polymorphisms in genes involved in lipid metabolism and regulation and by environmental factors such as smoking, sedentary lifestyle, stress, and diet. The main genes studied in relation to dyslipidemia are those that encode proteins, receptors, and enzymes related to lipid metabolism and regulation. Polymorphisms in the *LDLR*, *apoE*, *apo B*, *apo A-I, apo C-III*, *apo A-V*, *PCSK9*, *CETP*, and *LPL* genes are associated with changes in lipid profile.

Moreover, HIV-1-infected patients often have lipid disorders. The pathogenesis of these disorders is complex and multifactorial, involving viral and host factors and ART. By itself, HIV-1 by causes lipid disorders, and it acts synergistically with ART to generate dyslipidemia, insulin resistance, and lipodystrophy syndrome, especially in

patients who are treated with PIs.

The genetic causes of dyslipidemia in HIV-1-infected patients have been investigated because not all patients who use HAART exhibit metabolic disorders. Some polymorphisms in these patients are associated with lipid profile changes. Moreover, the genetic contribution to dyslipidemia alone explains up to 7.6% of the variation in HIV-1-infected patients, and HAART explains up to 6.2% of the variation. The combination of genotype and ART increases the risk of sustained dyslipidemia in HIV-1-infected individuals by up to 5-fold, with increased plasma concentrations of total cholesterol, LDL-C, and TG and decreased plasma HDL-C.

The genetic contribution to dyslipidemia is similar to or greater than the contribution of HAART. Thus, clinicians should consider genetics and the effects of ART when selecting an antiretroviral regimen for HIV-1 patients. Because gene polymorphisms cause dyslipidemia, they should be investigated in HIV-1-infected patients to identify individuals with an increased risk of developing dyslipidemia when treated with ART, especially those containing PIs. This knowledge could guide individualized treatment decisions and lead to new therapeutic targets for the treatment of dyslipidemia.

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DYSLIPIDEMIA IS ASSOCIATED WIH THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION, ANTIRETROVIRAL THERAPY, AND *PVU*II POLYMORPHISM OF *LOW DENSITY LIPOPROTEIN* IN SOUTHERN BRAZILIAN PATIENTS

Elaine Regina Delicato de ALMEIDA[1,2,] Andréa Name Colado SIMÃO[1], Helena Kaminami MORIMOTO[1,3], Ana Paula KALLAUR[3], Tamires FLAUZINO[4], Daniela Frizon ALFIERI[4], Jair Aparecido de OLIVEIRA[1], Luis Toshio UEDA[5] , Maria Angelica Ehara WATANABE[6]**,** Edna Maria Vissoci REICHE[1]

[1]Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, State University of Londrina, Av. Robert Koch, 60, CEP 86.038-440, Londrina, Paraná, Brazil. Phone/FAX number: + 55-43-3371-2619;

[2]Pathological Sciences Postgraduate Program, Biological Sciences Center, State University of Londrina, Av. Robert Koch, 60, CEP 86.038-440, Londrina, Paraná, Brazil. Phone number: + 55-43-3371-2378;

[3]Postgraduate Program of Health Sciences, Health Sciences Center, State University of Londrina, Av. Robert Koch, 60, CEP 86.038-440, Londrina, Paraná, Brazil. Phone/FAX number: + 55-43-3371-2619;

[4]Pharmacy Course, Health Sciences Center, State University of Londrina, Av. Robert Koch, 60, CEP 86.038-440, Londrina, Paraná, Brazil. Phone/FAX number: + 55-43-3371-2619;

[5] Integrated Center of Infectious Diseases of 17ª Secretariat of Health of Paraná, Londrina, Paraná State. Av. Manoel Ribas, 1, Londrina, Paraná, Brazil;

[6]Laboratory of Study and Applications of DNA Polymorphisms, Department of Pathological Sciences, Biological Sciences Center, State University of Londrina, Londrina; Campus Universitário CEP 86051-990, Londrina, Paraná, Brazil

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Short Title*:* Dyslipidemia and HIV-1 infection

Author for correspondence: Edna Maria Vissoci Reiche, Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, Londrina State University, Av. Robert Koch, 60, CEP 86.038-440, Londrina, Paraná, Brazil. Phone/FAX number: + 55-43-3371-2619. e-mail: reiche@sercomtel.com.br

ABSTRACT

The aim of this study was to evaluate the association between dyslipidemia and the human immunodeficiency type 1 (HIV-1) infection, the highly active antiretroviral therapy (HAART), and the *Pvu*II polymorphism in the intron 15 of *low density lipoprotein receptor (LDLR)* gene. The lipid profile was evaluated from 355 Southern Brazilian HIV-1-infected patients (100 antiretroviral-naïve and 255-HAART experienced), and 116 healthy controls. The *Pvu*II *LDLR* genotypes were determined from the genomic DNA using PCR-RFLP methods. The HIV-1-infected patients presented higher triglycerides (TG) and lower high-density lipoprotein cholesterol (HDL-C) levels than controls ($p<0.0001$). Those HAART-experienced showed higher TG ($p<0.0001$), total cholesterol ($p<0.0001$), and low-density lipoprotein cholesterol (LDL-C, $p=0.0003$) than those HAART-naïve. The frequency of patients with total cholesterol ≥ 200mg/dL, LDL-C ≥ 100mg/dL, and TG ≥ 150mg/dL was higher among those using HAART than those HAART-naïve ($p<0.0001$; $p=0.0248$; and $p=0.0269$; respectively). The frequencies of P1P1, P1P2, and P2P2 *Pvu*II *LDLR* genotypes among the HIV-1-infected patients and controls were 52.4%, 39.1%, and 8.5%; and 55.2%, 42.2%, and 2.6%, respectively. The frequency of high HDL-C was higher among those carrying the P2P2 genotype (11.8% *vs.* 5.6%, $p=0.0415$). The results underscore that HIV-1 infection *per se* and HAART are associated with dyslipidemia in HIV-1-infected patients, changing the total cholesterol, TG, and LDL-C levels. Moreover, the results suggest that the protective P2P2 genotype could be modulating, in part, the effect of HAART and the HIV-1 infection *per se* in the HDL-C levels.These results suggest this genotype as a beneficial genetic marker candidate associated with better lipid in these individuals.

INTRODUCTION

The introduction of the highly active antiretroviral therapy (HAART) in 1996, has dramatically reduced the mortality and morbidity of human immunodeficiency virus type 1 (HIV-1)-infected patients, leading to prolonged and improved quality of life, converting this infection into a chronic manageable disease [1].

It has become clear that HIV-1-infected patients have an increased risk for developing noninfectious consequences of this infection over time. Moreover, this longer life span has exposed them to the effects of aging, and other host and environmental factors known to increase the risk of obesity, diabetes, and cardiovascular disease (CVD) of the general population [2]. In the last few years, dyslipidemia, insulin resistance, central adiposity, oxidative stress, and lipodystrophy have been reported in several HIV-1-infected patients, and their relationship with HAART and HIV-1 infection *per se* have become a subject of debate and researches worldwide [3].

The pathogenesis of dyslipidemia in HIV-1 infection is complex and involves factors related to the virus, the host, and the antiretroviral therapy. This metabolic abnormality is frequently observed in HIV-1 infected patients in use of HAART, demonstrated by changes in lipid profile with increased levels of total cholesterol, low density lipoprotein cholesterol (LDL-C), triglycerides (TG), and decreased high density lipoprotein cholesterol (HDL-C), constituting a highly atherogenic lipid profile [4-7].

To various degrees, multiple HIV-1 viral proteins and antiretroviral drugs activate cell-signaling cascades, induce oxidative stress, disturb mitochondrial function, alter gene expression, and impair lipid metabolism. These changes occur in endothelial cells, in vascular muscle cells, macrophages, adipocytes, and in neuronal cells [8]. HIV-1 infection and HAART are also associated with accelerated atherosclerosis and an increased number of cases of myocardial infarction [9-10].

The type and severity of lipid abnormalities can vary according to the HAART regimen used. However, genetic factors may be involved with dyslipidemia, because not all patients exposed to same HAART regimen and comparable demographic, virological, and immunological, and characteristics develop variations in the lipid profile [11, 12].

Hypercholesterolemia is the major risk factor for atherosclerosis and its

premature cardiovascular complications. This dyslipidemic profile can be multifactorial or less frequent monogenic leading to autosomal dominant hypercholesterolemia (ADH) [13]. The first ADH causative gene identified was the *low density lipoprotein receptor (LDLR*), located at chromosome 19p13.2, and encoding the LDL receptor (LDLR) [14]. The uptake of cholesterol is mediated by the LDLR and plays a crucial role in lipoprotein metabolism. The LDLR is responsible for the binding and subsequent cellular uptake of apolipoprotein-B and E-containing lipoproteins (apo B and apo E, respectively) [15].

To date, over 1200 mutations in *LDLR* have been implicated in ADH [16]. Variations in *LDLR* can interfere to a varying extent with all the different stages of the posttranslational processing, binding, uptake, and subsequent dissociation of the LDL-particle-LDLR complex [15]. Individuals who are carriers for such mutations exhibit plasma total cholesterol levels two-fold or more above normal concentration and are at increased risk for developing atherosclerosis and coronary artery disease (CAD) [15].

The *Pvu*II polymorphism, located at the intron 15 of the *LDLR*, has been considered a genetic marker linked to one of the variations in *LDLR* expression that either structurally alters the receptor activity or alters its function in a regulatory manner [17]. This polymorphism has been associated with differences in LDL-C concentration in normo and hypercholesterolemic individuals from different countries [18].

Salazar et al [19] carried out the first study with the *LDLR Pvu*II intron 15 polymorphism in a Brazilian population and described a strong association between the P1P1 genotype with higher total cholesterol and LDL-C levels among the individuals with a lipid profile suggesting high risk for CAD and the normolipidemic individuals. Taken into account that not all patients exposed to same HAART regime develop dyslipidemia, the aim of the present study was to evaluate the association between dyslipidemia and factors such as HIV-1 infection *per se*, HAART, and *Pvu*II *LDLR* polymorphism at the intron 15 in a cohort of Southern Brazilian HIV-1-infected patients that include both antiretroviral naïve and treatment-experienced.

PATIENTS AND METHODS

Study design

The protocol of this cross-sectional study was approved by the Institutional Research Ethic Committee of the University of Londrina, Paraná State, Southern Brazil, and a written consent form was obtained from all of the individuals before the start of the study.

Patients and control cohorts

A total of 355 HIV-1-infected patients were consecutively recruited from Integrated Center of Infectious Diseases of 17ª Secretariat of Health of Paraná, Londrina, Paraná State, and from the Outpatient Clinical Hospital, of University of Londrina, Paraná State. Of them, 100 (28.2%) were antiretroviral naïve and 255 (71.8%) were treated with HAART including nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs). The control group consisted of 116 healthy individuals recruited from blood donors of the Blood Bank of Londrina and from the general population with similar demographic characteristics and from the same geographic area of the patients. All of the control individuals did not present either clinical symptoms or laboratory parameters of inflammation and blood-borne transmitted infections [20]. None of the control presented heart, thyroid, renal, hepatic, gastrointestinal or oncological diseases, or was receiving estrogen replacement therapy, drugs for hyperlipidemia, hyperglycemia, or antioxidant supplements. For all subjects included in the study, information on lifestyle factors and medical history were obtained at clinical evaluation. None of them was receiving a specific diet and reported no drink alcohol regularly. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist circumference was also measured and expressed as cm. Parameters such as age, gender, ethnicity, BMI, and waist circumference were controlled.

Pvu*II intron 15 polymorphism of the *LDLR

Peripheral blood cells were obtained with EDTA as anticoagulant, the samples were immediately centrifuged at 3,000 rpm for 15 min, and the plasma and buffy coats were stored at freezer -80^{o}C until use. The samples were consecutively identified by number to guarantee confidentiality. Genomic DNA was extracted (Biometrix Diagnóstica, Curitiba, Brazil) according to the manufacturer's instructions. A 800 base-pair (bp) fragment of the *LDLR* was amplified using polymerase chain

reaction (PCR) as previously reported [18, 20]. PCR products were completely digested with 0.3μL of *Pvu*II (Invitrogen, Life Technologies, Carlsbad, CA, USA) for 4h at 37^{o}C. The *Pvu*II *LDLR* intron 15 polymorphism was identified by restriction fragment length polymorphism (RFLP) analysis as previously reported [20]. The *Pvu*II cutting site (CAG↓CTG) is created by the substitution of a C to T within the sequence CAGCCG at a position about 900 bp from the 3' end of exon 16. P2 variant allele includes a restriction site for *Pvu*II which results in 200 bp and 600bp fragments after digestion, the P1 allele is identified by one fragment of 800 bp, and the heterozygous genotype P1P2 is identified by three fragments (800 bp, 600 bp, and 200bp). The PCR profiles were captured and recorded by photodocument system L-PIX-HE (Loccus Biotecnology, Cotia, Brazil).

Lipid profile measurements

Serum lipid levels were determined from blood samples obtained by venipuncture, without anticoagulant, following a 10 to 12 h overnight fast. Total cholesterol, LDL-C, HDL-C, and TG concentrations were measured by a biochemical analyzer Dimension Dade AR® (Dade Behring, Siemens Healthcare Diagnostics INC, Tarrytown, NY, United States). The results were expressed in mg/dL and categorized according to the cut-off values used to define metabolic syndrome [21, 22].

Statistical analysis

The sample size was calculated with the Statcalc Program from Epi Info version 6.04d and the data were evaluated using Graph Pad Prism version 5.0 (GraphPad Software Inc., San Diego, CA). Categorical variables were analyzed using the Chi-square test, and expressed as the absolute (n) and relative (%) numbers. Nonparametric continuous variables were analyzed using Mann-Whitney test and expressed as median and interquartile range 25%-75%.

The Hardy-Weinberg Equilibrium (HWE) was evaluated using the Chi-square test. The odds ratio (OR) and 95% confidence interval (95% CI) were also analyzed. All of the results were considered significant when $p < 0.05$.

RESULTS

The demographic, anthropometric, and biochemical characteristics of HIV-1-infected patients and controls are presented in Table 1. As expected, the individuals did not differ in any of the controlled parameters, such as gender, ethnicity, age, BMI, and waist circumference (p>0.05). The HIV-1-infected patients presented higher serum TG concentration than controls (p<0.0001). The patients, antiretroviral naïve and on HAART, exhibited lower serum HDL-C concentration than controls (p<0.0001).

Regarding the *Pvu*II *LDLR* intron 15 polymorphism, the frequencies of P1P1, P1P2, and P2P2 genotypes obtained among the HIV-1-infected patients and controls were 52.4%, 39.1%, and 8.5%; and 55.2%, 42.2%, and 2.6%, respectively, and were in HWE in both groups (x^2 test, p > 0.05). The allelic P1 and P2 frequencies were 0.76 and 0.24 among the HIV-1-infected patients and 0.72 and 0.28 among the controls, respectively.

The table 2 shows the characteristics of HIV-1 patients according to the use of HAART. Caucasian and younger individuals were more frequent among the antiretroviral naïve patients (67.0% *vs*. 55.3%, p=0.0440; median 32.0 *vs*. 42.0 years old, p<0.0001), respectively. The patients treated with HAART showed higher TG (167.0 *vs.* 119.5 mg/dL, p<0.0001), total cholesterol (207.0 *vs*. 175.0 mg/dL, p<0.0001), and LDL-C (124.2 *vs.* 102.9 mg/dL, p=0.0003) than those without HAART.

For univariate analysis, the lipid profile was categorized according to the cut-off values used to the metabolic syndrome criteria and the HIV-1 patients were matched for gender, ethnicity, age, BMI, and waist circumference (table 3). The number of individuals with total cholesterol levels ≥ 200mg/dL was higher among those using HAART (86.6% *vs*. 67.4%, OR=3.123, 95% CI=1.811-5.385, p<0.0001) and with NRTIs at the regimen (85.4% *vs.* 67.4%, OR=2.823, 95% CI=1.659-4.803, p<0.0001). The number of HIV-1 patients with LDL-C levels ≥ 100mg/dL was higher among those using HAART (74.4% *vs*. 62.6%, OR 1.739, 95% CI=1.070-2.825, p=0.0248), NRTIs (73.8% *vs*. 61.9%, OR=1.739, 95% CI=1.073-2.818, p=0.0239), and NNRTIs (31.4% *vs*. 19.4%, OR=1.898, 95% CI=1.118-3.224, p=0.0168). The number of HIV-1 patients with TG ≥ 150mg/dL was also higher among those treated with HAART (80.9% *vs*. 69.7%, OR 1.841, 95% CI=1.068-3.172, p=0.0269), PIs

(61.2% *vs*. 38.6%, OR=2.500, 95% CI=1.554-4.020, p=0.0001), and NRTIs (80.3% *vs*. 69.7%, OR=1.767, 95% CI=1.029-3.025, p=0.0378).

When HDL-C levels were categorized according to the gender of the HIV-1 patients and metabolic syndrome criteria, the frequency of individuals with low HDL-C levels did not differ among the HIV-1 infected patients antiretroviral naïve or on HAART (p=0.7375). Moreover, the frequencies of low HDL-C did not differ according to the PIs, NRTIs, and NNRTIs treatment (p=1.842, p=0.7242, and p=0.3228). However, when the HDL-C values were evaluated according to *LDLR Pvu*II intron 15 polymorphism, the frequency of individuals with HDL-C ≥ 40 mg/dL for men and ≥ 50 mg/dL for women was higher among those carrying the P2P2 genotype (11.8% *vs.* 5.6%, OR=0.4405, 95% CI=0.1968-0.9860, p=0.0415) (table 4).

DISCUSSION

This study reports, for the first time, the association between the lipid profile and the HIV-infection, HAART, and *LDLR Pvu*II intron 15 polymorphism among the Brazilian HIV-1-infected patients treatment naïve and HAART-experienced. The results obtained reinforced that both antiretroviral naïve and on HAART patients exhibit changes in lipid profile that are deemed as a risk factor for CVD, such as higher TG and lower HDL-C levels than controls. Moreover, the number of individuals with increased total cholesterol, LDL-C, and TG was higher among those using HAART.

Regarding the effect of HIV-1 infection by itself and HAART on the lipid profile, the results obtained are consistent with those carried out with HIV-1-infected patients from other countries [23-25]. We observed that dyslipidemia was more evident in patients with HIV-1 infection and those HAART-experienced, reinforcing the effects of these in the abnormalities of lipid metabolism.

HIV-1-associated dyslipidemia was recognized for years before the widespread use of PIs-based HAART and is characterized mainly by decreased total cholesterol, LDL-C, and HDL-C and lately elevated plasma TG [26-28]. The HIV-1 infection by itself is the main responsible of low HDL-C levels, because several steps of HIV-1 replication critically depend on cholesterol. HIV-1 decreases plasma HDL-C by impairing cholesterol-dependent efflux transporter ATP-binding cassette protein A1 (ABCA1) from human macrophages, a condition related to be highly atherogenic [29].

Additionally, the inflammation stimulates endothelial lipase and some acute phase proteins, such as serum amyloid A. The plasma level of endothelial lipase in humans was found to be inversely associated with HDL-C and the acute phase proteins accelerate the removal of HDL-C by macrophages [30].

Changes in lipid metabolism have been increasingly recognized among the HIV-1-infected patients since the introduction of HAART. Over 60% of the patients that are treated with PIs develop metabolic changes, such as hyperlipidemia with hypertriglyceridaemia in the majority of cases, endothelial dysfunction, hyperglycemia, and central obesity [31,32]. The elevation of TG levels in patients who received PIs suggested that select PIs stimulate TG synthesis in the hepatocytes [33].

NNRTIs show, in general, the best lipid profile of all antiretroviral drugs because they are associated with an increase in HDL cholesterol and a significant reduction in cholesterol total/HDL ratio. NNRTIs have also been associated with lower risk of myocardial infarction than other antiretroviral [34] that could hypothetically be associated with this good lipid profile. As regard the NNRTI nevirapine (NVP), the mechanism of HDL elevation may be an increase in the production of apolipoprotein-A1 [35].

PIs-associated dyslipidemia is a frequent class related event and can limit their use especially in patients with preexisting increase cardiovascular risk. PIs prevent the differentiation of preadipocytes by decreasing matrix metalloproteinase expression, inhibiting adiponectin secretion, and inhibiting TG and very low-density lipoprotein (VLDL) cholesterol clearance and catabolism [36]. Among the PIs, lopinavir/ritonavir (LVP/r), darunavir/ritonavir (DRV/r) and atazanavir (ATV) alone or with ritonavir (ATV/r) are the most extensively used PIs at present. A meta-analysis of major clinical trials [37] showed that patients randomized to LPV/r or fosamprevir/ritonavir (FPV/r) presented greater elevations of total cholesterol and TG than those assigned to saquinavir/ritonavir (SQV/r, ATV/r, or DRV/r, without differences in LDL-C or HDL-C.

A decreased expression of the LDLR may be explained by a direct effect of the PIs [38]. Other hypothesis is that PIs lead to dyslipidemia by inhibition of LDLR-related protein (LRP), which has homology to the catalytic site of HIV-1 protease, to which all PIs bind [31]. In addition, PIs also can modulate the function of certain LDLR family members. Among six different PIs evaluated, nelfinavir (NFV),

specifically, decreased mRNA and protein levels of the LDLR and LRP, which, in turn, decreased the functional activity of these two receptors [39]. A recent study showed that the expression of genes involved in cholesterol uptake (*LDLR*, *CD36*), synthesis (*HMGCR*), and regulation (*SREBP2*, *LXRA*) was significantly lower in both HAART-treated and HAART-naïve subjects than in HIV-1 negative controls, suggesting that both HIV and HAART affect monocyte cholesterol metabolism in a pattern consistent with accumulation of intramonocyte cholesterol [40]. These authors emphasize that this pattern of changes would be concerning for accelerated atherosclerosis given the role of intracellular cholesterol accumulation in monocyte-macrophages in the development of atherosclerosis.

The higher frequency of Caucasian and younger HIV-1 patients among the antiretroviral naïve than those treated with HAART included in the present study is consistent with other study [40] and a possible explanation for this result could be the time of HIV-1 infection diagnosis. Probably, the Caucasian and younger individuals had their HIV-1 infection more recently diagnosed or they are in an earlier phase of HIV-1 infection than non-Caucasian and older individuals, when the HAART was not recommended at that time, according to the Brazilian government guidelines [41]. Despite high and increasing coverage with HAART, the HIV infection represents a very clear example of the inequalities in access to health in the resource-limited countries, where, proportionally, more female are on antiretroviral treatment than men [42]. However, Brazil has a free and universal government program that guarantees access to HAART for all people with HIV/AIDS who need them and this may explain the similar gender distribution of patients on HAART therapy observed in the present study.

Despite Brazilians are one of the most genetically heterogeneous populations in the world, as the result of five centuries of interethnic crosses between peoples from three continents (Amerindians, Europeans and Africans) [43], the distribution pattern of the *Pvu*II *LDLR* intron 15 genotypes obtained in the present cohort of HIV-1-infected patients seems to be similar from controls and other Caucasian individuals worldwide; moreover, it is comparable to previously published among Brazilian individuals with a lipid profile suggesting high risk for CVD [19]. The relative frequencies of P1 and P2 alleles among the HIV-1 patients (0.72 and 0.28, respectively) are similar to that observed in hypercholesterolemic patients from Israel, Italy, Spain, Netherlands and Denmark, London, North America, Switzerland, and

Germany [18, 44-46].

Mutations were detected in the different domains of the *LDLR* [47] and have distinct impact on LDLR structure and function. The *Pvu*II polymorphism is located in an intronic region of the *LDLR* and may exert an indirect effect on cholesterol metabolism, probably by increasing the mRNA stability, activity or increasing the number of LDLR on the cell surface. The intron 15 C/T change is unlikely to be an allelic marker for a functional sequence elsewhere at the gene locus [17]. This polymorphism has been associated with differences in LDL-C concentration in normo and hypercholesterolemic individuals from different countries. Individuals carrying the P2P2 genotype exhibited LDL-C levels 10-20% lower than those with other genotypes and an association between the P2 allele with lower levels of plasma lipids than the P1 allele was also reported [17,18,48]. Salazar et al [19] described the strong association between the P1P1 genotype with higher total cholesterol and LDL-C levels among Brazilian individuals with a lipid profile suggesting high risk for CAD and normolipidemic individuals.

An interesting result was that the frequencies of low HDL-C observed among the HIV-1 patients HAART-experienced did not differ according to the PIs, NRTIs, and NNRTIs regimen; however, when the HDL-C values were evaluated according to *LDLR Pvu*II intron 15 polymorphism, the number of individuals with HDL-C ≥ 40 mg/dL for men and ≥ 50 mg/dL for women was higher among those carrying the P2P2 genotype, suggesting that the protective P2P2 genotype could be modulating in part, the effect of HIV-1 infection *per se* and HAART in reducing the HDL-C levels.

The complex polygenic trait of dyslipidemia among the HIV-1 has been demonstrated by a genome-wide study association (GWSA) that evaluated the contribution of 42 different SNPs and other variables, such as HAART, underlying conditions, sex, age, ethnicity, and HIV-1 disease parameters to dyslipidemia in 745 HIV-infected participants [49]. The results showed that the genetic background to dyslipidemia, alone, explained up to 7.6% of lipid variation in HIV-infected patients (7.6% non-HDL cholesterol, 6.2% HDL-C and 6.8% TG), and the HAART, alone, explained up to 6.2% of lipid variation (3.9% non-HDL cholesterol, 1.5% HDL-C and 6.2% TG). The authors concluded that an individual with the most dyslipidemic antiretroviral and genetic background exhibited three to five-fold increased risk of sustained dyslipidemia compared with an individual with the least dyslipidemic therapy and genetic background. Other GWAS involving > 100,000 individuals from

general population of European ancestry and non-European individuals (East Asians, South Asians, and African Americans) reported 59 loci with significant associations with lipid trait for the first time [50]. The authors reported that these loci contribute not only to the inter-individuals variation in serum lipid concentrations but also to extreme lipid phenotypes and impact lipid traits in the three non-European populations evaluated.

This study represents one of the first attempts at integrating genetic (*Pvu*II *LDLR* variant), environmental (HIV-1- infection by itself and HAART), on the lipid profile of the individuals that are living with HIV/AIDS among the Southern Brazilian population. Probably, the protector effect for dyslipidemia previously attributed to the P2P2 genotype could mitigate, in part, the deleterious effect of the HIV-1 infection and HAART among the carriers of this genetic variant. Other known and novel genetic factors associated with lipid metabolism pathways should be investigated in this cohort of HIV-1-infected individuals in an attempt to identify genetic markers candidates associated with dyslipidemia among the Brazilians. Gene products and pathways may provide new targets for development of more effective clinical strategies.

Therefore, the dyslipidemia must be actively investigated among all the HIV-1-infected patients, treatment naïve and HAART-experienced, aiming to prompt institution of pharmacological and non-pharmacological measures. These strategies associated with preventive measures and a continuously monitoring of this HAART adverse effect, mainly in those patients with a known genetic predisposition to the development of lipid disorders should be explicitly considered.

AUTHOR CONTRIBUTION

E.R.D. de Almeida recruited the patients, obtained the data and clinical samples, carried out the genotyping analysis, and wrote the manuscript; H K Morimoto recruited the patients, obtained the demographic, anthropometric, clinical, and clinical samples, and reported the results at database; J A de Oliveira carried out the serum lipid analysis; T Flauzino, and D F Alfieri carried out the genotyping analysis; A N C Simão conceived and designed the study, and carried out the statistical analysis; M A E Watanabe conceived, designed, and supervised the study; E M V Reiche conceived and supervised the study, discussed the results, and wrote

the manuscript.

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Table 1 Demographic, anthropometric, and biochemical characteristics of HIV-1-infected patients and healthy controls from Southern Brazil

	Healthy controls (n=116)	**HIV-1-infected patients (n=355)**	**p value**
Gender n (%)			
Female	66 (56.9)	174 (49.0)	0.1404*
Male	50 (43.1)	181 (51.0)	
Ethnicity n (%)			
Caucasian	77 (66.4)	208 (58.6)	0.1363*
No Caucasian	39 (33.6)	147 (41.4)	
Age (years)			
Median (IQR)	40.0 (31.0-47.0)	41.0 (32.0-46.0)	0.4343†
Body mass index (Kg/cm^2)			
Median (IQR)	24.8 (22.2-27.7)	24.2 (21.4-27.0)	0.1174†
Waist circumference (cm)			
Median (IQR)	89.0 (82.5-96.5)	90.0 (84.0-97.0)	0.6637†
Triglycerides (mg/dL)			
Median (IQR)	97.0 (68.5-129.0)	145.0 (97.0-215.0)	<0.0001†
Total cholesterol (mg/dL)			
Median (IQR)	194.0 (170.5-218.0)	194.0 (162.0-233.0)	0.7523†
LDL- C (mg/dL)			
Median (IQR)	115.0 (92.6-137.6)	117.8 (90.2-146.8)	0.5930†
HDL- C (mg/dL)			
Median (IQR)	55.0 (46.0-66.0)	43.0 (36.0-51.0)	<0.0001†

HIV-1: human immunodeficiency type 1; IQR: interquartile range (25%-75%); LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol;

*: Chi-square test ($p<0.05$); †: Mann–Whitney test ($p<0.05$)

Table 2: Demographic, anthropometric, and biochemical characteristics of HIV-1-infected patients from Southern Brazil, according to the therapy

	Without HAART (n=100)	**With HAART (n=255)**	**p value**
Gender n (%)			
Female	52 (52.0%)	122 (47.8%)	0.4810*
Male	48 (48.0%)	133 (52.2%)	
Ethnicity n (%)			
Caucasian	67 (67.0%)	141(55.3%)	0.0440*
No Caucasian	33 (33.0%)	114 (44.7%)	
Age (years)			
Median (IQR)	32.0 (26.0-42.0)	42.0 (36.0-48.0)	<0.0001†
Body mass index (Kg/cm^2)			
Median (IQR)	24.4 (21.3-26.9)	24.2 (21.5-27.2)	0.9872†
Waist circumference (cm)			
Median (IQR)	89.0 (80.3-96.0)	90.0 (84.0-98.0)	0.1714†
Triglycerides (mg/dL)			
Median (IQR)	119.5 (78.8-160.8)	167.0 (105.0-229.0)	<0.0001†
Total cholesterol (mg/dL)			
Median (IQR)	175.0 (152.3-193.5)	207.0 (168.0-246.0)	<0.0001†
LDL- C (mg/dL)			
Median (IQR)	102.9 (86.3-127.5)	124.2 (93.0-152.0)	0.0003†
HDL- C (mg/dL)			
Median (IQR)	42.5 (35.0-49.8)	43.0 (38.0-52.0)	0.2042†

HIV-1: human immunodeficiency type 1; HAART: highly active antiretroviral therapy; IQR: interquartile range (25%-75%); LDL-C: low- density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol;

*: Chi-square test (p<0.05); †: Mann–Whitney test (p<0.05)

Table 3: Demographic, anthropometric, and clinical characteristics of human immunodeficiency type 1(HIV-1)-infected patients from Southern Brazil, according to the serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels

	TC <200 mg/dL (n=184)	**TC ≥200 mg/dL (n=164)**	**LDL-C <100 mg/dL (n=139)**	**LDL-C ≥100 mg/dL (n=172)**	**TG <150 mg/dL (n=132)**	**TG ≥150 mg/dL (n=157)**
Gender n (%)						
Female	77 (41.8)	75 (45.7)	49 (35.2)	52 (30.2)	54 (40.9)	65 (41.4)
Male	107 (58.2)	89 (54.3)	90 (64.8)	120 (69.8)	78 (59.1)	92 (58.6)
Ethnicity n (%)						
Caucasian	108 (58.7)	100 (61.0)	71 (51.1)	88 (51.2)	78 (59.1)	97 (61.8)
No Caucasian	76 (41.3)	64 (39.0)	68 (48.9)	84 (48.8)	54 (40.9)	60 (38.2)
Age (years)						
Median	40.0	42.0	38.0	40.0	40.5	41.0
IQR	34.0-46.0	36.0-46.0	30.0-45.0	32.0-46.0	34.2-46.8	36.0-45.0
BMI (Kg/cm^2)						
Median	23.93	24.22	23.1	23.8	24.0	24.7
IQR	20.96-26.79	21.68-27.29	20.7-26.0	21.5-26.4	21.7-26.9	22.4-27.1
WC (cm)						
Median	89.5	90.0	88.0	89.0	90.0	90.0
IQR	82.5-94.8	86.0-98.0	81.0-97.0	84.0-95.0	84.0-97.0	87.0-98.0
HAART n (%)						
Yes	124 (67.4)	142 (86.6)*	87 (62.6)	128 (74.4)‡	92 (69.7)	127 (80.9)ll
No	60 (32.6)	22 (13.4)	52 (37.4)	44 (25.6)	40 (30.3)	30 (19.1)
PIs n (%)						
Yes	85 (46.2)	92 (56.1)	59 (42.4)	79 (45.9)	51 (38.6)	96 (61.2)¶
No	99 (53.8)	72 (43.9)	80 (57.6)	93 (54.1)	81 (61.4)	61 (38.8)
NRTIs n (%)						
Yes	124 (67.4)	140 (85.4)†	86 (61.9)	127 (73.8)§	92 (69.7)	126 (80.3)**
No	60 (32.6)	24 (14.6)	53 (38.1)	45 (26.2)	40 (30.3)	31 (19.7)
NNRTIs n (%)						
Yes	42 (22.8)	52 (31.7)	27 (19.4)	54 (31.4)*,¶	41 (31.1)	36 (22.9)
No	142 (77.2)	112 (68.3)	112 (80.6)	118 (68.6)	91 (68.9)	121 (77.1)

TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; BMI: body mass index; WC: waist circumference; IQR: interquartile range (25%-75%); HAART: highly active antiretroviral therapy; PIs: Protease inhibitors; NRTIs: nucleoside reverse transcriptase inhibitors; NNRTIs: non-nucleoside reverse transcriptase inhibitors;

*: Chi-square test, p<0.0001; odds ratio: 3.123; 95% confidence interval: 1.811-5.385;

†: Chi-square test, p<0.0001; odds ratio: 2.823; 95% confidence interval: 1.659-4.803;

‡: Chi-square test, p=0.0248, odds ratio: 1.739; 95% confidence interval: 1.070-2.825;

§: Chi-square test, p=0.0239, odds ratio: 1.739; 95% confidence interval: 1.073-2.818;

*,¶: Chi-square test, p=0.0168, odds ratio: 1.898; 95% confidence interval: 1.118-3.224;

ll: Chi-square test, p= 0.0269, odds ratio: 1.841; 95% confidence interval: 1.068-3.172;

¶: Chi-square test, p= 0.0001, odds ratio: 2.500; 95% confidence interval: 1.554-4.020;

**: Chi-square test, p=0.0378, odds ratio: 1.767; 95% confidence interval: 1.029-3.025.

Table 4: Demographic, genetic, clinical characteristics, and *Pvu*II *LDLR* intron 15 polymorphism results of HIV-1-infected patients from Southern Brazil, according to the serum high-density lipoprotein cholesterol (HDL-C) levels

	High HDL-C* **(n=152)**	**Low HDL-C†** **(n=179)**	**p value**
Gender n (%)			
Female	66 (43.4)	83 (46.4)	0.5912‡
Male	86 (56.6)	96 (53.6)	
Ethnicity n (%)			
Caucasian	102 (67.1)	108 (60.3)	0.2025‡
No Caucasian	50 (32.9)	71 (39.7)	
Age (years)			
Median (IQR)	40.0 (32.0-46.0)	40.0 (34.0-46.0)	0.8785§
Body mass index (Kg/cm^2) Median (IQR)	23.6 (21.2-26.3)	24.4 (21.7-27.0)	0.2827§
Waist circumference (cm)			
Median (IQR)	90.0 (84.2-98.0)	90.0 (84.0-96.0)	0.3801§
HAART n (%)			
Yes	107 (70.4)	129 (72.1)	0.7375‡
No	45 (29.6)	50 (27.9)	
PIs n (%)			
Yes	67 (44.1)	92 (51.4)	0.1842‡
No	85 (55.9)	87 (48.6)	
NRTIs n (%)			
Yes	106 (69.7)	128 (71.5)	0.7242‡
No	46 (30.3)	51 (28.5)	
NNRTIs n (%)			
Yes	42 (27.6)	41 (22.9)	0.3228‡
No	110 (72.4)	138 (77.1)	
Genotypic analysis n (%)			
P2P2	18 (11.8)	10 (5.6)	0.0415‡,‖
P1P1+P1P2	134 (88.2)	169 (94.4)	

*: HDL-C: high-density lipoprotein cholesterol was considered HIGH: men ≥ 40 mg/dL and women ≥ 50 mg/dL.;

† HDL-C: high-density lipoprotein cholesterol was considered LOW: men < 40mg/dL and women < 50mg/dL;

*Pvu*II *LDLR* intron 15 polymorphism: P1/P1: homozygous genotype for the P1 allele; P1/P2: heterozygous genotype with the P1 and P2 alleles; P2/P2: homozygous genotype for the P2 allele; IQR: interquartile range (25%-75%); HAART: highly active antiretroviral therapy; PIs: Protease inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NNRTIs: non-nucleoside reverse transcriptase inhibitors;

‡: Chi-square test, $p<0.05$; †: Mann-Whitney test, $p<0.05$;

ll: odds ratio: 0.4405; 95% confidence interval: 0.1968-0.9860

5 CONCLUSÃO

- Não foram observadas diferenças em relação ao sexo, IMC e circunferência abdominal entre os pacientes infectados pelo HIV-1 virgens de tratamento e em tratamento com HAART; entretanto, indivíduos mais jovens e caucasianos foram encontrados em maior frequência entre os pacientes infectados pelo HIV-1 virgens de tratamento antirretroviral.

- Os pacientes infectados pelo HIV-1 (virgens de tratamento antirretroviral e em tratamento com HAART) apresentaram maior concentração sérica de TG e menor concentração sérica de HDL-C do que os controles.

- Os pacientes com HIV-1 em uso de HAART apresentaram níveis aumentados de colesterol total, LDL-C e TG em relação aos pacientes infectados pelo HIV-1 virgens de tratamento antirretroviral.

- A frequência dos pacientes infectados pelo HIV-1 com níveis de colesterol total ≥ 200 mg/dL, LDL-C ≥ 100 mg/dL e TG ≥ 150 mg/dL foi maior entre aqueles que usavam HAART.

- Quando os pacientes com HIV-1 foram categorizados de acordo com os níveis de HDL-C, a frequência de indivíduos com baixos níveis de HDL-C (<40 mg/dL para homens e <50 mg/dL para mulheres) não diferiram entre os que estavam virgens de tratamento antirretroviral ou em tratamento com HAART. No entanto, quando os valores de HDL-C foram avaliados de acordo com o polimorfismo *Pvu*II do intron 15 do *LDLR*, a frequência de HDL-C ≥ 40 mg/dL para homens

e ≥ 50 mg/dL para mulheres foi maior entre os portadores do genótipo P2P2.

6 CONSIDERAÇÕES FINAIS

Este estudo forneceu, pela primeira vez, dados da associação entre o polimorfismo *Pvu*II localizado no intron 15 do *LDLR* e alterações no perfil lipídico dos pacientes brasileiros infectados pelo HIV-1, seja naqueles virgens de tratamento antirretroviral ou naqueles em tratamento com HAART. Embora o efeito desta variante alélica, isoladamente, não seja suficiente para explicar a dislipidemia observada nos pacientes infectados pelo HIV-1, a mesma poderia exercer uma influência adicional ou sinérgica com outros fatores genéticos e ambientais para uma maior vulnerabilidade à dislipidemia nestes indivíduos. O possível efeito protetor do genótipo P2P2 nos níveis séricos do HDL-C observados neste estudo pode ter minimizado os efeitos deletérios da infecção pelo HIV-1 *per se* e da terapia antirretroviral nos indivíduos que carregam esta variante alélica.

Outros polimorfismos genéticos associados à dislipidemia merecem ser investigados em pacientes infectados pelo HIV-1, como uma contribuição para a identificação de indivíduos com genótipos associados ao desenvolvimento de dislipidemia.

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Universidade Estadual de Londrina
COMITÊ DE ÉTICA EM PESQUISA

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná
Registro CONEP 268

Parecer PF Nº. 264/09 **CAAE Nº. 0206.0.268.000-09** **FOLHA DE ROSTO Nº. 306199**	Londrina, 20 de abril de 2010.

PESQUISADORA: **EDNA MARIA VISSOCI REICHE**
CCS/PAC

Prezada Senhora:

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná**" (Registro CONEP 268)– de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"AVALIAÇÃO DAS ALTERAÇÕES METABÓLICAS DO ESTRESSE OXIDATIVO E DO POLIMORFISMO GENÉTICO DO RECEPTOR DE LIPOPROTEÍNA DE BAIXA DENSIDADE (LDLR) EM PACIENTES INFECTADOS PELOS VÍRUS DA IMUNODEFICIÊNCIA HUMANA TIPO 1 (HIV-1), ATENDIDOS EM LONDRINA E REGIÁO, PARANÁ"

Situação do Projeto: **APROVADO**

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá apresentar ao CEP/UEL relatório final da pesquisa.

Atenciosamente,

Profª. Dra. Alexandrina Aparecida Maciel

Coordenadora
Comitê de Ética em Pesquisa-CEP/UEL

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Por favor, leia cuidadosamente este consentimento e não hesite em perguntar sobre qualquer dúvida que tenha.

Você está sendo convidado (a) a participar, voluntariamente, de um projeto de pesquisa com o título "Avaliação das alterações metabólicas, do estresse oxidativo e do polimorfismo genético do receptor de lipoproteína de baixa densidade (LDLR) em pacientes infectados pelo vírus da imunodeficiência humana tipo 1 (HIV-1), atendidos em Londrina e região, Paraná", coordenado pela professora Dra. Edna Maria Vissoci Reiche e com a participação de outros docentes pesquisadores da Universidade Estadual de Londrina. Cabe ao senhor (a) decidir se pretende participar ou não. Caso não tenha condições de ler e/ou compreender as informações contidas neste termo, o mesmo poderá ser assinado e datado por um membro da sua família ou responsável legal.

Você está tendo a opção de participar de uma pesquisa que tem o objetivo de saber quantos pacientes infectados com o vírus da imunodeficiência humana (HIV) apresentam alterações nos níveis de gordura no sangue, se esta alteração é em decorrência do uso de medicamentos antirretrovirais e a análise de um fator genético associado às alterações nos níveis de lipídeos (dislipidemias) como o gene do receptor da lipoproteína de baixa densidade (LDL), conhecido como o "colesterol ruim". Para participar do projeto, será necessária a coleta dos dados como peso, altura e cálculo do índice de massa corpórea (IMC = peso/altura^2); circunferência abdominal e medida de pressão arterial. Também será necessária a coleta uma pequena amostra de sangue para realização das provas bioquímicas (colesterol, glicose, insulina, ácido úrico e avaliação do estresse oxidativo) e genéticas (gene do receptor do LDL) . A coleta dos dados e da amostra de sangue será efetuada no mesmo dia em que o senhor (a) será atendido pelo serviço especializado para a realização dos exames de rotina do monitoramento do seu tratamento. Não haverá necessidade de agendar outros dias para coletas específicas para este projeto de pesquisa.

Os dados pessoais fornecidos e os resultados do exame realizado serão mantidos sob sigilo e somente serão utilizados para fins de pesquisa. Durante todas as etapas do projeto, os participantes serão identificados por um número codificado que será utilizado nas análises posteriores para garantir a preservação da integridade do indivíduo, garantir o anonimato e evitar a quebra de confidencialidade. Ao final do projeto, os resultados serão divulgados em forma de artigos científicos e comunicações em eventos científicos, sempre mantendo o sigilo da identidade dos participantes e as amostras de material biológico coletadas serão descartadas em local apropriado de descarte de material biológico (sangue, soro, plasma e DNA) dos laboratórios envolvidos, seguindo as normas de biossegurança padronizada no Hospital Universitário.

Declara que está completamente esclarecido sobre a forma como a pesquisa será realizada, não tenho nenhuma dúvida sobre sua natureza e os procedimento, sem risco, aos quais será submetido. Declara também que está ciente de que sua participação é voluntária, de que será informado sobre os resultados dos exames realizados, de não terá nenhum ônus e de que poderá se recusar ou abandonar a pesquisa em qualquer momento sem que haja penalização ou prejuízo algum para seu atendimento e tratamento. Está ciente também que o seu tratamento continuará sendo conduzido pelo seu médico e que nenhum pagamento ou benefício será feito ao participante ou aos familiares pela participação no presente estudo.

Eu estou disposto a participar dessa pesquisa e compreendi as condições acima descritas, concordo voluntariamente a participar desse estudo.

Assinaturas

Paciente ou representante legal (caso o paciente esteja impossibilitado de assinar ou compreender o conteúdo deste TCLE)

Nome:_____
Assinatura: _____
Local e data: Londrina, _____

Profissional que obteve o TCLE
Nome:_____
Assinatura: _____
Local e data: Londrina, _____

Pesquisador responsável: Nome: Professora Dra. Edna Maria Vissoci Reiche
Endereço: Departamento de Patologia, Análises Clínicas do Centro de Ciências da Saúde, Hospital Universitário de Londrina. Av. Robert Koch, 60, Vila Operária, CEP 86038-440 Fone: 43-3371-2321 ou 43-3371-2670

Comitê de Ética em Pesquisa em Seres Humanos da Universidade Estadual de Londrina Fone: 43-3371-2490

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Review Article

Provisional PDF

THE ROLES OF GENETIC POLYMORPHISMS AND HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION IN LIPID METABOLISM

Elaine Regina Almeida, Edna maria Reiche, Tamires Flauzino, Ana Paula Kallaur, and Maria Angélica Ehara Watanabe

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Complete List of Authors:	de Almeida, Elaine; University of Londrina, Department of Pathology, Clinical Analysis and Toxicology Simão, Andréa; University of Londrina, .Department of Pathology, Clinical Analysis and Toxicology Morimoto, Helena; University of Londrina, Department of Pathology, Clinical Analysis and Toxicology Kallaur, Ana; Postgraduate Program of Health Sciences, Health Sciences Center, State University of Londrina, Flauzino, Tamires; Pharmacy Course, Health Sciences Center, State University of Londrina, Alfieri, Daniela; Pharmacy Course, Health Sciences Center, State University of Londrina, Oliveira, Jair; Health Sciences Center, State University of Londrina,, Department of Pathology, Clinical Analysis and Toxicology, Ueda, Luiz; Integrated Center of Infectious Diseases of 17ª Secretariat of Health of Paraná, Londrina, Paraná State, Watanabe, Maria Angelica; Laboratory of Study and Applications of DNA Polymorphisms, Biological Sciences Center, State University of Londrina,, Department of Pathological Sciences, Reiche, Edna; State University of Londrina, Department of Pathology, Clinical Analysis and Toxicology
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