

# Elevated Lipoprotein(a) in Perinatally HIV-Infected Children Compared With Healthy Ethnicity-Matched Controls

Malon Van den Hof,<sup>1</sup> Mirthe J. Klein Haneveld,<sup>1</sup> Charlotte Blokhuis,<sup>1</sup> Henriette J. Scherpbier,<sup>1</sup> Hans P. G. Jansen,<sup>2</sup> Neeltje A. Kootstra,<sup>3</sup> Geesje M. Dallinga-Thie,<sup>2</sup> Sander J. H. van Deventer,<sup>4</sup> Sotirios Tsimikas,<sup>5</sup> and Dasja Pajkr<sup>1</sup>; for the NOVICE study group

<sup>1</sup>Pediatric Infectious Diseases, Emma Children's Hospital, <sup>2</sup>Department of Experimental Vascular Medicine and Vascular Medicine, and <sup>3</sup>Department of Experimental Immunology, Amsterdam University Medical Centers, location Academic Medical Center, Amsterdam, the Netherlands; <sup>4</sup>Department of Gastroenterology, Leiden University Medical Center, Leiden, the Netherlands; <sup>5</sup>Sulpizio Cardiovascular Center, Division of Cardiovascular Medicine, University California San Diego, La Jolla, California

**Background.** HIV-associated cardiovascular disease (CVD) risk in combination antiretroviral therapy (cART)-treated perinatally HIV-infected patients (PHIV+) remains unknown due to the young age of this population. Lipoprotein(a) (Lp(a)) has been established as an independent causal risk factor for CVD in the general population but has not been well established in the population of PHIV+.

**Methods.** We cross-sectionally compared lipid profiles, including nonfasting Lp(a), together with total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides between 35 cART-treated PHIV+ children aged 8–18 years and 37 controls who were matched for age, sex, ethnicity, and socioeconomic status. We explored associations between Lp(a) and disease- and treatment-related factors (inflammation, monocyte activation, and vascular), biomarkers, and neuroimaging outcomes using linear regression models.

**Results.** PHIV+ children had significantly higher levels of Lp(a) compared with controls (median, 43.6 [21.6–82.4] vs 21.8 [16.8–46.6] mg/dL;  $P = .033$ ). Other lipid levels were comparable between groups. Additional assessment of apolipoprotein B, apolipoprotein CIII, apolipoprotein E, and *APOE* genotype revealed no significant differences. Higher Lp(a) levels were associated with higher plasma apoB levels and with lower monocyte chemoattractant protein-1 and TG levels in PHIV+ children. Lp(a) was not associated with HIV- or cART-related variables or with neuroimaging outcomes.

**Conclusions.** cART-treated PHIV+ children appear to have higher levels of Lp(a) compared with ethnicity-matched controls, which may implicate higher CVD risk in this population. Future research should focus on the association between Lp(a) and (sub) clinical CVD measurements in cART-treated PHIV+ patients.

**Dutch Trial Register number.** NRT4074.

**Keywords.** cardiovascular disease risk; lipids; lipoprotein(a); MRI; perinatal HIV infection.

As a result of increased life expectancy in perinatally HIV-infected (PHIV+) patients using combination antiretroviral therapy (cART), HIV infection is now considered a chronic disease. The focus of research and care has increasingly shifted toward long-term complications, such as cardiovascular disease (CVD).

Compared with the general population, people who acquired HIV during adulthood are at elevated risk of premature atherosclerotic CVD [1]. Both HIV and cART are known for their long-term metabolic and cardiovascular complications, including lipodystrophy, dyslipidemia, and insulin resistance

[1–3]. Even when optimal systemic viral suppression has been achieved with cART, chronic immune activation may contribute to atherosclerosis [4, 5].

Due to the young age of the PHIV+ population, clinical CVD manifestations are not yet apparent, but evidence is accumulating that this population may be at increased risk of developing CVD later in life [6]. In PHIV+ patients, increased inflammatory markers and abnormal lipid profiles have been observed [3, 7] along with structural vascular changes, measured by carotid intima-media thickness (IMT), a surrogate marker for atherosclerosis [3, 6, 7].

During the past decade, elevated plasma lipoprotein(a) (Lp(a)) has been established as an independent causal risk factor for atherosclerotic CVD in the general population [8, 9]. Longitudinal studies in HIV-infected adults found an increase in Lp(a) levels after the initiation of cART, predominantly in patients with elevated Lp(a) before treatment, potentially increasing CVD risk [10–12]. Evidence concerning Lp(a) in HIV-infected adults showed a positive association between

Received 8 March 2019; editorial decision 21 June 2019

Correspondence: Malon Van den Hof, MD, Pediatric Infectious Diseases, Emma Children's Hospital, Amsterdam University Medical Centers, location Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands (m.vandenhof@amc.uva.nl).

Open Forum Infectious Diseases®

© The Author(s) 2019. Published by Oxford University Press on behalf of Infectious Diseases Society of America. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com. DOI: 10.1093/ofid/ofz301

Lp(a) and intima-media thickness (IMT), a surrogate marker for future cardiovascular disease [13].

Little is known about Lp(a) and its role in HIV-related CVD in the population of PHIV+ patients. To date, only 3 studies have measured Lp(a) levels in this population, reporting on elevated Lp(a) levels, either in comparison with an (ethnic) unmatched HIV-uninfected group or with regard to external reference values [10, 14, 15]. Due to being genetically determined, Lp(a) levels showed wide interethnic differences, with the most profound differences between those of African vs non-African descent [16, 17]. The lack of ethnicity-matched controls in these studies may have contributed to Lp(a) differences.

Inflammation plays an important role in the origin of cardiovascular disease [18]. In HIV-infected children, biomarkers associated with inflammation, endothelial dysfunction, and procoagulation have been shown to be higher in HIV-infected children compared with healthy controls [7, 19]. The association between Lp(a) and these biomarkers in presence of HIV infection remains unknown.

To date, no evidence is available on the association between Lp(a) levels and brain structure and function in PHIV+ children. We hypothesize that, due to the atherosclerotic properties of Lp(a) [20], Lp(a) may be associated with brain injury markers such as cerebral white matter hyperintensity volume.

As the population of PHIV+ patients surviving into adulthood is growing, investigating potential risk on future cardiovascular complications is highly important. A better understanding of the effect of long-term exposure to HIV and cART on Lp(a) may help us develop new strategies to reduce or prevent CVD in PHIV+ patients.

To investigate the potential effect of chronic perinatal HIV infection and cART exposure on cardiovascular disease risk, we performed a cross-sectional study comparing Lp(a) and other lipids between PHIV+ children aged 8–18 years and healthy age-, sex-, ethnicity-, and socioeconomic status (SES)-matched controls. We addressed the following research questions: Do Lp(a) and other lipid levels differ between cART-treated PHIV+ children and healthy matched controls? Second, is there an association between lipid abnormalities and HIV- and cART-related factors, plasma biomarkers of inflammation, monocyte activation and vascular dysfunction, and brain imaging outcomes?

## METHODS

### Study Design and Population

This study is part of the NOVICE study, investigating the possible effect of HIV infection and cART exposure on neurologic, cognitive, and visual performance in PHIV+ children, conducted at the Amsterdam University Medical Center, University of Amsterdam, the Netherlands, between 2012 and 2014. This study included PHIV+ children aged 8–18 years, as described earlier [21]. To limit potential sources

of bias, HIV-uninfected controls were recruited from the same communities as the PHIV+ children and matched for age, sex, ethnicity, and SES. SES was defined as parental education and occupational status. Parental education was scored according to the International Standard Classification of Education (ISCED) [22]. Occupational status was defined as 0, 1, or 2 caregivers with a paid job [21]. The ethics committee of the Amsterdam University Medical Center reviewed and approved the study protocol. We obtained written informed consent from all parents or legal guardians and all children aged 12 years and older. This study was registered with the Dutch Trial Register (Netherlands Trial Register) as NRT4074.

### Lipid Profiles

We collected nonfasting venous plasma blood samples from participants of the NOVICE study, which was stored at  $-80^{\circ}\text{C}$  until further analysis. For this substudy, we assessed Lp(a) levels using Vitalab Selectra E chemistry analyzer with reagents from Diasys (Diasys, Waterbury, CT), together with levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) using the Roche Cobas 8000 c702 chemistry system (Indianapolis, IN). We calculated low-density lipoprotein (LDL-C) using the Friedewald formula [23]. We defined abnormal lipid levels as follows: Lp(a)  $\geq 30$  mg/dL, TC  $\geq 5.2$  mmol/L (200 mg/dL), LDL  $\geq 3.4$  mmol/L (130 mg/dL), HDL  $\leq 1$  mmol/L (40 mg/dL), TG  $\geq 1.2$  mmol (110 mg/dL) for children aged  $< 10$  years, and  $\geq 1.7$  mmol (150 mg/dL) for children aged  $\geq 10$  years [24]. To obtain a full lipid profile, we further assessed apolipoprotein B (ApoB), apolipoprotein CIII (ApoCIII), apolipoprotein E (ApoE) levels, and APOE genotype. ApoCIII is known to be significantly associated with coronary artery disease risk, independent of traditional cardiovascular disease risk factors [25]. We used Vitalab Selectra E chemistry analyzer with reagents from Diasys for ApoB (Diasys, Waterbury, CT) and reagents from Randox for ApoCIII and ApoE (Randox, Crumlin, UK). We assessed APOE genotypes ( $\epsilon 2/\epsilon 2$ ,  $\epsilon 2/\epsilon 3$ ,  $\epsilon 2/\epsilon 4$ ,  $\epsilon 3/\epsilon 3$ ,  $\epsilon 3/\epsilon 4$ , and  $\epsilon 4/\epsilon 4$ ), as APOE genotypes are known to strongly influence Lp(a) levels [26]. We performed APOE genotyping by detecting the single nucleotide polymorphisms (SNPs) rs7412 and rs429358 with the TaqMan SNP Genotyping Assay of ThermoFisher (Waltham, MA), assessed with CFX96 Real-Time PCR detection system (Bio-Rad Laboratories, Hercules, CA).

### HIV- and Treatment-Related Characteristics

The Dutch HIV Monitoring Foundation provided data on historical HIV- and cART-related characteristics, as previously described [21]. We confirmed HIV-negative status in all controls.

### Inflammatory and Vascular Biomarkers

We assessed the following panel of biomarkers as biomarkers of inflammation and monocyte activation: interleukin-6 (IL-6), C-reactive protein (CRP), interferon gamma (IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), monocyte chemoattractant protein-1 (MCP-1), interferon gamma-induced protein 10 (IP-10), and soluble CD14 (sCD14). We assessed the following panel of biomarkers as biomarkers of endothelial activation and coagulation: soluble intracellular cell adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), D-dimer, thrombin-antithrombin complex (TAT), prothrombin fragment 1 + 2 (F1 + 2), von Willebrand factor antigen (vWF ag), and pro-von Willebrand factor (vWF pro). The details have been described previously [7].

### Neuroimaging Measures

We performed magnetic resonance imaging (MRI) and included the following measurements to investigate associations with lipid abnormalities: gray matter (GM) volume, white matter (WM) volume, white matter (WM) hyperintensity volume (based on fluid attenuation inversion recovery [FLAIR] imaging), WM integrity measurements such as fractional anisotropy (FA) and medial diffusivity (MD), which are based on diffusion tensor imaging (DTI), and cerebral blood flow (CBF), based on arterial spin labeling (ASL) imaging, all acquired through 3-Tesla magnetic resonance imaging (3-Tesla MRI) and processed as described previously [27, 28].

### Statistical Analysis

We compared relevant sociodemographic and lipid levels between PHIV+ children and healthy controls using the unpaired *t* test or Mann-Whitney *U* test for normally and non-normally distributed numeric variables, respectively. We used the Fisher exact test for categorical data.

We examined the relationships between abnormal lipid levels and HIV- or cART-related characteristics (inflammation, monocyte, coagulation, and endothelial activation), biomarkers, and neuroimaging outcomes using linear regression analysis. We logarithmically transformed skewed variables (Lp(a), TG, plasma biomarkers, and white matter hyperintensity volume) to approach a normal distribution. In the models in which we investigated the association between Lp(a) levels and lipid profiles, HIV- or cART-related characteristics, and biomarkers, we adjusted for ethnicity. As ethnicity highly determines Lp(a) levels, we did this to additionally adjust for the potential residual effect of ethnicity imbalance between groups.

In the model for volumetric neuroimaging measurements (such as GM and WM volume and WM hyperintensity volume), we adjusted for intracranial volume (ICV) [28]. For cerebral blood flow, we adjusted for sex, haematocrit levels, and age >16, as previously described [27]. We imputed missing biomarker values due to undetectably low values with the lower

limit of detection of the assay [7]. Variables with a *P* value <.20 in univariable analysis were included in multivariable regression analysis.

Post hoc, we performed a sensitivity analysis excluding PHIV+ children with a detectable viral load at study visit to investigate whether having a detectable viral load was driving the significant difference in Lp(a) levels between groups.

We performed all statistical analyses using R, version 3.3.3 (R Core Team, Vienna, Austria). We considered a *P* value <.05 statistically significant. We did not adjust for multiple comparison, as these analyses were considered exploratory.

## RESULTS

### Characteristics of PHIV+ and HIV-Uninfected Participants

Thirty-five PHIV+ and 37 HIV-uninfected children participated in this study. Table 1 shows the relevant demographic variables and HIV-related characteristics. PHIV+ children had a median age (interquartile range [IQR]) of 13.8 (12.2 to 15.9) years. Groups did not differ significantly in age, sex, ethnicity, SES, or in relevant clinical variables such as body mass index and blood pressure. The majority of children had black ethnicity (80% in PHIV+ and 76% in HIV-uninfected group). Most PHIV+ children were immigrants or adopted from Sub-Saharan Africa or Surinam (57%), whereas the majority of HIV-uninfected children were born in the Netherlands to immigrant parents (95%). Thirty-one children (89%) used cART, for a median duration (IQR) of 10.8 (5.3 to 13.7) years. At the time of the visit, 31 out of 35 children (89%) were on cART. Of the 4 children who did not use cART, 3 children (9%) had used cART previously, of whom 2 stopped therapy due to adherence problems less than 6 months before the study visit. The other child had stopped therapy at 4 years of age. One child (3%) had never been treated with cART. At the time of the visit, the majority of the PHIV+ children (61%) were on a regimen that included a backbone of 2 nucleoside reverse transcriptase inhibitors (NRTIs) and 1 non-nucleoside reverse transcriptase inhibitor (NNRTI).

### Lipoprotein Parameters

Table 2 shows the lipid results for PHIV+ and HIV-uninfected children. Lp(a) showed a highly right-skewed distribution. PHIV+ children had significantly higher (*P* = .033) Lp(a) levels (median [IQR], 43.6 [21.6 to 82.4] mg/dL; range, 2.3 to 212.7 mg/dL) compared with HIV-uninfected controls (median [IQR], 21.8 [16.8 to 46.6] mg/dL; range, 1.7 to 175.8 mg/dL). Twenty-one (60%) PHIV+ children had Lp(a) levels above the predefined cutoff of 30 mg/dL, compared with 15 (41%) children in the control group. This difference was not significantly different (*P* = .157). Lp(a) levels remained significantly higher in PHIV+ (median [IQR], 43 [20.1 to 82.3] mg/dL; range, 2.3 to 168.0 mg/dL) after we excluded 6 PHIV+ children (17%) who had any detectable viral load at the study visit (*P* = .049).

**Table 1. Demographics and Clinical Characteristics**

Characteristics	No.	PHIV+ (n = 35)	No.	HIV- (n = 37)	P
Age at blood draw, median (IQR), y	35	13.8 (12.2 to 15.9)	37	12.1 (11.5 to 15.7)	.156
Male gender, No. (%)	35	17 (49)	37	18 (49)	>.999
Ethnicity, No. (%)	35		37		.101
Black		28 (80)		28 (76)	
Caucasian		0 (0)		3 (8)	
Mixed		4 (11)		6 (16)	
Other <sup>a</sup>		3 (8)		0 (0)	
Region of birth, No. (%)	35		37		<.001
The Netherlands		11 (31)		35 (95)	
Sub-Saharan Africa		18 (51)		2 (5)	
Surinam		2 (6)		0 (0)	
Other		4 (11)		0 (0)	
Height, mean (SD), m	35	1.52 (0.14)	37	1.56 (0.12)	.213
Weight, mean (SD), kg	35	47 (13)	37	50 (15)	.250
Body mass index, median (IQR), kg/m <sup>2</sup>	35	18.6 (17.5 to 21.4)	37	19.6 (17.1 to 22.0)	.774
Blood pressure, median (IQR), mmHg					.079
Systolic		110 (100 to 114)		105 (95 to 112)	.811
Diastolic		65 (60 to 72)		65 (60 to 70)	
ISCED score, median (IQR) <sup>b</sup>	35	5 (4 to 6)	37	5 (5 to 6)	.671
Employment status parents, No. (%)	33		37		.461
Any parent employed		19 (52.8)		25 (67.6)	
Parents unemployed		14 (38.9)		12 (32.4)	
Unknown		3 (8.3)		0 (0)	
Age at HIV diagnosis, median (IQR), y	35	2.3 (0.6 to 4.9)			
Mode of transmission, No. (%)	35				
Perinatal		34 (97)			
Unknown		1 (3)			
CDC category, No. (%)	35				
N		4 (11)			
A		6 (17)			
B		16 (46)			
C		9 (26)			
Nadir CD4+ z-score, median (IQR)	33	-0.71 (-1.43 to -0.39)			
Age at initiation cART, median (IQR), y	32	2.6 (1.0 to 6.0)			
Current use of cART, <sup>d</sup> No. (%)	35	31 (89)			
Duration of cART, median (IQR), y	33	10.8 (5.3 to 13.7)			
cART regimen, No. (%)	31				
Backbone + NNRTI		19 (61)			
Backbone + PI		11 (35)			
Backbone + NNRTI + PI		1 (3)			
Exposure to PIs, No. (%)	35	21 (60)			
Duration of PI exposure, median (IQR), y	21	7.9 (5.8 to 9.3)			
CD4+ T cell z-score, median (IQR)	35	-0.09 (-0.28 to 0.20)			
HIV viral load (plasma), No. (%)	35				
Detectable		6 (17)			
Below lower limit of detection <sup>c</sup>		29 (83)			

Abbreviations: cART, combination antiretroviral treatment; CDC, Centers for Disease Control and Prevention (N, no symptoms, A, minimal symptoms, B, moderate symptoms, C, severe symptoms/AIDS); IQR, interquartile range; ISCED, International Standard Classification of Education; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

<sup>a</sup>Other children were of (mixed) Asian or Hispanic descent.

<sup>b</sup>Parent with the highest education.

<sup>c</sup>Less than 40 copies/mL.

<sup>d</sup>Three children were previously exposed to cART but had stopped treatment.

Levels of TC, HDL-C, LDL-C, TG, apoB, apoCIII, and ApoE were not statistically different between groups ( $P > .070$ ), nor was the number of children with TC, HDL-C,

LDL-C, or TG classified as abnormal ( $P > .103$ ). The distribution of APOE genotype did not significantly differ between groups ( $P = .454$ ).

**Table 2. Lipid Levels and APOE Genotype in Perinatally HIV-Infected Children and Healthy Ethnicity-Matched Controls**

	No.	PHIV+ (n = 35)	No.	HIV- (n = 37)	P
Lp(a), median (IQR), mg/dL	35	43.6 (21.6 to 82.4)	37	21.8 (16.8 to 46.6)	.033
Elevated Lp(a), No. (%)	35	21 (60)	37	15 (41)	.103
TC, mean (SD), mmol/L	34	4.20 (0.71)	36	4.25 (0.65)	.765
Elevated TC, No. (%)	34	2 (6)	36	3 (8)	>.999
HDL-C, mean (SD), mmol/L	35	1.51 (0.43)	36	1.55 (0.32)	.632
Elevated HDL-C, No. (%)	35	1 (3)	36	0 (0)	.493
LDL-C, mean (SD), mmol/L	33	2.35 (0.65)	36	2.38 (0.57)	.857
Elevated LDL-C, No. (%)	33	2 (6)	36	1 (3)	.603
TG, median (IQR), mmol/L	35	0.75 (0.48 to 1.02)	36	0.56 (0.42 to 0.86)	.070
Elevated TG, No. (%)	35	5 (14)	36	2 (6)	.260
ApoB, mean (SD), mg/dL	35	69.6.1 (16.3)	37	69.3 (14.1)	.930
ApoCIII, mean (SD), mg/dL	35	6.3 (2.5)	37	5.5 (2.0)	.150
ApoE, mean (SD), mg/dL	35	3.8 (1.1)	37	3.3 (0.9)	.089
APOE genotype, No. (%)	35		35		.454
ε2/ε2		0 (0)		0 (0)	
ε2/ε3		6 (17)		4 (11)	
ε2/ε4		1 (3)		4 (11)	
ε3/ε3		18 (51)		17 (49)	
ε3/ε4		10 (29)		9 (26)	
ε4/ε4		0 (0)		1 (3)	

We used the following cutoffs to define elevated values: lipoprotein(a)  $\geq 30$  mg/dL, total cholesterol  $\geq 5.2$  mmol/L (200 mg/dL), low-density lipoprotein cholesterol  $\geq 3.4$  mmol/L (130 mg/dL), high-density lipoprotein cholesterol  $\leq 1$  mmol/L (40 mg/dL), triglycerides  $\geq 1.2$  mmol (110 mg/dL) for children aged  $< 10$  years and  $\geq 1.7$  mmol (150 mg/dL) for children aged  $\geq 10$  years [24].

Abbreviations: ApoB, apolipoprotein B; ApoCIII, apolipoprotein CIII; ApoE, apolipoprotein E; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein a; PHIV+; perinatally HIV-infected patients; TC, total cholesterol; TG, triglycerides.

### Associations Between Lp(a) Levels and Other Lipid Profiles and HIV- and cART-Related Variables

Tables 3 and 4 show the association between Lp(a) and other lipid profiles, and between Lp(a) and HIV- and cART-related variables. In both groups, Lp(a) levels were significantly positively associated with ApoB levels (PHIV+ children: beta coefficient, 0.02; 95% confidence interval [CI], 0.00 to 0.04;  $P = .030$ ; HIV-uninfected children: beta coefficient, 0.07; 95% CI, 0.03 to 0.11;  $P = .003$ ). In PHIV+ children only, Lp(a) was inversely associated with TG levels (beta coefficient,  $-0.78$ ; 95% CI,  $-1.41$  to  $-0.19$ ;  $P = .023$ ). In HIV-uninfected children, Lp(a) was

significantly associated with LDL-C (beta coefficient,  $-1.27$ ; 95% CI,  $-2.35$  to  $-0.18$ ;  $P = .030$ ). Lp(a) levels were not significantly associated with HIV- and cART-related variables such as nadir CD4+ z-score, duration of cART or PI use, having a detectable HIV viral load at assessment, or CDC clinical stage in PHIV+ children.

### Associations Between Lp(a) Levels and Inflammatory and Vascular Biomarkers

PHIV+ children showed higher plasma levels of CRP, IFN- $\gamma$ , IP-10, and MCP-1 compared with controls, as described

**Table 3. Associations Between Lipid Profiles and Lipoprotein(a) Levels**

	No.	PHIV+				HIV-				
		Univariable Analysis		Multivariable Analysis		Univariable Analysis		Multivariable Analysis		
		Coefficient (95% CI)	P	Coefficient (95% CI)	P	Coefficient (95% CI)	P	Coefficient (95% CI)	P	
TC	34	0.09 (-0.51 to 0.69)	.771			36	0.25 (-0.19 to 0.68)	.281		
HDL-C	35	-0.07 (-1.00 to 0.85)	.878			36	-0.04 (-0.98 to 0.90)	.936		
LDL-C	33	0.12 (-0.51 to 0.76)	.701			36	0.38 (-0.11 to 0.87)	.138	-1.27 (-2.35 to -0.18)	.030
TG <sup>a</sup>	35	-0.83 (-1.48 to -0.18)	.017	-0.78 (-1.41 to -0.19)	.015	36	-0.26 (-0.88 to 0.35)	.521		
ApoB	35	0.03 (0.00 to 0.05)	.034	0.02 (0.00 to 0.04)	.030	37	0.03 (0.01 to 0.04)	.008	0.07 (0.03 to 0.11)	.003
ApoCIII	35	0.00 (-0.16 to 0.16)	.980			37	-0.01 (-0.16 to 0.15)	.938		
ApoE	35	0.13 (-0.21 to 0.47)	.451			37	-0.07 (-0.40 to 0.27)	.701		

Results of the ethnicity-adjusted linear regression analysis exploring associations between lipid profiles and lipoprotein(a) levels, shown as coefficient (95% confidence interval) and  $P$  value. Lipoprotein(a) levels were transformed on a 10 log scale. Variables with a  $P$  value  $< .20$  in univariable analysis were included in multivariable regression analysis.

Abbreviations: ApoB, apolipoprotein B; ApoCIII, apolipoprotein CIII; ApoE, apolipoprotein E; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

<sup>a</sup>Logarithmically transformed.

**Table 4. Association Between HIV- and Combination Antiretroviral Therapy–Related Factors and Lipoprotein(a) Levels**

	No.	Univariable Analysis		Multivariable Analysis	
		Coefficient (95% CI)	<i>P</i>	Coefficient (95% CI)	<i>P</i>
Nadir CD4+ z-score	34	−0.40 (−0.96 to 0.16)	.171	−0.42 (−0.99 to 0.15)	.190
Detectable viral load <sup>b</sup>	36	0.26 (−0.75 to 1.26)	.617		
Duration of cART use, y <sup>a</sup>	33	−0.03 (−0.13 to 0.07)	.560		
Duration of PI use, y <sup>a</sup>	35	−0.06 (−0.14 to 0.03)	.207		
CDC category	35				
B		0.33 (−0.58 to 1.25)	.482		
C		0.47 (−0.58 to 1.54)	.383		

Results of the ethnicity-adjusted linear regression analysis exploring associations between HIV- and cART-related characteristics and lipoprotein(a) levels in perinatally HIV-infected children. Shown as coefficient (95% confidence interval) and *P* value. Lipoprotein(a) levels were transformed on a 10 log scale. Variables with a *P* value <.20 in univariable analysis were included in multivariable regression analysis.

Abbreviations: cART, combination antiretroviral treatment; CDC, Centers for Disease Control and Prevention (N, no symptoms, A, minimal symptoms, B, moderate symptoms, C, severe symptoms/AIDS); CI, confidence interval; PI, protease inhibitor.

<sup>a</sup>Also adjusted for age.

<sup>b</sup>At the time of study visit.

previously [7]. In PHIV+ children but not in the HIV-uninfected group, Lp(a) levels were inversely associated with the level of MCP-1 (beta coefficient, 1.17; 95% CI, −2.16 to −0.18; *P* = .027) (Table 5). In HIV-uninfected children only, Lp(a) was positively associated with sCD14 (beta coefficient, 1.52; 95% CI, 0.24 to 2.79; *P* = .27) and negatively associated with sICAM-1 (beta coefficient, −0.44; 95% CI, −0.86 to −0.02; *P* = .047).

#### Associations Between Lp(a) Levels and Neuroimaging Outcomes

PHIV+ children showed lower GM and WM volume, higher volume of WM hyperintensity, poorer WM integrity, and higher CBF in WM, the basal ganglia, and the thalamus, as compared

with healthy matched controls [21, 27, 28]. In PHIV+ and HIV-uninfected children, Lp(a) levels were not significantly associated with any of these neuroimaging outcomes (Supplementary Table 1).

#### DISCUSSION

In this cross-sectional study, we aimed to investigate cardiovascular disease risk by means of Lp(a) levels and other lipid levels, in long-term cART-treated PHIV+ children. We found higher levels of Lp(a) in cART-treated PHIV+ children compared with healthy matched controls, which may be associated with lower

**Table 5. Associations Between Plasma Biomarkers and Lipoprotein(a) Levels in Perinatally HIV-Infected Children and Healthy Matched Controls**

	PHIV+					HIV-				
	No.	Univariable Analysis		Multivariable Analysis		No.	Univariable Analysis		Multivariable Analysis	
		Coefficient (95% CI)	<i>P</i>	Coefficient (95% CI)	<i>P</i>		Coefficient (95% CI)	<i>P</i>	Coefficient (95% CI)	<i>P</i>
CRP	34	0.21 (−0.06 to 0.47)	.134	.23 (−0.01 to 0.48)	.076	36	0.03 (−0.26 to 0.33)	.824		
IL-6	34	0.13 (−0.27 to 0.54)	.524			37	−0.04 (−0.38 to 0.29)	.792		
IFN-γ	34	−0.08 (−0.55 to 0.38)	.725			37	0.05 (−0.28 to 0.38)	.775		
TNF-α	34	−0.30 (−1.22 to 0.63)	.531			37	−0.05 (−0.95 to 0.85)	.914		
MCP-1	34	−1.09 (−2.12 to −0.07)	.045	−1.17 (−2.16 to −0.18)	.027	37	0.62 (−0.35 to 1.60)	.220		
IP-10	34	−0.16 (−0.65 to 0.32)	.519			37	0.14 (−0.40 to 0.67)	.624		
sCD14	34	0.08 (−0.52 to 0.69)	.221			37	−0.33 (−0.77 to 0.11)	.148	1.52 (0.24 to 2.79)	.027
sICAM-1	34	0.61 (−0.81 to 2.02)	.654			37	1.21 (−0.10 to 2.51)	.166	−0.44 (−0.86 to −0.02)	.047
sVCAM-1	34	−0.50 (−1.63 to 0.63)	.389			37	0.62 (−0.44 to 1.68)	.259		
D-dimer	34	0.25 (−0.42 to 0.92)	.473			37	0.24 (−0.29 to 0.77)	.374		
TAT complex	34	−0.82 (−2.33 to 0.69)	.297			37	0.00 (−0.82 to 0.83)	.997		
F1 + 2	34	0.77 (−0.62 to 2.15)	.285			37	−0.16 (−0.98 to 0.66)	.707		
vWF ag	34	0.35 (−0.67 to 1.37)	.509			37	0.48 (−0.33 to 1.29)	.258		
vWFpro	34	−0.17 (−1.62 to 1.28)	.820			37	−0.15 (−1.10 to 0.81)	.765		

Results of the ethnicity-adjusted linear regression analysis exploring associations between inflammatory and endothelial biomarkers and lipoprotein(a) levels. Shown as coefficient (95% confidence interval) and *P* value. Lipoprotein(a) levels and biomarker levels were logarithmically transformed. Variables with a *P* value <.20 in univariable analysis were included in multivariable regression analysis.

Abbreviations: CI, confidence interval; CRP, C-reactive protein; F1 + 2, prothrombin fragment 1 + 2; IL-6, interleukin-6; IFN-γ, interferon-gamma; IP-10, interferon-gamma-inducible protein 10; MCP-1, monocyte chemoattractant protein-1; PHIV+, perinatally HIV-infected patients; sCD14, soluble cluster of differentiation 14; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TAT complex, thrombin-antithrombin III complex; TNF-α, tumor necrosis factor-alpha; vWF ag, Von Willebrand factor antigen; vWF pro, von Willebrand factor propeptide.

levels of inflammation marker MCP-1. This study suggests no associations between Lp(a) levels and HIV- and cART-related factors, and no associations with neuroimaging outcomes.

Over the years, Lp(a) has been established as an independent causal risk factor for atherosclerotic CVD in the general population [8, 9]. Based on wide interethnic differences in Lp(a) levels, an ethnicity-matched control group is crucial to studying Lp(a) levels in PHIV+ patients [16, 17]. Although interethnic differences may potentially have confounded results from earlier studies [10, 14, 15], the current study—including an ethnicity-matched control group—consistently found higher levels of Lp(a) in PHIV+ children compared with healthy peers.

We investigated whether a difference in *APOE* genotype could potentially have contributed to a difference in Lp(a) caused by a difference between the groups. A large study recently reported that *APOE* genotype not only influences LDL-C levels, but also strongly affects Lp(a) levels [26]. As the genotypes were evenly distributed in PHIV+ children and controls, we expect these genetic influences to be limited.

We found that Lp(a) levels were inversely associated with monocyte chemoattractant protein-1 (MCP-1) in PHIV+ children but not in controls. MCP-1 regulates the migration and infiltration of monocytes/macrophages, which is required for immunological surveillance of tissues, and adequate response to inflammation [29]. We do not have a clear explanation for this finding. A possible explanation for this inverse association might be the binding of Lp(a) to MCP-1, as demonstrated by previous *in vivo* and *in vitro* studies, thereby possibly affecting the detection of MCP-1 at higher Lp(a) concentrations [30]. We found a significant inverse association between Lp(a) and sICAM-1 and a significant positive association between Lp(a) and sCD14 in the healthy control group but not in the HIV-infected group. Our findings regarding ICAM-1 do not support previous evidence, which has shown that Lp(a) induces ICAM-1 expression by endothelial cells [31, 32]. Lp(a) has been demonstrated to recruit and activate monocytes [33], which is in agreement with the observed association between Lp(a) and sCD14, a marker of monocyte activation. It is difficult to explain that these significant associations only occur in healthy controls, but the associations might be explained by a type I error, as we did not correct for multiple testing. Recent evidence in treated PHIV+ children found distinct gut microbiota to be associated with markers of vascular endothelial activation and inflammation, potentially leading to CVD [34].

The current study investigated and did not find associations between Lp(a) levels and brain imaging outcomes, such as GM and WM volume, white matter hyperintensity volume, and cerebral blood flow. White matter hyperintensities on MRI are indicative of vascular disease; therefore, an association with Lp(a) levels might be plausible [20]. Research studying this association is, however, scarce. One cross-sectional study in

patients with acute ischemic stroke reported a positive association between Lp(a) and deep and subcortical white matter hyperintensities [35]. A possible explanation for the lack of association between Lp(a) levels and brain imaging abnormalities in this study might be the young age of our study population. Future (longitudinal) studies will further elucidate the relationship between Lp(a) and neuroimaging outcomes in PHIV+.

Although our study uniquely compares lipid profiles between PHIV+ children with an ethnicity-matched control group, several limitations need to be acknowledged. We matched controls to PHIV+ children as best as possible regarding important variables, such as ethnicity. However, we cannot completely rule out genetic differences as the cause of higher Lp(a) in PHIV+ children, as we did not assess Lp(a) genotype, including the copy number variation of the KIV-2 coding region and known SNPs, directly. Due to the cross-sectional design, it was not possible to study the temporal direction of associations or to infer causality. The small sample size may have hampered the detection of associations between Lp(a) levels and HIV- and treatment-related characteristics (inflammation, monocyte activation, and vascular), biomarkers, and neuroimaging outcomes. Further, the small sample size did not allow for larger multivariable regression models or subgroup analyses, for example, to investigate the association between Lp(a) levels and cART regimens. We assessed all lipid levels using nonfasting blood samples. Although the evidence indicates that Lp(a) levels remain stable after normal food intake, using nonfasting blood samples may have affected TG and LDL-C levels [36].

The clinical relevance of elevated Lp(a) levels in PHIV+ remains unclear. Few studies have investigated the association between Lp(a) levels in children and parental history of heart attack as a proxy for future CVD risk; they have found a positive association between pediatric Lp(a) levels and family history of premature heart attack [16, 37]. In this study, we found that 60% of the PHIV+ children had elevated Lp(a) levels compared with 41% of the controls, using a cutoff of 30 mg/dL. Debate is still ongoing as to whether the cutoff is appropriate to use across different ethnic groups, as it remains inconclusive whether the association between Lp(a) levels and CVD in black individuals is similar to that in Caucasian individuals [37, 38]. As the majority of our study population is black, this needs to be established in future studies.

This study is the first to describe elevated Lp(a) levels in PHIV+ children compared with healthy ethnicity-matched controls, which potentially contribute to CVD risk in HIV-positive individuals. Despite the highly heritable nature of Lp(a), these findings, together with existing evidence, indicate that Lp(a) levels can be modulated by HIV and/or cART [10, 39]. The effect of these findings on long-term clinical outcomes remains unknown and needs to be monitored. In the long term, this could provide us with prevention and treatment strategies to reduce HIV-associated CVD burden.

## Acknowledgments

We thank all the study participants; Annouschka Weijnsfeld and Atie van der Plas, pediatric HIV nurses of the Emma Children's Hospital, for their efforts to recruit study participants; and the HIV Monitoring Foundation for their additional data collection and management. We thank Joseph L. Witztum for critically reviewing the manuscript and John J.P. Kastelein for discussions on the study setup.

**Financial support.** This work was supported by AIDSfonds (grant number 2015009).

**Potential conflicts of interest.** MvdH: none. MKH: none. CB: none. HS: none. HJ: none. NK: none. GD: none. SvD: none. Dr. Tsimikas reports personal fees from Ionis Pharmaceuticals, Boston Heart Diagnostics, Oxitope, Inc., and Kleanthi Diagnostics outside the submitted work. In addition, Dr. Tsimikas has a patent Co-inventor of patents on oxidation-specific antibodies via the University of California San Diego with royalties paid. DP: none. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**NOVICE study group.** M. Van den Hof, C. Blokhuis, S. Cohen, D. Pajkrt, H.J. Scherpbier, T.W. Kuijpers, A. van der Plas, A. Weijnsfeld, J.S. ter Stege (Emma Children's Hospital, Amsterdam University Medical Centers, location Academic Medical Center, Amsterdam); N.A. Kootstra, M.W.A. Caan, H.J.M.M. Mutsaerts, C.B.L.M. Majoie, N. Demirkaya, F.D. Verbraak, B. Schmand, G. Geurtsen, R.A.A. Mathot (Amsterdam University Medical Centers, location Academic Medical Center, Amsterdam); F.W.N.M. Wit, P. Reiss (Stichting HIV Monitoring and Department of Internal Medicine, Amsterdam University Medical Centers, location Academic Medical Center); C.E. Teunissen (Amsterdam University Medical Centers, location VU, Amsterdam); J. Kuhle (University Hospital Basel); J.C.M. Meijer (Sanquin, Amsterdam).

## References

- Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of cardiovascular disease among people living with HIV: a systematic review and meta-analysis. *HIV Med* **2012**; 13:453–68.
- Friis-Moller N, Sabin CA, Weber R, et al; Data Collection on Adverse Events of Anti HIVDSG. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med* **2003**; 349:1993–2003.
- Barlow-Mosha L, Eckard AR, McCormsey GA, Musoke PM. Metabolic complications and treatment of perinatally HIV-infected children and adolescents. *J Int AIDS Soc* **2013**; 16:18600.
- Di Biagio A, Rosso R, Maggi P, et al. Inflammation markers correlate with common carotid intima-media thickness in patients perinatally infected with human immunodeficiency virus 1. *J Ultrasound Med* **2013**; 32:763–8.
- Duprez DA, Neuhaus J, Kuller LH, et al; INSIGHT SMART Study Group. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. *PLoS One* **2012**; 7:e44454.
- Idris NS, Grobbee DE, Burgner D, et al. Cardiovascular manifestations of HIV infection in children. *Eur J Prev Cardiol* **2015**; 22:1452–61.
- Blokhuis C, Peeters CFW, Cohen S, et al. Systemic and intrathecal immune activation in association with cerebral and cognitive outcomes in paediatric HIV. *Sci Rep* **2019**; 9:8004.
- Bennet A, Di Angelantonio E, Erqou S, et al. Lipoprotein(a) levels and risk of future coronary heart disease: large-scale prospective data. *Arch Intern Med* **2008**; 168:598–608.
- Kamstrup PR, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. Extreme lipoprotein(a) levels and risk of myocardial infarction in the general population: the Copenhagen City Heart Study. *Circulation* **2008**; 117:176–84.
- Périard D, Telenti A, Sudre P, et al. Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. The Swiss HIV Cohort Study. *Circulation* **1999**; 100:700–5.
- Koppel K, Bratt G, Eriksson M, Sandström E. Serum lipid levels associated with increased risk for cardiovascular disease is associated with highly active antiretroviral therapy (HAART) in HIV-1 infection. *Int J STD AIDS* **2000**; 11:451–5.
- Mauss S, Berger F, Schmutz G, et al. Lipoprotein(a) in patients initiating antiretroviral therapy. *HIV Med* **2008**; 9:415–20.
- Enkhmaa B, Anuurad E, Zhang W, et al. Lipoprotein(a) and HIV: allele-specific Apolipoprotein(a) levels predict carotid intima-media thickness in HIV-infected young women in the Women's Interagency HIV Study. *Arterioscler Thromb Vasc Biol* **2017**; 37:997–1004.
- Charakida M, Donald AE, Green H, et al. Early structural and functional changes of the vasculature in HIV-infected children: impact of disease and antiretroviral therapy. *Circulation* **2005**; 112:103–9.
- Lainka E, Oezbek S, Falck M, et al. Marked dyslipidemia in human immunodeficiency virus-infected children on protease inhibitor-containing antiretroviral therapy. *Pediatrics* **2002**; 110:e56.
- Srinivasan SR, Dahlen GH, Jarpa RA, et al. Racial (black-white) differences in serum lipoprotein (a) distribution and its relation to parental myocardial infarction in children. Bogalusa Heart Study. *Circulation* **1991**; 84:160–7.
- Howard BV, Le NA, Belcher JD, et al. Concentrations of Lp(a) in black and white young adults: relations to risk factors for cardiovascular disease. *Ann Epidemiol* **1994**; 4:341–50.
- Libby P, Ridker PM, Hansson GK; Leducq Transatlantic Network on Atherothrombosis. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* **2009**; 54:2129–38.
- Miller TL, Somarriva G, Orav EJ, et al. Biomarkers of vascular dysfunction in children infected with human immunodeficiency virus-1. *J Acquir Immune Defic Syndr* **2010**; 55:182–8.
- Wardlaw JM, Valdés Hernández MC, Muñoz-Maniega S. What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. *J Am Heart Assoc* **2015**; 4:001140.
- Cohen S, Ter Stege JA, Geurtsen GJ, et al. Poorer cognitive performance in perinatally HIV-infected children versus healthy socioeconomically matched controls. *Clin Infect Dis* **2015**; 60:1111–9.
- UNESCO. International Standard Classification of Education ISCED 2011. Montreal: UNESCO Institute for Statistics; **2012**.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **1972**; 18:499–502.
- Expert panel on Integrated guidelines for cardiovascular health and risk reduction in children and adolescents. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics* **2011**; 128(Suppl 5):S213–56.
- van Capelleveve JC, Bernelot Moens SJ, Yang X, et al. Apolipoprotein C-III levels and incident coronary artery disease risk: the EPIC-Norfolk prospective population study. *Arterioscler Thromb Vasc Biol* **2017**; 37:1206–12.
- Moriarty PM, Varvel SA, Gordts PL, et al. Lipoprotein(a) mass levels increase significantly according to APOE genotype: an analysis of 431 239 patients. *Arterioscler Thromb Vasc Biol* **2017**; 37:580–8.
- Blokhuis C, Mutsaerts HJ, Cohen S, et al. Higher subcortical and white matter cerebral blood flow in perinatally HIV-infected children. *Medicine (Baltimore)* **2017**; 96:e5891.
- Cohen S, Caan MW, Mutsaerts HJ, et al. Cerebral injury in perinatally HIV-infected children compared to matched healthy controls. *Neurology* **2016**; 86:19–27.
- Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* **2009**; 29:313–26.
- Wiesner P, Tafelmeier M, Chittka D, et al. MCP-1 binds to oxidized LDL and is carried by lipoprotein(a) in human plasma. *J Lipid Res* **2013**; 54:1877–83.
- Takami S, Yamashita S, Kihara S, et al. Lipoprotein(a) enhances the expression of intercellular adhesion molecule-1 in cultured human umbilical vein endothelial cells. *Circulation* **1998**; 97:721–8.
- Riches K, Porter KE. Lipoprotein(a): cellular effects and molecular mechanisms. *Cholesterol* **2012**; 2012:923289.
- Schnitzler JG, Dallinga-Thie GM, Kroon J. The role of (modified) lipoproteins in vascular function: a duet between monocytes and the endothelium. *Curr Med Chem* **2019**; 26:1594–609.
- Sessa L, Reddel S, Manno E, et al. Distinct gut microbiota profile in antiretroviral therapy-treated perinatally HIV-infected patients associated with cardiac and inflammatory biomarkers. *AIDS* **2019**; 33:1001–11.
- You CJ, Liu D, Liu LL, et al. Correlation between fibrinogen and white matter hyperintensities among nondiabetic individuals with noncardiogenic ischemic stroke. *J Stroke Cerebrovasc Dis* **2018**; 27:2360–6.
- Langsted A, Kamstrup PR, Nordestgaard BG. Lipoprotein(a): fasting and nonfasting levels, inflammation, and cardiovascular risk. *Atherosclerosis* **2014**; 234:95–101.
- Obisesan TO, Aliyu MH, Adediran AS, et al. Correlates of serum lipoprotein (A) in children and adolescents in the United States. The third National Health Nutrition and Examination Survey (NHANES-III). *Lipids Health Dis* **2004**; 3:29.
- Paré G, Çaku A, McQueen M, et al; INTERHEART Investigators. Lipoprotein(a) levels and the risk of myocardial infarction among 7 ethnic groups. *Circulation* **2019**; 139:1472–82.
- Enkhmaa B, Anuurad E, Zhang W, et al. Effect of antiretroviral therapy on allele-associated Lp(a) level in women with HIV in the Women's Interagency HIV Study. *J Lipid Res* **2018**; 59:1967–76.