

Association of *PLA2G2A* single nucleotide polymorphism and cardiometabolic diseases in an HIV-infected population

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Abstract. While single nucleotide polymorphisms (SNPs) of the *phospholipase A2 (PLA2)* gene have been associated with cardiometabolic diseases (CMDs), such associations are yet to be demonstrated in a human immunodeficiency virus (HIV)-infected African population. Therefore, the association between SNPs of the *PLA2* gene and CMDs in a South African population with HIV was investigated. This cross-sectional study included 716 participants recruited from HIV clinics in Cape Town, South Africa. Hardy-Weinberg equilibrium (HWE) was assessed for the SNPs investigated. CMDs were compared across the genotypes of the SNPs in HWE. Linear and logistic regression analyses, adjusting for age and sex were used to assess the risk of CMDs across the genotypes. Among the SNPs investigated, rs4744 with GG (dominant) and AA (recessive) genotypes were in HWE ($P=0.998$). High-density lipoprotein-cholesterol (HDL-C; $P=0.044$) and a BMI ≥ 30 kg/m² ($P=0.037$) were significantly higher in participants with the dominant genotype compared with those with the recessive genotype of the rs4744 SNP. Moreover, the rs4744 recessive genotype was associated with dyslipidemia characterized by low HDL-C in linear regression ($\beta=0.366$; $P=0.024$) and logistic regression ($OR=0.19$; $P=0.049$), and also associated with MetS in logistic regression ($OR=0.14$; $P=0.036$). In

conclusion, the phospholipase A2 group IIA (*PLA2G2A*) gene has a potential utility for CMD risk assessment in African individuals with HIV.

Introduction

South Africa is facing a fourfold burden of health issues including maternal, newborn and child health conditions, human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) and tuberculosis (TB), non-communicable diseases (NCDs), alongside violence and injuries (1). Despite measures taken to curb these infectious diseases, HIV/AIDS remain prominent globally, with South Africa accounting for 20% of all new infections worldwide in 2021. Moreover, the prevalence of HIV was increased from an estimated 13% (~7.8 million) in 2020 to 13.9% in 2022 (8.45 million) in South Africa (2). Having the largest number of people enrolled on antiretroviral (ARV) therapy (ART) worldwide, South Africa has been able to reduce premature deaths from AIDS, increasing the life expectancy of people living with HIV (PLWH). However, the ageing population of HIV-infected individuals are more prone to cardiometabolic diseases (CMDs) and other age-associated diseases including Alzheimer's disease, Parkinson's disease and cancer (3). CMDs are the leading causes of mortality in the NCD category and include diabetes mellitus (DM), hypertension, cerebrovascular diseases, and other forms of heart diseases such as cardiomyopathies, coronary artery disease and heart failure (4).

The pathway linking HIV/AIDS and CMDs has previously been investigated (5). The virus activates inflammatory responses, cellular apoptosis and mitochondrial dysfunction, which enhances the production of proinflammatory cytokines [tumor necrosis factor (TNF)- α , interleukins (ILs) and C-reactive protein (CRP)], while suppressing the release of the anti-inflammatory cytokine adiponectin, leading to the development of insulin resistance, dyslipidemia, obesity and DM (6). Conversely, ARVs, more specifically non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease

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inhibitors (PIs) after long term use alter glucose homeostasis, causing dyslipidemia, lipodystrophy and mitochondrial dysfunction (6). These dysregulations lead to the development of CMDs (7). The underlying pathways are regulated by various genes, including the phospholipase A2 (*PLA2*) gene.

The *PLA2* gene belongs to the family of phospholipases which encode for enzymes involved in the hydrolysis of phospholipid substrates at specific sn-2 bonds to produce free fatty acids and lysophospholipids. Among the free fatty acids, arachidonic acid, which is a precursor of eicosanoids, including the inflammatory markers prostaglandins and leukotrienes, is the most widely produced. Several isoforms of the *PLA2* enzyme have been discovered with the secreted *PLA2* (s*PLA2*) and cytosolic *PLA2* (c*PLA2*) being the most studied (8). These two differ in that, c*PLA2* is specific to arachidonic acid while s*PLA2* produces various fatty acids (9). A total of about 14 isoforms of s*PLA2* have been identified, with 10 characterized in the human genome including group IB, IIA, IID-F, III, V, X, XIIA and XIIB (10).

Studies on the expression of s*PLA2* have mostly been carried out in knockout mice and other animal models with the expression of s*PLA2* group IIA observed to be upregulated by IL-1, IL-6, TNF- α , lipopolysaccharides (LPS) (11,12) and a high fat diet (HFD) in male Wistar rats (13). When the phospholipase A2 group IIA (*PLA2G2A*) inhibitor KH064 was administered orally to these rats, there was a reduction in weight gain, fat mass and improvement in glucose tolerance and insulin sensitivity, indicating that the increase in expression was associated with metabolic abnormalities (13). However, overexpression of human *PLA2G2A* in male C57BL/6 mice protected them from weight gain on an HFD, enhanced energy expenditure and oxygen consumption, and improved glucose clearance and insulin sensitivity, as determined using glucose tolerance tests and insulin tolerance tests, thereby alleviating obesogenic symptoms in response to an HFD (14,15). While s*PLA2* contributes to dyslipidemia, insulin resistance and obesity through the breakdown of oxidized lipid contained in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (16), c*PLA2* contributes to metabolic diseases through the expansion of lipid droplets and adipogenesis (17,18). Therefore, alteration in the expression of *PLA2* genes will affect their corresponding proteins and downstream targets, leading to the development of CMDs (19).

Single nucleotide polymorphism (SNP) is the most common type of genetic variation among humans. It occurs when a base in a given portion of a gene is substituted by another. These changes have been shown to affect mRNA expression, modify the activity of a protein, leading to the development of a disease. Regarding *PLA2*, a previous study showed that SNPs of this gene are associated with a lower risk of hypertension and CVD, weight loss in patients with chronic obstructive pulmonary disease, stroke, dyslipidemia, and atherosclerosis (19). Specifically, the rs4744 SNP has been associated with increased serum *PLA2G2A* levels and with increased CVD events (20). However, there is sparse literature from Africa despite the high prevalence of HIV and cardiometabolic diseases. The present study therefore aimed to examine the association of nine SNPs in the *PLA2* gene (*PLA2G2A*, *PLA2G2C* and *PLA2G4E*) with CMDs in South African adults living with HIV infection.

Patients and methods

Study type and population. The present study was a cross-sectional study consisting of 716 HIV-infected individuals aged ≥ 18 years and receiving ART. The participants were recruited from primary health care facilities in the Western Cape province of South Africa between March 2014 and February 2015. A total of 42 facilities in Cape Town and 20 in the surrounding rural municipalities met the criteria of provision of ART to a minimum of 325 patients per month and were included in the present study. Among these 62 facilities, 13 urban and 4 rural were randomly selected, with 15-60 participants recruited from each facility. Approval was obtained from the South African Medical Research Council Ethics Committee (approval no. EC021-11/2013) in Cape Town, South Africa and the study was conducted in accordance with the principles of the Declaration of Helsinki. The Health Research Office of the Western Cape Department of Health and the selected healthcare facilities in Cape Town, South Africa granted permission for the recruitment of participants. Written informed consent was obtained from all participants before inclusion in the study.

Inclusion and exclusion criteria. HIV-positive adults (18 years and older) attending facilities with directed HIV clinics, and who were willing to participate and provided informed consent were included in the present study. Participants were excluded from the study if they were: Bedridden, patients with active malignancy or currently undergoing treatment for malignancy, patients on chronic corticosteroid treatment, pregnant or breastfeeding women, and patients unwilling or unable to provide informed consent.

Data collection and sampling. A structured interviewer-administered questionnaire adapted from the WHO STEP-wise approach surveillance tool (21) was used for data collection. On recruitment day, sociodemographic information, anthropometric and blood pressure measurements, medical history of HIV infection including duration of diagnosis of HIV infection, CD4 counts and ARV regimen were recorded in the questionnaire by trained fieldworkers. Socio-demographic information, medical history of HIV infection, anthropometric and blood pressure measurements were obtained as previously described (22). All participants who consented for the study were invited for blood sample collection the following day. Blood samples were collected in EDTA tubes and tubes without anti-coagulant after participants had fasted for at least 8 h, and a portion was processed for biochemical analysis. Plasma glucose (hexokinase method), serum creatinine (Cayman Chemical) and gamma glutamyl transferase (Abcam) were measured using colorimetric methods according to the manufacturers' protocols. Estimated glomerular filtration rate (eGFR) was calculated using the IDMS-traceable Modification of Diet in Renal Disease (MDRD) Study equation (23). Total cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides were measured in serum samples by colorimetric methods using enzymatic techniques (24-26), LDL-cholesterol (LDL-C) was calculated using the formula described by Friedewald *et al* (27), and non HDL-C was calculated using the formula: TC-HDL-C. Liver function enzymes (alanine transaminase and aspartate transaminase) were quantified using

Table I. Primer sequences for SNP genotyping.

SNP	Forward primer (5'-3')	Reverse primer (5'-3')
rs11573156	TTGCATATCCCCACACTGGA	TTGGTAGTCCCTTTGGGTGTG
rs4744	CTGAGACCTCTGCGCCCATC	CCCATCACCAGACAACTCCCA
rs10732279	ATAACTGAGTGCGGCTTCCTT	GGTAAGAGCTGACCCTGACCT
rs6426616	GGCCTGTTGGGGATGATCTG	TCCTTAACCCTTGGCCCCTT
rs2301475	TGATAGACCCAGGACACAAAC	CCTCCCCTGTCAAAGTCCAAA
rs12139100	CCCATCCTCCAAATCCCGTT	CTAGCTGTGTTACAGGGGCA
rs193222555	CTTCTTCTCCACTGCGAGCC	TACTGTGCTTGGTGCTAGGG
rs149056482	GCTCACCTGGTGCCTTGTATTT	GCTGAGTTGTTAGCTGGGGAA
rs116431025	TGGGAAGAAAGTCACGATGGG	GCTGAGTTGTTAGCTGGGGA

SNP, single nucleotide polymorphism.

standardized methods according to the manufacturer's protocols (Thermo-Fisher Scientific, Inc.). All colorimetric assays were performed using a Beckman Coulter AU 500 spectrophotometer (Beckman Coulter, Inc.). Plasma insulin was quantified by chemiluminescence immunoassay (Human Insulin CLIA kit; Abnova Corporation) and glycated haemoglobin (HbA1c) was determined using high-performance liquid chromatography in accordance with the National Glycohaemoglobin Standardization Programme. Highly sensitive CRP (hs-CRP), TNF- α , IL-2 and IL-10 were measured by ELISA (Biomatik kit). All biochemical analyses were performed at an ISO 15189 accredited pathology laboratory (PathCare, Reference Laboratory, Cape Town, South Africa) which had no access to the clinical information of the participants.

The remaining portion of blood samples were frozen at -80°C for DNA extraction and SNP genotyping. DNA was extracted from whole blood samples by the salt extraction method (28).

Genotyping of SNPs was carried out using the TaqMan® Genotyping Master Mix Protocol from ThermoFisher Scientific, Inc. A total of nine SNPs of the *PLA2* gene were genotyped (rs11573156, rs4744, rs10732279, rs6426616, rs2301475, rs12139100, rs116431025, rs193222555 and rs149056482). The *PLA2* gene was selected as it encodes for enzymes which are involved in the regulation of inflammation and have the potential to be used as markers of respiratory, neurodegenerative and cardiometabolic diseases (20). The SNPs were selected from the dbSNP-polymorphism repository (<https://www.ncbi.nlm.nih.gov/snp>). In total, six secreted *PLA2* SNPs (three *PLA2G2A* and three *PLA2G2C* SNPs) and three c*PLA2* SNPs (three *PLA2G4E* SNPs), which are widely studied, and the minor allele have been reported to be associated with increased or reduced serum/activity levels (20,29,30).

A sufficient amount of PCR Master Mix (Applied Biosystems®; Thermo Fisher Scientific, Inc.) for the requisite number of reactions was produced in a 1.5-ml tube containing 5 μ l of 2X Genotyping Mix (cat. no. 4381656; Applied Biosystems®; Thermo Fisher Scientific, Inc.), 0.125 μ l of 40X SNP assay mix (cat. no. 4331349; Applied Biosystems®; Thermo Fisher Scientific, Inc.; containing the forward and reverse primers specific for each SNP; Table I), and 2.875 μ l ddH₂O for each reaction. The mixture was pulse vortexed and then briefly centrifuged (1,000 x g for 30 sec at room temperature). Subsequently, 8 μ l of the Master

Mix were pipetted into each of the wells of the 96-well plate. Then, 2 μ l of the template DNA sample (5 ng/ μ l concentration) were pipetted into the appropriate well. For quality control purposes, a non-template control was also included in each PCR run, with 2 μ l of ddH₂O used in place of template DNA. When all components of the PCR had been pipetted into the appropriate wells, the 96-well plate was covered with MicroAmp optical caps (Applied Biosystems®; Thermo Fisher Scientific, Inc.), and PCR was performed using Applied Biosystems® Quant Studio™ 7 Flex Real-time PCR system (ThermoFisher Scientific, Inc.) as follows: Initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 90 sec and extension at 60°C for 90 sec.

Genotypes were confirmed by randomly selecting 20 samples which were sequenced by Inqaba Biotec using the Sanger sequencing method. The chromatograms showing the GG, GA and AA genotypes of the rs4744 SNP are provided as Figs. S1-S3, respectively.

Calculations and definitions. Body mass index (BMI) was calculated as weight (kg)/height (m²). Participants were categorized according to BMI as normal weight (BMI <25 kg/m²), overweight (BMI $\geq$ 25 kg/m² and BMI <30 kg/m²) and obese (BMI $\geq$ 30 kg/m²) (31). Central obesity was determined using the following criteria: Waist circumference (WC) >94 cm in men and >80 cm in women (32). Hypertension was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg or diastolic blood pressure (DBP) $\geq$ 90 mmHg or known hypertension on treatment (33). Dyslipidemia was defined as TC >5 mmol/l, triglycerides >1.5 mmol/l, HDL-C <1.2 mmol/l, LDL-C >3.0 mmol/l and non-HDL-C >3.37 mmol/l or taking anti-lipid agents (34). Diabetes was defined as fasting plasma glucose $\geq$ 7.0 mmol/l and/or 2-h post glucose load $\geq$ 11.1 mmol/l, previously diagnosed or taking antidiabetic medications (35).

Insulin resistance (IR) was based on the homeostasis model assessment (HOMA) using the formula:

$$\text{HOMA - IR} = \frac{\text{Fasting Glucose (nmol/l)} \times \text{Fasting Insulin (}\mu\text{U/l)}}{22.5}$$

Beta cell function was determined by HOMA- β using the formula (36):

$$\text{HOMA-}\beta = \frac{20 \times \text{Fasting Insulin (}\mu\text{U/ml)}}{\text{Fasting Glucose (}\frac{\text{mmol}}{\text{ml}}) - 3.5}$$

Table II. Clinical characteristics of study participants across MetS status.

Variable ^a	N	Total: 716 (100%)	With MetS: 197 (27.5%)	Without MetS: 519 (72.5%)	P-value
Age (years)	716	38.0 (32.0-45.0)	39.0 (34.0-45.5)	37.0 (30.5-42.0)	<0.001
Weight (kg)	715	68.4 (58.1-81.8)	80.0 (70.3-93.5)	65.9 (56.0-80.3)	<0.001
Height (cm)	715	160.6 (156.0-166.3)	161.3 (156.4-165.6)	160.1 (155.5-165.1)	0.012
BMI (kg/m ²)	715	26.3 (22.1-32.1)	30.5 (27.5-36.3)	24.9 (21.5-31.7)	<0.001
Waist circumference (cm)	715	88.0 (77.5-98.0)	98.5 (92.6-106.8)	84.7 (76.5-96.3)	<0.001
Hip circumference (cm)	715	102.1 (92.6-112.1)	109.2 (103.0-120.2)	100.5 (92.0-111.3)	<0.001
Waist to hip ratio	715	0.9 (0.8-0.9)	0.9 (0.8-1.0)	0.8 (0.8-0.9)	<0.001
Waist to height ratio	715	0.6 (0.5-0.6)	0.6 (0.6-0.7)	0.5 (0.5-0.6)	<0.001
SBP (mmHg)	715	117.0 (107.0-129.5)	124.3 (116.0-135.8)	114.0 (105.0-124.3)	<0.001
DBP (mmHg)	725	82.0 (75.0-90.5)	89.8 (82.3-97.3)	80.3 (73.5-86.5)	<0.001
Heart rate (beats/min)	725	74.5 (66.5-82.5)	76.0 (70.0-82.8)	75.0 (67.0-82.5)	0.016
CD4 count (cells/mm ³)	371	395.0 (241.0-604.0)	429.5 (275.0-670.5)	358.0 (225.0-558.0)	0.187
HIV diagnosis (duration in months)	NA	60.0 (24.0-108.0)	74.5 (48.0-120.0)	53.0 (24.0-96.0)	<0.001
Alanine transaminase (IU/l)	712	23.0 (17.0-34.0)	24.0 (17.5-36.5)	22.0 (16.0-32.0)	0.132
Aspartate transaminase (IU/l)	712	29.0 (24.0-38.0)	29.5 (24.0-37.5)	29.0 (24.5-38.0)	0.916
Total cholesterol (mmol/l)	711	4.3 (3.7-5.1)	4.3 (3.7-5.0)	4.4 (3.8-5.2)	0.024
HDL-C (mmol/l)	711	1.3 (1.0-1.5)	1.1 (1.0-1.2)	1.4 (1.1-1.7)	<0.001
Triglycerides (mmol/l)	710	1.0 (0.8-1.4)	1.2 (0.9-1.8)	0.9 (0.7-1.2)	<0.001
LDL-C (mmol/l)	711	2.5 (2.0-3.1)	2.5 (2.1-3.2)	2.5 (2.0-3.0)	<0.001
Non-HDL-C (mmol/l)	665	3.0 (2.5-3.7)	3.1 (2.6-3.8)	3.0 (2.4-3.5)	<0.001
Glucose (mmol/l)	711	5.0 (4.6-5.4)	5.3 (4.7-6.0)	4.9 (4.6-5.2)	<0.001
Insulin (μ U/l)	679	6.1 (4.0-9.6)	8.3 (6.0-12.3)	5.4 (3.6-8.8)	<0.001
HOMA- β (μ U/mmol)	677	81.3 (48.6-130.1)	99.2 (55.6-155.7)	81.0 (49.7-120.8)	0.220
HOMA-IR (μ U x mmol/l ²)	678	1.4 (0.9-2.3)	2.0 (1.4-3.4)	1.2 (0.8-1.9)	<0.001
HbA1c (%)	712	5.4 (5.2-5.7)	5.6 (5.3-6.0)	5.4 (5.2-5.7)	<0.001
Creatinine (μ mol/l)	710	58.0 (51.0-67.0)	59.5 (51.0-65.5)	58.0 (51.0-66.0)	0.456
GGT (U/l)	711	39.0 (26.0-66.0)	47 (26.5-73.5)	37 (24.0-61.0)	0.019
hs-CRP (mg/l)	711	5.4 (2.4-14.0)	6.4 (2.8-15.4)	5.0 (1.7-12.3)	0.075
INF- γ (pg/ml)	660	14 (12-16)	13.5 (12.0-16.0)	14 (12.0-16.0)	0.104
IL-10 (pg/ml)	660	18 (14.5-24)	17 (14.0-22.0)	18.5 (14.8-25)	0.062
IL-2 (pg/ml)	660	9.5 (9-10)	9 (9-10)	9.5 (9-10)	0.402
TNF- α (pg/ml)	660	19 (15-23.5)	19 (15.5-22.5)	19 (15-24)	0.954
eGFR (ml/min)	701	91.0 (91.0-91.0)	91.0 (91.0-91.0)	91.0 (91.0-91.0)	NA
Sex					<0.001
Female	710	562 (79.2)	174 (31.0)	388 (69.0)	
Male		148 (20.8)	25 (16.9)	123 (83.1)	
Obese (BMI $\geq$ 30 kg/m ²)	715	246.0 (34.4)	107.0 (54.3)	139.0 (26.8)	<0.001
WC (cm): men >94; women >80	715	440.0 (61.5)	186.0 (94.4)	254.0 (49.0)	<0.001
Hypertension	715	173.0 (24.2)	75.0 (38.1)	98.0 (18.9)	<0.001
Type 2 diabetes	709	60 (8.5)	46 (23.1)	14 (2.7)	<0.001
Hyperglycemia	711	148.0 (20.8)	100.0 (50.8)	48.0 (9.3)	<0.001
Total cholesterol >5.0 mmol/l	711	181.0 (25.5)	55.0 (28.1)	126.0 (24.5)	0.325
HDL-C <1.2 mmol/l	711	265.0 (37.3)	115.0 (58.7)	150.0 (29.1)	<0.001
Triglycerides >1.5 mmol/l	710	129.0 (18.2)	85.0 (43.4)	44.0 (8.6)	<0.001
LDL-C >3.0 mmol/l	711	199.0 (28.0)	81.0 (41.3)	118.0 (22.9)	<0.001
Non-HDL-C >3.37 mmol/l	665	243.0 (36.5)	94.0 (50.5)	149.0 (31.1)	<0.001

The median test was used to compare medians, and the chi squared test for independence was used to compare proportions. ^aValues are provided as the median (25-75th percentiles) or count (percentage). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; HOMA- β , homeostasis model assessment of β -cell dysfunction; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycated haemoglobin; GGT, gamma glutamyl transferase; hs-CRP, highly sensitive C-reactive protein; IL, interleukin; TNF- α , tumor necrosis factor- α ; eGFR, estimated glomerular filtration rate; WC, waist circumference; NA, not applicable.

Table III. Frequency of the PLA2 gene SNP genotypes and minor allele frequency.

SNP	Homozygous for major allele, n (%)	Heterozygous, n (%)	Homozygous for minor allele, n (%)	Minor allele frequency (%)	Deviation from HWE (P-value)
rs11573156 (n=589)	304.0 (51.6)	283.0 (48.1)	2.0 (0.3)	24.4	<0.0001
rs4744 (n=661)	549.0 (83.1)	106.0 (16.0)	6.0 (0.9)	8.9	0.940
rs10732279 (n=702)	312.0 (44.4)	379.0 (54.0)	11.0 (1.6)	28.0	<0.0001
rs6426616 (n=684)	50.0 (7.3)	580.0 (84.8)	54.0 (7.9)	49.7	<0.0001
rs2301475 (n=688)	313.0 (45.5)	333.0 (48.4)	42.0 (6.1)	30.3	0.00067
rs12139100 (n=703)	346.0 (49.2)	344.0 (48.9)	13.0 (1.9)	26.1	<0.0001
rs116431025 (n=680)	273.0 (40.1)	407.0 (59.9)	0.0 (0.0)	29.9	<0.0001
rs193222555 (n=694)	4.0 (0.6)	349.0 (50.3)	341.0 (49.1)	25.7	<0.0001
rs149056482 (n=706)	5.0 (0.7)	445.0 (63.0)	256.0 (36.3)	32.3	<0.0001

Chi-square goodness-of-fit test to access deviation from HWE. PLA2, phospholipase A2; SNP, single nucleotide polymorphism; Hardy-Weinberg equilibrium.

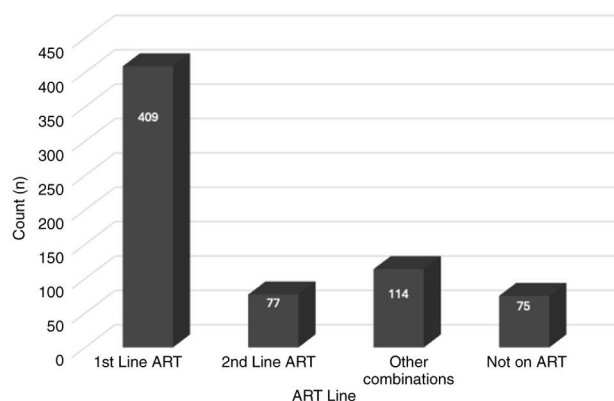


Figure 1. Antiviral therapy regimens used. ART, antiretroviral therapy.

Metabolic syndrome (MetS) was defined using the Joint Interim Statement (JIS) criteria (37) when three of the following conditions were met: i) A waist circumference ≥ 80 cm for women or ≥ 94 cm for men; ii) triglyceride level ≥ 1.7 mmol/l; HDL-C level < 1.04 mmol/l in men or < 1.3 mmol/l in women; iii) high blood pressure defined by SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg or receiving hypertensive medication; and iv) fasting plasma glucose ≥ 5.6 mmol/l or receiving diabetic medications.

The three genotypes were determined from the PCR amplification output as follows: A sample was homozygous for the dominant allele when amplification curves were observed only in the VIC channel, amplification signal in the ROX channel was an indication for homozygous recessive and amplification in both channels was an indication of heterozygous genotype.

Statistical analysis. Data were entered onto an Excel spreadsheet and exported into the IBM Statistical Package for Social Sciences (SPSS) version 25 software (IBM Corp.) for analysis. Continuous variables which were skewed, are reported as median (25-75th percentile) and compared using the median test, while categorical variables are reported as ratio and percentages and compared using the chi squared test. Hardy

Weinberg equilibrium (HWE) was assessed for all nine SNPs, and the rs4744 which was in HWE was used for further analysis. The interactions between genotypes of the rs4744 SNP and cardiometabolic risk profile were determined using linear and logistic regression analysis, by incorporating the dominant, recessive and additive models on the predictive variable, as well as their interaction with age and sex. All analysis were carried out at 95% confidence interval and a 2-tailed $P < 0.05$ was considered to indicate a statistically significant difference.

Results

General characteristics of study participants. A total of 716 participants were involved in the present study, with 72.9% being women and the median duration of HIV was 60 months (25-75th percentile: 24-108) (Table II). The majority of participants (60.6%, $n=409$) were on first-line ART, 11.4% (77 participants) were on second-line ART, 16.9% (114 participants) were on other ART combinations and 11.1% of participants ($n=75$) were not on HIV medications (Fig. 1). The prevalence of type 2 diabetes, obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$), hypertension and metabolic syndrome were 8.5, 34.4, 24.2 and 27.5%, respectively (Table II). Age, weight, height, BMI, waist circumference, hip circumference, waist-to-hip ratio, waist-to-height ratio, SBP, DBP, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), glycated haemoglobin (HbA1c), and gamma glutamyl transferase (GGT) were significantly higher in participants with MetS when compared with those without MetS (Table II). Moreover, the prevalence of MetS was significantly higher amongst the female participants (31.0%) compared with the male participants (16.9%); $P < 0.001$ (Table II).

Genotypic distribution and minor allele frequency of single nucleotide polymorphisms. The genotypic distribution and minor allele frequency of the nine SNPs are presented in Table III. The percentage of successful genotyping of the nine SNPs ranged between 82.3 and 98.6%. All three genotypes were present for rs11573156 (C/G), rs4744 (G/A), rs10732279 (A/G), rs6426616 (G/A), rs2301475 (A/G), rs12139100 (C/T),

Table IV. Clinical characteristics compared across PLA2G2A rs4744 genotypes.

Variable ^a	N	GG (n=536)	AA (n=6)	GA (n=105)	P-value
Age (years)	716	37.5 (31.0-43.0)	46.0 (37.0-55.0)	36.0 (31.0-43.0)	0.350
Weight (kg)	715	71.3 (59.2-88.4)	73.3 (73.2-73.5)	67.5 (56.2-84.6)	0.165
Height (cm)	715	160.8 (155.7-165.5)	165.9 (160.6-171.3)	159.9 (156.8-163.5)	0.898
BMI (kg/m ²)	715	27.7 (22.9-33.7)	26.7 (25.0-28.4)	27.6 (21.8-31.8)	0.202
Waist circumference (cm)	715	89.5 (79.5-101.6)	95.5 (91.5-99.5)	88.0 (78.2-97.8)	0.325
Hip circumference (cm)	715	104.5 (94.4-115.7)	107.23 (100.3-114.3)	103.0 (90.5-111.3)	0.081
Waist to hip ratio	715	0.9 (0.8-0.9)	0.9 (0.8-0.9)	0.8 (0.8-0.9)	8.813
Waist to height ratio	715	0.6 (0.5-0.6)	0.6 (0.6-0.6)	0.6 (0.5-0.6)	0.438
SBP (mmHg)	715	117.0 (107.5-128.5)	120.8 (114.5-127.0)	112.8 (104.0-124.0)	0.263
DBP (mmHg)	725	82.5 (75.5-89.5)	83.8 (80.0-87.5)	82.0 (71.5-88.5)	0.185
Heart rate (beats/min)	725	75.3 (67.5-81.5)	71.5 (67.0-76.0)	78.0 (66.5-85.5)	0.176
CD4 count (cells/mm ³)	371	375.0 (229.0-594.0)	660.0 (476.0-884.0)	408.5 (254.0-545.0)	0.358
Duration of HIV in months	659	60 (24-108)	66.0 (12-84)	60 (36-96)	0.846
Alanine transaminase (IU/l)	712	23.0 (17.0-34.0)	19.0 (16.0-22.0)	23.0 (16.0-34.0)	0.790
Aspartate transaminase (IU/l)	712	30.0 (25.0-38.0)	26.5 (20.0-33.0)	30.0 (24.0-37.0)	0.619
Total cholesterol (mmol/l)	711	4.4 (3.8-5.1)	3.9 (3.4-4.4)	4.3 (3.5-5.0)	0.792
HDL-C (mmol/l)	711	1.3 (1.1-1.6)	1.2 (1.1-1.3)	1.3 (1.0-1.5)	0.044 ^b
Triglycerides (mmol/l)	710	1.0 (0.8-1.3)	0.8 (0.7-0.9)	0.9 (0.6-1.3)	0.259
LDL-C (mmol/l)	711	2.5 (2.0-3.1)	2.2 (1.7-2.7)	2.3 (1.9-2.8)	0.531
Non-HDL cholesterol (mmol/l)	665	3.0 (2.52-3.67)	2.7 (2.2-3.1)	2.9 (2.5-3.2)	0.388
Glucose (mmol/l)	711	5.0 (4.6-5.4)	5.1 (4.5-5.6)	4.9 (4.6-5.2)	0.776
Insulin (μ U/l)	679	6.2 (4.0-9.80)	4.9 (3.6-6.1)	5.4 (4.0-8.7)	0.073
HOMA- β (μ U/mmol)	677	86.7 (50.0-130.8)	78.1 (34.3-122.0)	86.0 (60.0-145.0)	0.247
HOMA-IR (μ IU x mmol/l ²)	678	1.4 (0.9-2.3)	1.1 (0.9-1.2)	1.2 (0.9-1.8)	0.207
HbA1c (%)	712	5.4 (5.2-5.7)	5.4 (5.3-5.5)	5.5 (5.2-5.6)	0.693
Creatinine (μ mol/l)	710	58.0 (51.0-65.0)	48.0 (44.0-52.0)	56.0 (49.0-67.0)	0.240
GGT (U/l)	711	39.0 (25.0-66.0)	73.0 (19.0-127.0)	27.0 (19.0-40.0)	0.281
hs-CRP (mg/l)	711	5.1 (1.9-12.9)	3.1 (2.0-4.2)	4.8 (1.8-10.1)	0.992
INF- γ (pg/ml)	613	14 (12-16.3)	16 (11-17)	13.5 (12-15)	0.323
IL-10 (pg/ml)	613	18.5 (14.5-24)	16 (16-43)	18 (14-23)	0.414
IL-2 (pg/ml)	613	9.5 (9.5-10)	9 (8.5-10)	9 (9-10)	0.882
TNF- α (pg/ml)	613	19 (15-23)	22.5 (21.5-28)	19 (16-23)	0.041 ^b
eGFR (ml/min)	701	91.0 (91.0-91.0)	91.0 (91.0-91.0)	91.0 (87.0-91.0)	NA
Sex (Female)	647	429 (80.0)	6 (100)	79 (75.2)	0.246
BMI $\geq$ 30 kg/m ²	646	195 (36.4)	0 (0.0)	27 (26.0)	0.037 ^b
WC (cm): men >94; women >80	646	335 (62.5)	3 (50.0)	59 (56.7)	0.459
Hypertension	646	144 (26.9)	0 (0.0)	30 (28.8)	0.30
Type 2 diabetes	643	46 (8.6)	0 (0.0)	7 (6.7)	0.367
Total cholesterol >5.0 mmol/l	643	138 (25.9)	0 (0.0)	25 (23.8)	0.322
HDL-C <1.2 mmol/l	643	234 (44.0)	6 (100)	47 (44.8)	0.023 ^b
Triglyceride >1.5 mmol/l	642	73 (13.7)	2 (33.3)	12 (11.4)	0.297
LDL-C >3.0 mmol/l	643	150 (28.2)	1 (16.7)	28 (15.6)	0.787
Non HDL-C >3.37 mmol/l	598	184 (37.2)	2 (33.3)	30 (30.9)	0.49

The median test was used to compare medians, and the chi squared test for independence was used to compare proportions. ^aValues are provided as the median (25-75th percentiles) or count (percentage). ^bP<0.05. PLA2G2A phospholipase A2 group IIA; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; HOMA- β , homeostasis model assessment of β -cell dysfunction; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycated haemoglobin; GGT, gamma glutamyl transferase; hs-CRP, highly sensitive C-reactive protein; IL, interleukin; TNF- α , tumor necrosis factor- α ; eGFR, estimated glomerular filtration rate; WC, waist circumference.

rs193222555 (T/C) and rs149056482 (T/C), while rs116431025 (C/T) contained the CC and CT genotype. Amongst the nine

SNPs investigated, only rs4744 was in HWE (Table III). All the other eight SNPs significantly deviated from HWE

Table V. Simple linear regression analysis of rs4744 SNP genotypes and cardiometabolic traits.

Variable	Age	Sex (Female=Reference)	BMI	rs4744 major	rs4744 hetero	R2 Model 1	R2 Model 2
SBP (mmHg)	0.735 (0.079) ^a	7.841 (1.941) ^a	0.173 (0.113)	0.586 (1.933)	9.431 (7.439)	0.002	0.155
DBP (mmHg)	0.275 (0.053) ^a	2.466 (1.292)	0.227 (0.075) ^b	7.142 (4.951)	0.972 (1.287)	0.004	0.059
TC (mmol/l)	0.026 (0.004) ^a	-0.211 (0.109) ^c	0.011 (0.006)	0.615 (0.418)	-0.072 (0.109)	0.002	0.06
HDL-C (mmol/l)	0.004 (0.02) ^c	-0.176 (0.042) ^a	-0.013 (0.002) ^a	0.366 (0.162) ^c	0.371 (0.165) ^c	0.001	0.053
TG (mmol/l)	0.012 (0.003) ^a	0.275 (0.063) ^a	0.016 (0.004) ^a	-0.253 (0.241)	-0.068 (0.063)	-0.001	0.077
LDL-C (mmol/l)	0.020 (0.004) ^a	-0.110 (0.094)	0.021 (0.005) ^a	0.390 (0.395)	-0.077 (0.093)	0.002	0.075
Non HDL-C (mmol/l)	0.022 (0.004) ^a	-0.028 (0.101)	0.025 (0.006) ^a	0.233 (0.377)	-0.077 (0.102)	0.000	0.072
Glucose (mmol/l)	0.029 (0.007) ^a	0.233 (0.181)	0.038 (0.010) ^a	0.310 (0.691)	-0.016 (0.180)	-0.002	0.040
Insulin	-0.062 (0.032)	-0.961 (0.790)	0.350 (0.045) ^a	2.144 (3.198)	-1.480 (0.786)	0.008	0.128
HOMA-β	-0.627 (0.927)	-20.258 (22.993)	4.495 (1.324) ^a	19.828 (103.649)	-4.870 (22.878)	-0.003	0.011
HOMA-IR	-0.004 (0.010)	-0.139 (0.239)	0.104 (0.014) ^a	0.540 (0.968)	-0.379 (0.238)	0.005	0.166
HbA1c	0.015 (0.003) ^a	0.086 (0.083)	0.020 (0.005) ^a	-0.072 (0.318)	0.046 (0.083)	-0.003	0.052

Data analysis using simple linear regression. Data are reported as coefficients (standard error). Model 1=R2 for covariates only; Model 2=R2 adjusted for age, sex and BMI. ^aP<0.001, ^bP<0.01 and ^cP<0.05. SNP, single nucleotide polymorphism; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; HOMA-β, homeostasis model assessment of β-cell dysfunction; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycated haemoglobin.

Table VI. Logistic regression analysis of rs4744 SNP genotypes and cardiometabolic diseases.

Variable	Age	Sex (Female=Reference)	BMI	rs4744 major	rs4744 hetero
Diabetes	1.07 (1.03-1.10) ^a	1.49 (0.68-3.26)	1.07 (1.02-1.11) ^b	Undetermined	Undetermined
Obesity (WC)	0.96 (0.92-0.99) ^b	13.0 (5.37-31.5) ^a	0.51 (0.45-0.58) ^a	2.70 (0.09-85.6)	3.53 (0.10-119.5)
Hypertension	1.02 (1.04-1.08) ^a	1.17 (0.85-2.21)	1.02 (1.00-1.05)	Undetermined	Undetermined
MetS	1.07 (1.04-1.09) ^a	0.70 (0.39-1.27)	1.13 (1.09-1.16) ^a	0.14 (0.02-0.88) ^c	0.10 (0.02-0.67) ^c
High TC (mmol/l)	1.05 (1.03-1.07) ^a	0.89 (0.54-1.46)	1.02 (0.99-1.05)	Undetermined	Undetermined
Low HDL-C (mmol/l)	0.98 (0.96-1.00) ^c	2.54 (1.62-3.97) ^a	1.05 (1.02-1.08) ^a	0.19 (0.03-1.07) ^c	0.19 (0.03-0.99) ^c
High TG (mmol/l)	1.05 (1.03-1.08) ^a	3.08 (1.79-5.31) ^a	1.06 (1.02-1.09) ^c	0.19 (0.03-1.18)	0.18 (0.03-1.20)
High LDL-C (mmol/l)	1.05 (1.03-1.07) ^a	0.84 (0.51-1.38)	1.04 (1.01-1.07) ^b	1.52 (0.17-13.96)	1.48 (0.16-14.05)
High non-HDL-C (mmol/l)	1.05 (1.03-1.07) ^a	1.00 (0.63-1.61)	1.04 (1.01-1.07) ^b	0.85 (0.14-5.06)	0.67 (0.11-4.19)

Data analysis using binary logistic regression. Data are reported as the odds ratio (95% confidence interval). ^aP<0.001, ^bP<0.01 and ^cP<0.05. SNP, single nucleotide polymorphism; BMI, body mass index; MetS, metabolic syndrome; WC, waist circumference; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein cholesterol.

(all P<0.001). Although positive controls were used during genotyping and the genotypes were confirmed by sequencing 20 samples, the absence of genotyping error or copy number variation for SNPs deviating from HWE cannot be excluded. As such, SNPs not in HWE were excluded from further analysis, and only rs4744 was investigated on its association with cardiometabolic diseases.

Participants characteristics across rs4744 genotypes. In the present study, 647 participants with anthropometric and biochemical measurements were successfully genotyped for rs4744. The GG genotype was the most prevalent representing 83.1% followed by the heterozygous GA representing 16.0%, while the AA genotype was found in 0.9% of the participants (Table III). When clinical measurements were compared across genotypes of the rs4744 SNP of the *PLA2G2A* gene, the recessive AA genotype was associated with low median HDL-C (P=0.044), high median TNF- α (P=0.041), HDL-C <1.2 mmol/l (P=0.023) and the dominant GG genotype was associated with BMI $\geq$ 30 kg/m² (P=0.037), while no significant differences were observed with all other measurements (Table IV). No significant differences were observed with type 2 diabetes and traits of dysglycemia, high blood pressure and other markers of dyslipidemia between the genotypes (Table IV).

Linear and logistic regression analysis for the association of rs4744 SNP genotypes with cardiometabolic traits. Linear regression adjusting for age, sex and BMI was used to explore the relationship between the rs4744 recessive genotype of the *PLA2G2A* gene and cardiometabolic traits. The results showed that the recessive genotype was associated with HDL-C (β =0.366; P=0.024) when compared with the homozygous dominant genotype and (β =0.371; P=0.025) when compared with the heterozygous genotype. There was no association between the genotypes and all other cardiometabolic traits (Table V).

Binary logistic regression was used to examine whether the recessive genotype of the rs4744 SNP of the *PLA2G2A* gene

could be used to predict CMDs and traits including obesity, diabetes, hypertension, MetS or abnormal lipid levels in the study population. The odds of prevalent MetS for individuals with the recessive genotype were 7 times higher (P=0.017) and 10 times higher (P=0.036) when compared with individuals of the heterozygous genotype and dominant genotype, respectively (Table VI). The odds of dyslipidemia characterized by low HDL-C were 5 times higher for individuals with the recessive genotype compared with those with the heterozygous and dominant genotypes (P=0.049).

Discussion

The present study sought to examine the association between nine SNPs of the *PLA2* gene and cardiometabolic diseases including diabetes, obesity, hypertension and MetS in a black South African population with HIV. This population consisted of more female participants (79.3%) than male participants (20.7%), a disparity which cannot be explained by the sex distribution in Cape Town, where females comprised 51% and males 49% in 2015 (38). The high representation of females could result from the high prevalence of HIV as well as the willingness of women to participate in research, and the reluctance of providing blood samples by potential male participants. The prevalence of these cardiometabolic diseases were 8.4, 34.4, 24.2 and 27.5% for diabetes, obesity, hypertension and MetS, respectively. Amongst the nine SNPs examined, only the rs4744 SNP was in HWE equilibrium. Given that non-random mating and genotyping errors may result to deviation from HWE and lead to spurious associations, all SNPs not in HWE were not analyzed for their association with cardiometabolic traits. As such, only rs4744 SNP of the *PLA2G2A* gene was investigated for its association with cardiometabolic diseases. The recessive (AA) genotype of the rs4744 SNP was significantly associated with low HDL-C and high TNF- α levels. Moreover, linear and logistic regression adjusting for age, sex and BMI revealed that carrying the AA genotype of the SNP was associated with low HDL-C levels and an increased risk of MetS.

Non-synonymous SNPs can influence the expression of a gene and mRNA levels leading to an increase or a decrease in the level of the translated protein/enzyme (30,39). The association between SNPs in the *PLA2* gene and its corresponding enzyme activity has been extensively studied (40-42). In Caucasians with and without coronary artery disease (CAD) and diabetes, two SNPs of the *PLA2G2A* gene (rs11573156, and rs1774131 rare alleles) were associated with high enzyme activity and three SNPs (rs3767221, rs3753827 and rs2236771 rare alleles) were associated with low enzyme activities (40). Similarly, sPLA2-IIa levels were almost 200% higher in carriers of the rs4744 recessive genotype when compared with carriers of the wild type homozygous genotype in patients with stable CAD (20). Similarly, the rare allele of the rs11573156 SNP was associated with high enzyme activity in French patients with myocardial infarction (41). Moreover, altered activities and levels of PLA2G2A proteins have been observed to be associated with cardiometabolic traits and diseases including dyslipidemia, insulin resistance (42), obesity, CVD, stroke and type 2 diabetes (43). Lower levels of sPLA2 enzyme activity and sPLA2-IIA mass were associated with a reduced risk of cardiovascular events in the general European population (29). Similarly the A/A genotype of rs4744 SNP was associated with a higher risk of acute cardiovascular events (acute coronary syndrome, myocardial infarction, coronary revascularization) (20). As such, changes in the expression of genes resulting from SNPs could possibly alter downstream pathways which are associated with the development of cardiometabolic diseases. The present findings could therefore be indicative that the recessive genotype of the rs4744 SNP of the *PLA2G2A* gene alters downstream targets leading to the development of dyslipidemia characterized by low HDL-C and MetS.

The various forms of the *PLA2* gene have been reported to be associated differently with insulin sensitivity and adiposity. The expression of *PLA2G1B* and *PLA2G2E* was shown to be positively associated with the risk of obesity and insulin resistance (17,44). Similarly, an inhibitor of the *PLA2G2A* gene reduced the overexpression of the gene and attenuated visceral adiposity, and reversed most characteristics of MetS, including insulin sensitivity, glucose intolerance and cardiovascular abnormalities in male Wistar rats (13). Conversely, overexpression of *PLA2G2A* improved insulin sensitivity, glucose tolerance, and adiposity in IIA⁺ mice (mice expressing the human *PLA2G2A* gene), indicating that the metabolic effect is dependent on the SNP studied (44). Given that rs4744 SNP has been associated with increased serum PLA2G2A levels leading to the development of CVD (20), a similar mechanism might be postulated in this present study whereby the recessive genotype contributed to the dysregulation of the enzyme or activity level leading to the development of MetS.

Another pathway through which the recessive genotype of the rs4744 SNP of the *PLA2G2A* gene could increase the risk of MetS is the inflammatory pathway and dyslipidemia. Notably, 100% of participants with the minor AA genotype had low HDL-C levels, characteristic of dyslipidemia compared with 44% of the major (GG) genotype carriers. The dyslipidemia could also result from the ARVs, given that >80% of the study participants were on either protease inhibitors, NRTIs, NNRTIs or a combination of these ARVs, which

contribute to abnormal lipid metabolism. Moreover, the virus could contribute to increased inflammation in carriers of the minor genotype. This is because the inflammatory marker, TNF- α was higher in carriers of the recessive genotype when compared with carriers of the dominant genotype. The absence of association with IL-10 might indicate that the SNP investigated induced inflammation by targeting the pro-inflammatory pathway without affecting the anti-inflammatory pathway.

The limitations of the present study include the small sample size and low representation of male participants. Due to the limited sample size, only 6 participants with the recessive genotype were genotyped, and none were found in the diabetes, obesity, and hypertension group. As such, logistic regression to predict diabetes, obesity, and hypertension could not be computed. Furthermore, the present study did not assess the expression and activity levels of serum PLA2G2A, making it impossible to establish a correlation between the genotypes and the expression levels of the enzyme. Moreover, the levels of prostaglandins and leukotrienes, which are the main inflammatory markers produced from hydrolysis of phospholipids by PLA2G2A enzymes, were not determined. Additionally, the study only involved HIV participants, limiting the possibility to explore and compare the effects between HIV and non-HIV populations. Therefore, further studies to mitigate these limitations are warranted.

In conclusion, in a black South African population with HIV, the minor AA genotype of the rs4744 SNP of the *PLA2G2A* gene could potentially contribute to cardiometabolic risk evaluation. This minor genotype was revealed to be associated with a high PLA2 enzyme activity level. In addition, this enzyme mediates lipid signaling (20), and in the South African population may contribute to CMDs by inducing inflammation and dyslipidemia. Given that, to the best of our knowledge, this is the first study to report such findings and no independent validation was carried out, further studies in independent cohorts are warranted to confirm these results, while investigating other SNPs and protein expression levels. Furthermore, functional studies in animal models will contribute to identify the molecular pathways which may be essential in the establishment of therapeutic targets.

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Availability of data and materials

The data SNP dataset is available at: https://esango.cput.ac.za/articles/dataset/_b_Investigating_PLA2G2A_SNPs_and_cardiometabolic_diseases_in_South_Africa_b_/29480720?file=55989242.

Authors' contributions

TEM and APK conceived the study and acquired the funding. NEN performed the single nucleotide polymorphism genotyping. NEN and UN confirm the authenticity of all the raw data, performed the data analysis and interpretation, and wrote the original draft of the manuscript. All authors participated in the revision of the manuscript, and read and approved the final version.

Ethics approval and consent to participate

Approval was obtained from the South African Medical Research Council Ethics Committee (approval no. EC021-11/2013) in Cape Town, South Africa and the study was conducted in accordance with the principles of the Declaration of Helsinki. The Health Research Office of the Western Cape Department of Health and the selected healthcare facilities in Cape Town, South Africa granted permission for the recruitment of participants. Written informed consent was obtained from all participants before inclusion in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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