Journal of Acute Disease

Original Article

doi: 10.4103/2221-6189.307388 jadweb.org

The −250G>A polymorphism in the hepatic lipase gene promoter influences plasma lipid profile and lipoprotein ratio in patients with ischemic stroke

Johnson Oshiobugie Momoh[1,2]✉, Akinniyi Adediran Osuntoki[2], Osaretin Albert Taiwo Ebuehi[2], Olusola Ajibaye[3]

[1]*Department of Chemical Sciences (Biochemistry Unit), School of Pure and Applied Sciences, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria*

[2]*Department of Biochemistry, College of Medicine, University of Lagos, PMB 12003, Lagos, Nigeria*

[3]*Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research, Lagos, Nigeria*

ABSTRACT

Objective: To evaluate the influence of –250G>A (rs2070895) polymorphism in hepatic lipase gene (*LIPC*) promoter on plasma lipid parameters of ischemic stroke patients.

Methods: A total of 100 stroke patients and 100 control subjects matched for sex (59 men and 41 women) and age were selected. Hepatic lipase activity and lipid profiles were measured while lipoprotein ratios were calculated. Genotyping of the –250G>A promoter polymorphism of the *LIPC* was performed by the polymerase chain reaction and restriction fragment length polymorphism method combined with 2% gel electrophoresis and then confirmed by direct sequencing. The *LIPC* promoter gene sequencing data were compared with refseqNG011465.1 *LIPC* from GenBank.

Results: The frequencies of GG, GA and AA genotypes of *LIPC* rs2070895 polymorphism were 39%, 45% and 16% for the control, 10%, 37% and 53% for the stroke subjects (P<0.000 1), respectively. The frequencies of G and A alleles were 61.5% and 38.5% for the control, and 28.5% and 71.5% for the stroke subjects (P<0.000 1). Our study shows that the mutant allele of the *LIPC* promoter was associated with dyslipidemia, lower hepatic lipase activity, and this variation contributed to the increased defective plasma high-density lipoprotein-cholesterol (HDL-C), HDL_2-C and HDL_3-C concentration for both subjects. The control subjects had 6 single nucleotide polymorphism and 6 amino acid substitutions while the stroke subjects had 32 single nucleotide polymorphism and 20 amino acid substitutions.

Conclusions: *LIPC* –250G>A polymorphism can influence plasma lipid profiles and lipoprotein ratios in patients with ischemic stroke.

KEYWORDS: –250G>A polymorphism; rs2070895; Hepatic lipase gene promoter; Lipid parameters; Ischemic stroke

1. Introduction

Epidemiological studies have indicated that dyslipidemia, include a high concentration of plasma triglycerides (TG)[1], cholesterol (TC)[1], apolipoprotein B (ApoB)[2] and low-density lipoprotein-cholesterol (LDL-C)[1] together with low concentration of high-density lipoprotein-cholesterol (HDL-C)[1] and apolipoprotein A1 (ApoA1)[2] are risk factors for stroke and cardiovascular diseases[1]. Some studies have shown clearly that plasma lipid concentrations are controlled and influenced by factors such as exercise[3], demographics[4], diet[5], alcohol consumption[6], cigarette smoking[6,7], hypertension[8], obesity[9] and genetic factors. In our previous study, we explained that lipoprotein ratios can be used to predict the risk of cardiovascular diseases[1]. These ratios include TC/HDL-C, LDL-C/HDL-C, TG/HDL-C, log TG/HDL-C, HDL-C/TC, HDL-C/LDL-C and atherogenic coefficient[1]. Jeppesen *et al.*

✉To whom correspondence may be addressed. E-mail: mjohnson_2008@yahoo.com

How to cite this article: Momoh JO, Osuntoki AA, Ebuehi OAT, Ajibaye O. The –250G>A polymorphism in the hepatic lipase gene promoter influences plasma lipid profile and lipoprotein ratio in patients with ischemic stroke. J Acute Dis 2021; 10(1): 28-35.

Article history: Received 24 April 2020; Revision 20 November 2020; Accepted 25 November 2020; Available online 25 January 2021

showed that the ability of these lipid lipoprotein ratios to predict cardiovascular disease is clinically important and may explain the association of lipid ratios with cardiovascular risk factors unrelated to TC metabolis[10].

Hepatic lipase (HL) (Triacylglycerol lipase, EC 3.3.3.3) enzyme is a glycoprotein responsible for the modulation of the metabolism of LDL, intermediate-density lipoprotein, and HDL[11]. High plasma HL activity is associated with low HDL-C level, and HL converts large TG-rich HDL_2-C into small dense HDL_3-C[12]. The enzyme can facilitate hepatic uptake of lipoproteins[13,14]. The genetic variation in the HL gene can affect the concentration of these lipid lipoproteins and then raise the risk of ischemic cardiovascular disease (myocardial infarction, ischemic heart disease, and angina pectoris) and ischemic cerebrovascular disease (transient ischemic attack, amaurosis fugax and ischemic stroke)[15].

HL gene is located on chromosome 15q21, and is comprised of 9 exons and 8 introns. It spans over 30 kb of DNA and encodes a protein of 449 amino acids[16,17]. Eller *et al.* showed that 20%-30% of variations in HL activity are due to the presence of polymorphism in the promoter region of the lipase gene (*LIPC*)[18]. Studies have identified four different promoter polymorphisms of the *LIPC* (–250G>A, –514C>T, –710T>C, and –763A>G), and they are in complete linkage disequilibrium[19,20]. These polymorphisms are associated with a significant decrease in plasma HL activity and increased HDL-C concentration. Furthermore, five relatively common HL amino acid substitutions have been reported with either defective HL activity (T383M, L334F, S267F) or normal activity (N193S, V73M)[21,22]. The study aims to explore the effect of *LIPC* –250G>A promoter polymorphisms on plasma lipid profiles, lipoprotein ratios, diastolic blood pressure, systolic blood pressure and body mass index (BMI) in ischemic stroke subjects.

2. Materials and methods

2.1. Chemicals

Genomic DNA was isolated from blood leukocytes using DNA Qiagen kits according to manufacturer instructions. The quality and quantity of extracted DNA were determined using the spectrophotometric method with NANODROP 1000R (Thermo Fisher Scientific, USA). The *LIPC* genes were amplified using PCR machine (TECHNE TC-4000) with the necessary PCR reagents and primers. The PCR products were digested with *Dra* Ⅰ restriction enzyme followed by electrophoresis on 2% w/v agarose gel stained with ethidium bromide, 0.5 mg/mL in Tris-borate EDTA. The PCR amplicons were sequenced using AB13777 automatic DNA sequencer (Applied Biosystem). HL reagents contain: glycerol trioleate (2.9 μmol) (Amersham, England U.K.), glyceryl tri [1-^{14}C] oleate (0.13 μmol), gum arabic (10 mg), and bovine serum albumin (20 mg), 0.9% NaCl (0.1 mL) and 2 M NaCl (0.5 mL). ApoA1 and ApoB reagents from Bayer Diagnostic SpA, Italy contain: antibody reagent for ApoA1 (Anti-human ApoA1 antiserum, Tris 0.05 mol/L at pH 8.0, 50 g of polyethylene glycol, 1 g of surfactant and 1 g of sodium azide contained per litre), antibody reagent for ApoB (Anti-human ApoB antiserum, Tris 0.05 mol/L at pH 8.0, 50 g of polyethylene glycol, 1 g of surfactant and 1 g of sodium azide contained per litre). TG, TC, HDL-C and HDL_3-C, were measured using commercially available test kits from Randox Laboratories Ltd, England UK.

2.2. Study subjects

Clinical and laboratory data of 100 ischemic stroke subjects (59 men and 41 women) who visited Lagos University Teaching Hospital Lagos, Nigeria were obtained for the analysis. All the stroke subjects underwent cerebral computerized tomography test and they were confirmed to have ischemic stroke. Besides blood from 184 control subjects was collected and assayed initially, and 100 samples matched for sex and age (59 men and 41 women) met the study inclusion criteria. Blood was placed in lithium heparin vacutainer and EDTA tubes from subjects who had been fasting for 12 to 16 h. Altogether, 84 samples were excluded due to risk factors associated with cardiovascular diseases; history of myocardial infarction, heart attack, congestive heart failure, stroke, diabetes, HIV, fasting blood glucose ⩾7.0 mmol/L; and drug administrations which affects plasma (lipid-lowering medication like: beta-blockers, fibrates or statins, diuretics among others). There was no significant difference in age and socioeconomic status between the control subjects and the stroke patients.

2.3. Ethical approval

All the control and stroke subjects were given consent forms and questionnaires. The research ethical approval was obtained from Lagos University Teaching Hospital Research and Ethical Committee with Healthy Research Committee assigned no: ADM/DCST/HREC/100. Healthy individuals and stroke patients who were not willing to participate in the research were excluded from the study.

2.4. Blood pressure and BMI

Blood pressure of all subjects was measured using a mercurial sphygmomanometer after 5 min rest. In addition, height and weight were measured to calculate BMI. Obesity of the subjects was defined as BMI ⩾30 kg/m^2.

2.5. HL activity

HL activity was assayed in post-heparin plasma using an immunochemical method[12]. An immunochemical method is a process utilizing highly specific affinity of an antibody for its antigen to detect the distribution of a given protein (antigen) in tissues or cells. This methodology is highly important in clinical context, as the normal localizations of a large number of proteins are known to be altered in various disease states. HL was measured within one month

after the clinic visit. For the assay of HL activity, 10 μL postheparin plasma was incubated at 28 ℃ for 90 min with a substrate mixture. Both substrates contained per milliliter, glycerol trioleate (2.9 μmol), glyceryl tri [1-^{14}C] oleate (0.13 μmol), gum arabic (10 mg), and bovine serum albumin (20 mg). The substrate for total postheparin plasma HL activity also contains 0.9% NaCl (0.1 mL) and a high salt concentration (2 M NaCl, 0.5 mL). The assays were run at 28 ℃ for 90 min. The released ^{14}C free fatty acid was separated from glycerides by the liquid partition system and its radioactivity determined in a Packard liquid scintillation counter. The assay includes two blanks containing saline instead of postheparin plasma and two reference standards of postheparin plasma taken from two normal subjects and kept frozen in small aliquots. The lipase activities were calculated from the difference of FFA radioactivity between sample and blank, and they are expressed as μmoles FFA released per millilitre of postheparin plasma per hour.

2.6. ApoA1 and ApoB

ApoA1 and ApoB were determined by the immunoturbidimetric method[23]. It is based on the reaction of sample containing human apolipoprotein A-1 and apolipoprotein B and specific antiserum to form an insoluble complex which can be measured turbidimetrically at 340 nm.

2.6.1. ApoA1 assay

Antibody reagent preparation: The anti-human ApoA1 antiserum was diluted in buffer (Tris, 0.05 mol/L, pH 8.0) containing per litre, 50 g of polyethylene glycol, 1 g of surfactant and 1 g of sodium azide. The reagent was later stored at 5 ℃.

Preparation of diluent: The diluent contained tris buffer (0.01 mol/L, pH 8.0) contained per litre, 9 g of sodium chloride, 1 g of bovine serum albumin, 22 g of surfactant and 1 g of sodium azide. The ApoA1 was assayed by mixing 10 μL of diluted plasma with 50 μL of distilled water, plus 200 μL of antibody reagent and allowed to stand for 5 min at 37 ℃. The absorbance was measured at 340 nm against the reagent blank.

2.6.2. ApoB assay

Antibody reagent for ApoB was preparation using Anti-human ApoB antiserum instead of the anti-human ApoA1 antiserum and follow the procedure mentioned above. The ApoB was assayed by mixing 20 μL of diluted plasma with 40 μL of distilled water, plus 200 μL of antibody reagent and allowed to stand for 5 min at 37 ℃. The absorbance was measured at 340 nm against the reagent blank. ApoB/ApoA1 ratio was calculated.

2.7. Lipid parameters and lipoprotein ratios

The blood samples were collected in lithium heparinize tubes, and the plasma was separated from the blood cells by centrifugation at 1 500×*g* for 15 min at 4 ℃. TG, TC, HDL-C and HDL_3-C were assayed by using Randox kits according to manufacturer instructions. And very LDL-C (VLDL-C), LDL-C, HDL_2-C, and non-HDL-C were calculated as follows[1,24]:

VLDL-C=TG/5

LDL-C = TC – HDL-C – TG/5

HDL_2-C = HDL-C – HDL_3-C

Non-HDL-C = TC – HDL-C

2.8. Atherogenic ratios

Castellis risk index Ⅰ and Ⅱ, atherogenic index of plasma, atherogenic coefficient were calculated as follows:

Castellis risk index Ⅰ = TC/HDL-C[1]

Castellis risk index Ⅱ = LDL-C/HDL-C[1]

Atherogenic index of plasma = log TG/HDL-C[1]

Atherogenic coefficient = (TC – HDL-C)/HDL-C[1]

TG/HDL-C and HDL-C/LDL-C were also calculated.

Lipoprotein ratios shown above were determined by using the method described by Momoh *et al.*[1]. Dyslipidemia was defined by the presence of one or more abnormal serum lipid indexes as described in our previous study[1].

2.9. DNA analysis

The genomic DNA was isolated from blood leukocytes using DNA Qiagen kits following the manufacturer's instructions. The purified extracted DNA was later stored in the refrigerator at 4 ℃ until analysis. The quality and quantity of the purified extracted DNA were determined using the spectrophotometric method with NANODROP 1000^R (Thermo Fisher Scientific, United States of America), which quantified the amount of extracted DNA in nanogram per microlitre (ng/μL) and assessed the quality (purity) based on the ratio of absorbance at 260 nm : 280 nm for all the samples[25]. The isolated DNA samples were genotyped by using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. The genotyping of the *LIPC* at position –250 was done by polymerase chain reaction. The primers were as follows F:5' CCTACCCCGACCTTTGGCAG-3' and R: 5'-GGGGTCCAGGCTTTCTTGG-3'. The PCR cycling conditions were as follows: initial denaturation at 94 ℃ for 2 min, followed by 35 cycles of denaturation at 94 ℃ for 30 s, annealing at 64 ℃ for 30 s, and extension at 72 ℃ for 30 s, with a final extension at 72 ℃ for 2 min. The polymerase chain reaction products were then digested with 1 μL restriction enzyme *Dra* Ⅰ at 36 ℃, followed by electrophoresis on 2% agarose gel containing ethidium bromide, 0.5 mg/mL in Tris-borate EDTA. Gel images were captured and DNA bands were visualized with the gel documentation system. The sizes of the fragments obtained were estimated by comparing with the 50 bp to 1 kb DNA ladder (Jena Bioscience GmbH Germany) marker. The absence of the cutting site indicates the –250G allele while the presence of the cutting site indicates the –250A allele. To confirm the results, the experiments were repeated and direct sequencing was performed. The genotyping of the PCR amplicons were completely consistent. The 411 bp PCR amplicons were

sequenced in both forward and reverse direction using AB13777 automatic DNA sequencer (Applied Biosystem) with the following primers: RhS-F: 5'-CCTACCCCGACCTTTGGCAG-3'; RhS-R: 5'-GGGGTCCAGGCTTTCTTGG-3' for 10% of the samples.

2.10. Statistical analyses

GraphPad prism with version 5.01 was used. The data are expressed as Mean ± SD. Allele frequency was determined *via* direct counting and the standard-goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. One-way ANOVA *post hoc* Turkey's test was used for the analysis to compare the significant difference between wild type and mutant genotypes. Two-way ANOVA *post hoc* Turkey's test was used to compare the significant difference between genotypes for both separate subjects. The significant level of the test was set at α=0.05.

3. Results

3.1. Electrophoresis and genotyping

The target gene of 411 bp was found in all blood samples for the control and the stroke subjects (Figure 1). The genotypes were named according to the absence or presence of the enzyme restriction sites; when a G to A base substitution at 250 nucleotide position of the *LIPC*. AA genotypes were homozygote with bands of 170 bp. The GA genotypes were heterozygote with bands of 170 and 411 bp while GG genotypes were homozygote with band of 411 bp (Figure 2).

3.2. Genotypic and allelic frequencies

The observed and expected genotypes of the examined –250 G>A *LIPC* single nucleotide polymorphism (SNP) for the control and the stroke subjects were examined according to Hardy-Weinberg equilibrium, and no significant difference were found between the observed frequence and the expected Hardy-Weinberg frequence in both subjects. The result shows that the frequencies of G and A alleles were 61.5% and 38.5% for the control and 28.5% and 71.5% for the stroke subjects, respectively (Table 1).

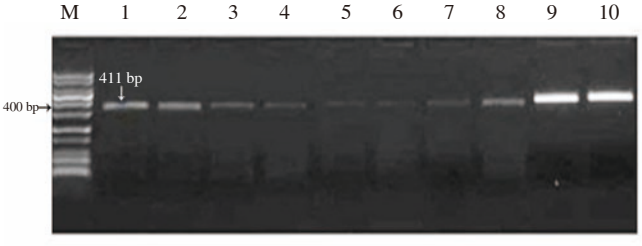

Figure 1. Agarose gel electropherogram for *LIPC* –250G>A PCR products. Lane M: 50 bp to 1kb marker ladder; Lanes 1-10: the DNA samples of the 411 bp band of the target genes.

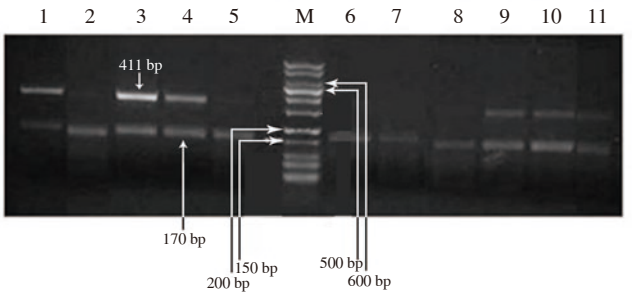

Figure 2. Agarose gel electropherogram for genotyped PCR products. Lane M: 50 bp to 1 kb marker ladder; Lanes 1-4 and 9-11: GA genotypes (411 bp and 170 bp); and Lanes 5 and 6-8: AA genotypes (170 bp).

3.3. Plasma lipid parameters for both the control and stroke subjects with different genotypes

The HL activity, AC, TC/HDL-C and LDL-C/HDL-C were significantly (P<0.05) higher in the GG genotype when compared to the mutants genotypes (GA and AA) and other parameters were significantly (P<0.05) lower in the GG genotype for both subjects. TC, TG, HDL-C, HDL_2-C, HDL_3-C and VLDL-C were significantly (P<0.05) lower in the GA genotype compared to the AA genotype for both subjects (Table 2).

3.4 Sequencing

The PCR-RFLP for the AA, GA and GG genotypes were confirmed by sequencing (Supplementary figure 1).

4. Discussion

In this study, a base variation (–250G>A) in the *LIPC* promoter region was determined by PCR-RFLP and DNA sequencing. The polymorphism in this study was presented in 90% (GA=37% and AA=53%) of patients with ischemic stroke and 61% (GA=45% and AA=16%) of the non-symptomatic control subjects. The A allele was significantly more abundant in the ischemic stroke subjects compared with the control. For all the control subjects, HL activities were significantly higher in GG genotype compared with the heterozygous –250A allele (P=0.008 5) and homozygous –250A allele (P=0.000 1). The control subjects with homozygous AA genotype had lower HL activity (P=0.000 9) compared with the heterozygous A allele (GA genotype). For the stroke subjects, HL activity was higher in patients with GG genotype compared with the GA genotype (P=0.0 014) and AA genotype (P=0.000 1). The stroke subjects with GA genotype had significantly higher HL activity compared with the AA genotype (P=0.000 2). The significant difference (P=0.008 5-0.000 1) in the HL activities was as a result of genetic mutation in (–250 G→A) their nucleotide sequence. This polymorphism was responsible for the lower HL activity in patients with the mutant A allele.

The *LIPC* –250A allele was shown to be associated with higher concentration of TC, VLDL, TG and dyslipidemia for both the

Table 1. Distribution of different genotypes of –250G>A of the *LIPC* gene.

Genotype/Allele frequency	Control subjects		Stroke subjects	
	Observed frequency	Expected H-W frequency	Observed frequency	Expected H-W frequency
GG	39 (39%)	37.82 (37.82%)	10 (10%)	8.12 (8.12%)
GA	45 (45%)	47.36 (47.36%)	37 (37%)	40.75 (40.75%
AA	16 (16%)	14.82 (14.82%)	53 (53%)	51.12 (51.12%)
P-value	0.6190		0.3569	

Table 2. *LIPC* –250 G>A genotypes and plasma lipid parameters for both subjects.

Parameters	Control subjects			Stroke subjects		
	GG (*n*=39)	GA (*n*=45)	AA (*n*=16)	GG (*n*=10)	GA (*n*=37)	AA (*n*=53)
HL (μmol/h/mL)	32.32 ± 0.63^{am}	30.19 ± 0.50^{bn}	26.94 ± 0.69^{co}	24.71 ± 1.07^{am}	21.24 ± 0.45^{bn}	18.64 ± 0.47^{co}
TC (mg/dL)	168.49 ± 13.86^{abmn}	164.28 ± 16.98^{bn}	179.47 ± 19.45^{am}	210.31 ± 19.54^{bn}	214.63 ± 21.98^{bn}	234.32 ± 24.56^{am}
TG (mg/dL)	82.05 ± 3.82^{co}	91.81 ± 3.01^{bn}	99.26 ± 3.97^{am}	168.88 ± 7.15^{co}	186.79 ± 8.09^{bn}	198.48 ± 9.53^{am}
HDL-C (mg/dL)	96.71 ± 3.01^{co}	101.31 ± 2.64^{bn}	110.25 ± 3.06^{am}	44.17 ± 1.93^{co}	55.92 ± 2.51^{bn}	63.66 ± 2.14^{am}
HDL_2-C (mg/dL)	20.77 ± 1.14^{co}	22.38 ± 1.13^{bn}	28.77 ± 0.83^{am}	8.26 ± 0.85^{co}	19.50 ± 0.79^{bn}	23.27 ± 1.18^{am}
HDL_3-C (mg/dL)	75.94 ± 0.99^{co}	78.90 ± 0.56^{bn}	81.54 ± 0.97^{am}	35.91 ± 0.37^{co}	36.42 ± 0.19^{bn}	40.39 ± 0.41^{am}
LDL-C (mg/dL)	51.20 ± 2.59^{am}	45.75 ± 1.48^{cn}	49.04 ± 5.75^{bm}	132.40 ± 12.21^{am}	121.50 ± 5.80^{bn}	131.30 ± 2.48^{am}
VLDL-C (mg/dL)	16.46 ± 0.36^{co}	18.51 ± 0.35^{bn}	20.10 ± 0.32^{am}	33.77 ± 0.43^{co}	37.27 ± 0.38^{bn}	39.36 ± 0.56^{am}
Non-HDC (mg/dL)	71.80 ± 4.33^{am}	63.00 ± 1.64^{co}	69.14 ± 5.82^{bn}	166.20 ± 12.23^{bm}	158.70 ± 5.78^{cn}	170.70 ± 2.54^{am}
AC	0.766 ± 0.059^{am}	0.628 ± 0.020^{bn}	0.656 ± 0.075^{bn}	3.798 ± 0.301^{am}	2.868 ± 0.119^{bn}	2.749 ± 0.085^{co}
TC/HDL-C	1.77 ± 0.059^{am}	1.63 ± 0.020^{bn}	1.66 ± 0.075^{bn}	4.80 ± 0.301^{am}	3.87 ± 0.12^{bn}	3.75 ± 0.085^{co}
LDL-C /HDL-C	0.531 ± 0.057^{am}	0.445 ± 0.020^{bn}	0.457 ± 0.069^{bn}	3.025 ± 0.295^{am}	2.196 ± 0.116^{bn}	2.048 ± 0.073^{co}
HDL-C /LDL-C	2.044 ± 0.143^{co}	2.348 ± 0.097^{bn}	2.986 ± 0.507^{am}	0.365 ± 0.041^{bn}	0.505 ± 0.028^{am}	0.497 ± 0.015^{am}
TG/HDL-C	0.864 ± 0.028^{bn}	0.916 ± 0.018^{am}	0.924 ± 0.033^{am}	3.867 ± 0.138^{am}	3.358 ± 0.061^{bn}	3.152 ± 0.083^{co}
AIP	0.072 ± 0.013^{bn}	0.042 ± 0.009^{am}	0.039 ± 0.015^{am}	0.585 ± 0.016^{am}	0.524 ± 0.009^{bn}	0.492 ± 0.011^{co}
ApoA1 (g/L)	1.342 ± 0.095^{am}	1.382 ± 0.089^{am}	1.400 ± 0.113^{am}	1.079 ± 0.279^{am}	1.105 ± 0.397^{am}	1.233 ± 0.285^{am}
ApoB (g/L)	0.746 ± 0.067^{am}	0.759 ± 0.080^{am}	0.774 ± 0.096^{am}	0.788 ± 0.092^{am}	0.815 ± 0.097^{am}	0.856 ± 0.106^{am}
ApoB/ApoA1	0.566 ± 0.032^{am}	0.556 ± 0.029^{am}	0.565 ± 0.024^{am}	0.744 ± 0.179^{am}	0.752 ± 0.118^{am}	0.700 ± 0.075^{an}
BMI (kg/m^2)	22.74 ± 0.30^{co}	23.80 ± 0.37^{bn}	24.54 ± 0.62^{am}	28.89 ± 1.27^{co}	30.16 ± 0.51^{bn}	31.79 ± 0.49^{am}
SBP (mmHg)	112.70 ± 2.27^{co}	117.80 ± 2.95^{bn}	124.40 ± 2.73^{am}	160.50 ± 3.37^{co}	168.20 ± 2.78^{bn}	183.90 ± 2.29^{am}
DBP (mmHg)	71.72 ± 3.45^{bn}	72.96 ± 2.21^{am}	73.44 ± 3.19^{am}	116.70 ± 4.44^{co}	120.80 ± 1.70^{bn}	124.10 ± 1.10^{am}

HL: hepatic lipase; TC: cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; VLDL-C: very low-density lipoprotein-cholesterol; non-HDL-C: non-high-density lipoprotein-cholesterol; AC: atherogenic coefficient; AIP: atherogenic index of plasma; ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. One-way ANOVA *post hoc* Turkey's test was used for the analysis to compare the significant difference among the different genotypes (GG, GA, AA) for both subjects separately. Those genotypes that have different superscript letters (a, b, c) are significant ($P<0.05$) while those that have the same superscript letters are not significant ($P>0.05$). Two-way ANOVA *post hoc* Turkey's test was used for comparing the significant difference among the different genotypes (GG, GA, AA) for both subjects respectively. Those genotypes that have the same superscript letters (m, n, o) are not significant ($P>0.05$) while those that have different superscript letters are significant ($P<0.05$).

control and the stroke subjects. Tahvanainen *et al.* observed that carriers of the A allele had significantly higher level of TG in intermediary density lipoprotein, LDL, and HDL particles, making them more buoyant among subjects with lower HL activities[26]. This may be due to heterogeneity of the study populations or the presence of impaired glucose tolerance, which may have masked the effect of the –250G>A polymorphism on lipid parameters concentration. The present study showed that the frequencies of G and A alleles were 61.5% and 38.5% for the control, and 28.5% and 71.5% for the stroke subjects ($P<0.0001$). We found that the AA genotype was responsible for higher HDL-C, HDL_2-C, and HDL_3-C ($P<0.0001$) levels for both the control and the stroke subjects. The SNPs in the *LIPC* promoter is responsible for the higher ($P<0.0001$) increase in the production of defective HDL-C, HDL_2-C, and HDL_3-C levels as a result of dysfunctional HL activity. This mean that increased concentration of these lipoproteins may lead to an impaired reverse TC transport and paradoxically increase the risk of ischemic stroke for both subjects. Clinical laboratory studies have found an inverse relationship between hepatic lipase activity and plasma HDL-C concentrations[19,27,28]. Some drugs like anabolic steroids that increase hepatic lipase activity causes a reduction in plasma levels of HDL-C[29]. A study has reported that *LIPC* promoter SNPs are linked with increased levels of HDL-C concentration[30], and paradoxically elevated the risk of ischemic heart disease[30] and other cerebrovascular diseases. Similar results were obtained using defective cholesteryl ester transfer protein[31]. In such cases, defective elevated HDL-C may be a marker of dysfunctional reverse TC transport and may increase the development of atherosclerosis and stroke. In addition, a study showed that genotype score of nine SNPs was linked with alteration in the levels of HDL or LDL-C and was an independent risk factor for cardiovascular disease[32]. Several other studies have indicated significant association between

LIPC –250G>A polymorphisms and HDL-C levels[33,34]. Zambon *et al.* showed that the promoter polymorphism significantly (P<0.05) influence HDL_2-C but not HDL-C[35], Hegele *et al.* did not find a significant effect of *LIPC* polymorphisms on HDL-C levels[36]. Jiménez-Gómez[37], showed no significant linkage between the –250G>A polymorphism and serum HDL-C levels because young healthy and normolipidemic subjects were included in their study. They also explained that the effect of the polymorphism may be cushioned by multiple genes that influence HDL-C. To support their result, Ko *et al.* found significant associations between –514C>T and –250G>A polymorphisms of the *LIPC* promoter and HDL-C levels in obese men[34]. Studies have shown that in addition to genetic polymorphisms, other different factors like: cigarette smoking[6,7], demographics[4], obesity[9], diet[5], hypertension[8], alcohol consumption[6], and exercise modulate plasma lipid parameters level[3]. These different factors may partly explain why the relationship between HDL-C levels and the –250G>A polymorphism was not observed in several other previous studies. For example, Craig *et al.* showed that people who smoke heavily have 9% lower HDL-C levels than non-smokers[7]. Juo *et al.* showed that 38% of the changes of the HL activity was associated with the *LIPC* promoter haplotypes, while; only 25% of the changes in the HDL-C was associated with HL activity[20]. Thus, with different other factors influencing HDL-C levels; one would not expect to observe significant associations between HDL-C and the *LIPC* promoter haplotypes in all studies. We observed that the genotypic and allelic frequencies of *LIPC* –250A were higher in the overweight/obese stroke subjects compared to the normal-weight control. The mutant allele was significantly higher in the homozygous and heterozygous –250A allele compared to the homozygous –250G allele for both the control and stroke subjects. The allelic frequencies of *LIPC* –250G>A between white Americans and African Americans were different in several previous studies. The –250A of the *LIPC* polymorphisms in white Americans was less common compared to allele in African Americans[35,38]. The frequencies of the *LIPC* –250A allele were 15%-21% among Caucasians[35], 39% among Chinese[34], 47% among Japanese-Americans[35], 32% among Brazilian[39], and 45%-53% among African Americans[35,38]. The ischemic stroke subjects had significantly lower (P<0.0001) ApoA1 level, higher ApoB level and ApoB/ApoA1 ratio compared with the control subjects. Breckenridge *et al.* showed that HL activity was associated with ApoB levels[40]. However, Jiménez-Gómez *et al*. did not show an increase in ApoB with a deficiency in hepatic activity[37]. In our study, we observed no association between ApoB levels and the –250G/A polymorphism for both subjects. The control and stroke subjects *LIPC* promoter sequences were compared with refseqNG011465.1 *LIPC* from GenBank. For the control subjects, 6 SNPs were observed and 6 amino acid substitutions were obtained. The stroke subjects had 32 SNPs and 20 amino acid substitutions when compared with refseqNG011465.1 *LIPC* from GenBank. The stroke subjects had more SNPs compared with the control. Comparing the control and the stroke subjects *LIPC* promoter from the same geographical location (Lagos, Nigeria), 40 SNPs and 21 amino acid substitutions were obtained. The nucleotides sequence of the control subjects were deposited in GenBank with accession number KY436389-KY436395.

In conclusion, this research study provides evidence that –250G>A *LIPC* promoter polymorphism can influence plasma HL activity and lipid parameters, and it is a significant risk factor responsible for the development of ischemic stroke.

Conflict of interest statement

The authors report no conflict of interest.

Acknowledgements

The authors are grateful to all the subjects who participated in the study and the Staff of Lagos University Teaching Hospital (LUTH), neurologists in the Department of Basic medicine in LUTH, the ethical unit of LUTH for granting us approval, and Mr. M.A. Ekum from the Department of Mathematics/Statistic Lagos State Polytechnic for his assistance in the statistical analysis. Our sincere gratitude goes to Dr. O.I. Ayantola, Mr. A.P. Ayantola and other member of staff of Aishat Specialist hospital Ltd Idi-Araba, Lagos, Nigeria for their assistance and support. Special greetings to Dr. Oyesola Ojewunmi and colleagues from Sickle cell foundation centre, Room B08-15, DNA Lab, Idi-Araba, Lagos, Nigeria for their assistance when carrying out this research work. We are also grateful to Mr. Musa Abdullahi Aiyegbeni for his assistance in bleeding our control and stroke subjects.

Authors' contributions

All the authors conceived and designed the plan of the entire study, provided research materials, organized the data for analysis and interpretation, organized and prepared the initial and final draft of all the content of the research paper. These authors are responsible for the content and originality of the manuscript.

References

[1] Momoh JO, Osuntoki AA, Ebuehi OAT. Hepatic lipase influences plasma lipid profiles and lipoprotein ratios in regional hospital patients with ischemic stroke. *Int J Biochem Res Rev* 2018; **21**(3): 1-13.

[2] Kwiterovich Jr PO, Coresh J, Smith HH, Bachorik PS, Derby CA, Pearson TA. Comparison of the plasma levels of apolipoproteins B and A-1, and other risk factors in men and women with premature coronary artery disease. *Am J Cardiol* 1992; **69**(12): 1015-1021.

[3] Durstine JL, Grandjean PW, Davis PG, Ferguson MA, Alderson NL, DuBose KD. Blood lipid and lipoprotein adaptations to exercise: A quantitative analysis. *Sports Med* 2001; **31**(15): 1033-1062.

[4] Yin RX, Chen YM, Pan SL, He FP, Liu TW, Yang DZ, et al. Effects of demographic, dietary and other lifestyle factors on the prevalence of hyperlipidemia in Guangxi Hei Yi Zhuang and Han populations. *Eur J Cardiovasc Prev Rehabil* 2006; **13**(6): 977-984.

[5] Mann JI. Dietary effects on plasma LDL and HDL. *Curr Opin Lipidol* 1997; **8**(1): 35-38.

[6] Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, et al. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the lipid research clinics follow-up study. *Am J Epidemiol*1987; **126**(4): 629-637.

[7] Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: An analysis of published data. *BMJ* 1989; **298**(6676): 784-788.

[8] Yin RX, Wu JZ, Lin WX, Chen YM, Yang DZ, Pan SL. The environmental and genetic evidence for the association of hyperlipidemia and hypertension. *J Hypertens* 2009; **27**(2): 251-258.

[9] Berns MA, de Vries JH, Katan MB. Increase in body fatness as a major determinant of changes in serum total cholesterol and high density lipoprotein cholesterol in young men over a 10-year period. *Am J Epidemiol* 1989; **130**(6): 1109-1122.

[10]Jeppesen J, Facchini FS, Reaven GM. Individuals with high total cholesterol/HDL cholesterol ratios are insulin resistant. *J Intern Med* 1998; **243**(4): 293-298.

[11]Santamarina-Fojo S, Haudenschild C, Amar M. The role of hepatic lipase in lipoprotein metabolism and atherosclerosis. *Curr Opin Lipidol* 1998; **9**(3): 211-219.

[12]Kuusi T, Saarinen P, Nikkilä EA. Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein2 in man. *Atherosclerosis* 1980; **36**(4): 589-593.

[13]Thuren T. Hepatic lipase and HDL metabolism. *Curr Opin Lipidol* 2000; **11**(3): 277-283.

[14]Jansen H, Verhoeven AJM, Sijbrands EJG. Hepatic lipase: a pro- or anti-atherogenic protein? *J Lipid Res* 2002; **43**(9): 1352-1362.

[15]Johannsen TH, Kamstrup PR, Andersen RV, Jensen GB, Sillesen H, Tybjaerg-Hansen A, et al. Hepatic lipase, genetically elevated high-density lipoprotein, and risk of ischemic cardiovascular disease. *J Clin Endocrinol Metabl* 2009; **94**(4): 1264-1273.

[16]Cai SJ, Wong DM, Chen SH, Chan L. Structure of the human hepatic triglyceride lipase gene. *Biochemistry* 1989; **28**(23): 8966-8971.

[17]Ameis D, Stahnke G, Kobayashi J, McLean J, Lee G, Büscher M, et al. Isolation and characterization of the human hepatic lipase gene. *J Biol Chem* 1990; **265**(12): 6552-6555.

[18]Eller P, Schgoer W, Mueller T, Tancevski I, Wehinger A, Ulmer H, et al. Hepatic lipase polymorphism and increased risk of peripheral arterial disease. *J Intern Med* 2005; **258**(4): 344-348.

[19]Van't Hooft FM, Lundahl B, Ragogna F, Karpe F, Olivecrona G, Hamsten A. Functional characterization of 4 polymorphisms in promoter region of hepatic lipase gene. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1335-1339.

[20]Juo SH, Han Z, Smith JD, Colangelo L, Liu K. Promoter polymorphisms of hepatic lipase gene influence HDL_2 but not HDL_3 in African American men: CARDIA study. *J Lipid Res* 2001; **42**(2): 258-264.

[21]Knudsen P, Antikainen M, Uusi-Oukari M, Ehnholm S, Lahdenperä S, Bensadoun A, et al. Heterozygous hepatic lipase deficiency, due to two missense mutations R186H and L334F, in the HL gene. *Atherosclerosis* 1997; **128**(2): 165-174.

[22]Knudsen P, Antikainen M, Ehnholm S, Uusi-Oukari M, Tenkanen H, Lahdenperä S, et al. A compound heterozygote for hepatic lipase gene mutations Leu334-Phe and Thr383-Met: correlation between hepatic lipase activity and phenotypic expression. *J Lipid Res* 1996; **37**(4): 825-834.

[23]Domenico B, Mauro M, Francesco A, Francesco Z, Glordano T, Glovanni B. Immunoturbidimetric method for routine determinations of apolipoproteins A-1 and B. *Clin Chem* 1991; **37**(5): 742-747.

[24]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**(6): 499-502.

[25]Hyeyoung K, Won K. Synalpheus jejuensis n. sp. (Crustacea: Decapoda: Alpheidae) from Korea based on morphological and molecular study. *Animal Cells Syst* 2014; **18**(5): 351-357.

[26]Tahvanainen E, Syvanne M, Frick MH, Murtomaki-Repo S, Antikainen M, KesaniemiYA, et al. Association of variation in hepatic lipase activity with promoter variation in the hepatic lipase gene. *J Clin Invest* 1998; **101**(5): 956-960.

[27]Lambert G, Amar MJ, Martin P, Fruchart-najib J, Föger B, Shamburek RD, et al. Hepatic lipase deficiency decreases the selective uptake of HDLcholesteryl esters *in vivo*. *J Lipid Res* 2000; **41**(5): 667-672.

[28]Hedrick CC, Castellani LW, Wong H, Lusis AJ. *In vivo* interactions of apoA-II, apoA-I, and hepatic lipase contributing to HDL structure and antiatherogenic functions. *J Lipid Res* 2001; **42**(4): 563-570.

[29]Kantor MA, Bianchini A, Bernier D, Sady SP, Thompson PD. Androgens reduce HDL_2-cholesterol and increase hepatic triglyceride lipase activity. *Med Sci Sports Exerc*1985; **17**(4): 462-465.

[30]Andersen RV, Wittrup HH, Tybjærg-Hansen A, Steffensen R, Schnohr P, Nordestgaard BG. Hepatic lipase mutations, elevated high-density lipoprotein cholesterol, and increased risk of ischemic heart disease: the Copenhagen city heart study. *J Am Coll Cardiol* 2003; **41**(11): 1972-1982.

[31]Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Jensen G, Tybjærg-Hansen A. Elevated HDL cholesterol is a risk factor for ischemic heart disease in white women when caused by a common mutation in the cholesteryl ester transfer protein gene. *Circulation* 2000; **101**(16): 1907-1912.

[32]Kathiresan S, Melander O, Anevski D, Guiducci C, Burtt NP, Roos C, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. *N Engl J Med* 2008; **358**(12): 1240-1249.

[33]Jiménez-Gómez Y, Pérez-Jiménez F, Marín C, Gómez P, Moreno R, Delgado J, et al. The –250G/A polymorphism in the hepatic lipase gene promoter influences the postprandial lipemic response in healthy men. *Nutr Metab Cardiovasc Dis* 2008; **18**(3): 173-181.

[34]Ko YL, Hsu LA, Hsu KH, Ko YH, Lee YS. The interactive effects of hepatic lipase gene promoter polymorphisms with sex and obesity on high-density-lipoprotein cholesterol levels in Taiwanese-Chinese. *Atherosclerosis* 2004; **172**(1): 135-142.

[35]Zambon A, Deeb SS, Hokanson JE, Brown BG, Brunzell JD. Common variants in the promoter of the hepatic lipase gene are associated with lower levels of hepatic lipase activity, buoyant LDL, and higher HDL_2

cholesterol. *Arterioscler Thromb Vasc Biol* 1998; **18**(11): 1723-1729.

[36]Hegele RA, Harris SB, Brunt JH, Young TK, Hanley AJ, Zinman B, et al. Absence of association between genetic variation in the *LIPC* gene promoter and plasma lipoproteins in three Canadian populations. *Atherosclerosis* 1999; **146**(1): 153-160.

[37]Jiménez-Gómez Y, Pérez-Jiménez F, Marín C, Gómez P, Moreno R, Delgado J, et al. The –250G/A polymorphism in the hepatic lipase gene promoter influences the postprandial lipemic response in healthy men. *Nutr Metab Cardiovasc Dis* 2006; **18**(3): 173-181.

[38]Vega GL, Clark LT, Tang A, Marcovina S, Grundy SM, Cohen JC. Hepatic lipase activity is lower in African American men than in white American men: effects of 50 flanking polymorphism in the hepatic lipase gene (*LIPC*). *J Lipid Res* 1998; **39**(1): 228-232.

[39]De-Andrade FM, Silveira FR, Arsand M, Antunes ALS, Torres MR, Zago AJ, et al. Association between –250G/A polymorphism of the hepatic lipase gene promoter and coronary artery disease and HDL-C levels in a Southern Brazilian population. *Clin Genet* 2004; **65**(5): 390-395.

[40]Breckenridge WC, Little JA, Alaupovic P, Wang CS, Kuksis A, Kakis G, et al. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis* 1982; **45**(2): 161-179.