Effect of Single Nucleotide Polymorphism rs1044925 in Acyl-CoA: Cholesterol Acyltransferase-1 Gene on Plasma Lipid Parameters in Patients with Ischemic Stroke

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Original Research Article

ABSTRACT

Acyl-coenzyme A: cholesterol acyltransferase-1 (ACAT-1) catalyzes the synthesis of cholesteryl esters from cholesterol and fatty acyl-CoA in tissues and the enzyme plays a major role in atherosclerosis and cellular cholesterol homeostasis. The study shows the effect of single nucleotide polymorphism rs1044925 in acyl-CoA:cholesterol acyltransferase-1 gene on plasma lipid parameters in patients with ischemic stroke. 100 patients with ischemic stroke and 100 controls matched for sex and aged 46-87 were selected for the study. Lipid profiles were measured using Randox kits and lipoprotein ratios were calculated using Excel software. The genotyping of the acyl-coenzyme A: cholesterol acyltransferase-1 rs1044925 SNP were performed by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) combined with 2% gel electrophoresis. There were significant difference (P<0.05) in the genotypic and allelic frequencies of ACAT-1 rs1044925 SNP between the normolipidemic and patients with ischemic stroke. The frequencies of AC, CC and AA genotypes of the ACAT-1 gene were 33%, 3% and 64% for the

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control and 57%, 5% and 38%, for the stroke subjects respectively. The frequencies of C and A alleles were 19.50% and 80.50% for the control and 33.50% and 66.50% for the ischemic stroke subjects (P < 0.0001) respectively. The effects of genotypes on plasma lipid profiles and lipoprotein ratios were found for both control and stroke subjects. The A allele carriers of ACAT-1 rs1044925 SNP had lower plasma TG, TC, VLDL-C and other lipid parameters as compared to the C allele carriers for both subjects. The C allele carriers were responsible for the increase in dyslipidemia for both subjects. The results of this study show that the polymorphism of rs1044925 in the ACAT-1 gene as effect on plasma lipid profiles and lipoprotein ratios in ischemic stroke patients.

1. INTRODUCTION

Stroke is the second-leading causes of mortality worldwide and is the second most common causes of death and disability in developed countries [1]. Approximately 80% of all strokes are ischemic. They result from occlusion of a major cerebral artery by a thrombus or embolism which results in reduced blood flow and a major decrease in the supply of glucose, oxygen and all other nutrients as well as disrupting the nutrient and waste exchange process required to support brain metabolism [1]. If cerebral arterial blood flow is not restored within a short period, cerebral ischemia will result, with subsequent neuron death within the perfusion territory of the affected blood vessels [1]. Studies have shown that dyslipidemia include a high concentration of plasma total cholesterol (TC), triglycerides (TG), apolipoprotein B (ApoB), low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) [2] together with low concentration of high density lipoprotein-cholesterol (HDL-C) [2] and apolipoprotein A1(ApoA1) [2,3] are risk factors for stroke and cardiovascular diseases [2]. Different studies have shown that plasma or serum lipid concentrations are controlled and influenced by factors such as diet [4], obesity [5], demographics [6], exercise [7], alcohol consumption [8], cigarette smoking [8,9], hypertension [10], and genetic factors [2]. In my previous study, we showed that lipoprotein ratios can be used to predict the risk of cardiovascular diseases [1]. These ratios include: TG/HDL-C, log TG/HDL-C, LDL-C/HDL-C, TC/HDL-C, HDL-C/TC, HDL-C/LDL-C and TC – HDL-C/HDL-C [1, 2].

Cholesterol is found in tissues and in plasma or serum lipoproteins either as free cholesterol or cholesteryl esters. Cellular cholesterol exists both as esterified cholesterol and as free sterol.

Acyl-coenzyme A: cholesterol acyltransferase (ACAT) (ACAT, E.C.2.3.1.26) is also known as sterol *o*-acyltransferase (SOAT). The enzyme is an intracellular enzyme that synthesizes cholesteryl esters from long-chain fatty acyl-CoA and cholesterol in various body tissues [11]. It helps in cellular cholesterol homeostasis. The enzyme has two isoforms, ACAT-1 and ACAT-2, with different intracellular localizations and membrane topology in mammalian species [12,13,14]. ACAT-1 is expressed in various cells, tissues and organs like: brain, kidney, adrenal glands [11], macrophages [15] and is responsible for foam cell formation in macrophages. ACAT-2 is expressed only in the intestine and liver [12,13,16] and is responsible for cholesterol absorption process in intestinal mucosal cells [17]. The inhibition of ACAT activity has been associated with decreased plasma cholesterol levels by suppressing cholesterol absorption and by diminishing the assembly and secretion of apolipoprotein B containing lipoproteins such as very low density lipoprotein (VLDL). ACAT inhibition prevents the conversion of macrophages into foam cells in the arterial walls, a critical event in the development of atherosclerosis [18,19].

ACAT-1 and ACAT-2 are members of the membrane bound acyltransferase (MBOAT) family [20]. MBOAT enzymes contain multiple trans-membrane domains (TMDs) and share the first active site histidine located in a long hydrophobic region, and the second active site asparagine located in a long hydrophilic region. Based on biochemical reactions, the MBOAT family contains 3 subgroups: the first group (comprises of ACAT-1, ACAT-2, and diacylglycerol acyltransferase-1 (DGAT-1)) acylates the -OH moiety of cholesterol or diacylglycerol; the second group acylates an amino acid residue within a protein or a peptide hormone [21,22]; the third group acylates a

lysophopholipid to reform a phospholipid [23]. ACAT-1 polypeptide contains 550 amino acids, and is located mainly at the endoplasmic reticulum. The enzyme contains 9 trans-membrane domains (TMD), with 5 loops located at the cytoplasmic side and 3 loops located at the luminal side of the endoplasmic reticulum. The first large loop not conserved in ACAT-2 or DGAT-1, is not needed for enzyme activity, but plays a key role in forming a dimerization domain [24]. The active site histidine (H460 in human ACAT-1) is located within TMD #7, while the other active site asparagine (N421 in human ACAT-1) is located within the 4th large cytoplasmic loop [25]. The helical coil rich domains present in TMD #7 and TMD #8 can be dissected into two distinct functional sides: one side is involved in substrate binding and catalysis, while the other side is involved in subunit interaction [26]. Key residues in TMD #7 in ACAT-1 are conserved in ACAT-2, and DGAT-1, while key residues in TMD #8 in ACAT-1 are conserved in ACAT-2, but not in DGAT-1.

Within the C-terminal half of ACAT-1, numerous residues are conserved with those of ACAT-2; the functions of these conserved residues are largely unknown at present. Within the coding region of human ACAT-1 cDNA, a single nucleotide polymorphism (SNP) exists at residue 526: the codon is either CAG for glutamine (Q), or CGG for arginine (R). 100% African American, between 75 to 88% of European are homozygous with Q526 while 14% Chinese and approximately 13% Japanese populations are homozygous with R526. Q/R526 is located in the C-terminal loop of ACAT-1. Within this region, it has been shown that C546 and C528 form a disulfide bond; the lack of disulfide bond formation significantly decreases ACAT-1 protein content [27].

Studies have shown that two single nucleotide polymorphisms (SNPs) of rs1044925 and -77G→A in the ACAT-1 gene were associated with modifications of serum lipid concentrations [28,29] and with low cerebrospinal fluid levels of cholesterol [30], but one SNP (R526G) in the ACAT-1 gene was not associated with serum lipid parameters [29]. The study explores the effect of the association between single nucleotide polymorphism rs1044925 in the acyl-CoA:cholesterol acyltransferase-1 gene and plasma lipid parameters in ischemic stroke subjects.

2. MATERIALS AND METHODS

2.1 Study Subjects

Clinical and laboratory data of 100 adult Ischemic stroke subjects who visited Lagos University Teaching Hospital (LUTH) in South-west, Nigeria were obtained for the analysis.

100 ischemic stroke patients' (41 women and 59 men) blood samples were collected and assayed. All the ischemic stroke patients had cerebral computerized tomography taken which showed cerebral infarction and they were confirmed by neurologists in LUTH to have ischemic stroke. The control subjects consist of 100 individuals within the same age range and socio-economic status as the stroke patients. Blood samples were obtained in EDTA and heparin vacutainer bottles from stroke patients and healthy individuals who have been fasting for 12 to 16 hours. All the healthy individuals and stroke patients were given consent forms and questionnaire. Ethical approval was also obtained from the Research and Ethical Committee of the Institution. Stroke and control subjects not willing to participate in the study were excluded from the study.

2.2. Determination of Lipid Parameters

2.2.1 Determination of ApoA1 and ApoB

The ApoA1 and ApoB were determined in the sample by immunoturbidimetric method as described by Domenico et al. [31].

2.2.2 Determination of plasma lipid profiles and lipoprotein ratios

Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) to give a final concentration of 0.1%. Plasma was separated from red blood cells by centrifugation at 1500 X g for 15 minutes at 4°C. The Total Cholesterol (TC), Triglyceride (TG) and HDL-Cholesterol were assayed using Randox kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Antrim, United Kingdom).

VLDL and LDL- Cholestrol were calculated by method described by Momoh et al. [1].

VLDL-C =TG/5, Non- HDL-Cholesterol = Total Cholesterol — HDL-Cholesterol

LDL-C=TC - HDL-C –TG/5

2.2.3 The atherogenic ratios were calculated as follows

Castellis risk index I (CRI-1) = TC/HDL-C

Castellis risk index II (CRI-11) = LDL-C/HDL-C

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

Atherogenic index of plasma (AIP) value
= log TG/HDL-C

Atherogenic coefficient (AC) value
= (TC - HDL-C)/HDL-C

Dyslipidemia was defined according to Momoh et al. [1]; National Cholesterol Education Program [32] and Sposito et al. [33]. Dyslipidemia was defined by the presence of one or more abnormal plasma lipid indexes.

2.3 DNA Analysis

2.3.1 Amplification of ACAT-1 gene

Genomic DNA was isolated from peripheral blood leukocytes using DNA Qiagen kits according to manufacturer instructions. The extracted DNA was stored at 4°C until analysis. The quality and quantity of 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 nanogramme per microlitre (ng/µL) and assessed the quality (purity) based on the ratio of absorbance at 260nm:280nm for all the samples [34]. Genotyping of the ACAT-1 rs1044925 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) [28,35].

The ACAT-1 gene was amplified using PCR machine (VERITI 96 WELL THERMAL) with the necessary PCR reagents and primers. The amplification at position 389 of the ACAT-1 gene was done by Polymerase Chain Reaction with the use of primer pairs. The primers of ACAT-1 polymorphism used for the analysis were F:5' TATATTAAGGGGATCAGAAGT-3' and R: 5'-CCACCTAAAAACATACTACC-3' [36]. The PCR Cycling conditions for the ACAT-1 SNPs were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 64°C for 30 sec, and extension at 72°C for 30 sec, with a final extension at 72°C for 2 min. All PCR reactions were carried out in a total volume of 50 µl containing 0.2 mMdNTPs, 2 nM MgCl$_2$, 1µl of each primer, and 1 unit of AmpliTaq polymerase. Negative controls were included in each set of reaction.

2.3.2 Agarose Gel electrophoresis of amplified ACAT-1 gene

Amplification was confirmed by electrophoresis of PCR amplicons on 2% w/v agarose gel (stained with ethidium bromide, 0.5 mg/mL) in Tris-borate EDTA. Electrophoresis was carried out at 120 V, 50 W, and 300 mA for 30 minutes at 160°C. On completion of the electrophoresis, bands were visualized with the gel documentation system (Infinity 3026, France). The sizes of the fragments obtained were estimated by comparison with the 50 bp DNA ladder (Jena Bioscience GmbH, Germany) run alongside with the negative control. The genomic DNA of the control and the stroke subjects after amplification with PCR and imaged by 2% agarose gel electrophoresis, the purpose gene of 389 bp nucleotide sequences were found in all the blood samples before genotyping.

2.3.3 Genotyping of ACAT-1 gene

The PCR products were then digested with 0.5µL restriction enzyme *Rsa1* at 37°C. Briefly, 0.5µLof Restriction enzyme (*Rsa1*) was added to 1 µL of PCR amplicon and 10x TBE Buffer (5 µL) in a reaction volume of 50 µL. The solution was mixed properly and incubated for 37°C for maximum of 15 min. The digested PCR products were followed by electrophoresis on 2% agarose gel containing ethidium bromide. To validate the results, genotyping experiments were repeated for all samples twice. The genotyping of these samples were completely consistent. The genotypes identified were named according to the absence or presence of the enzyme restriction sites, when A to C transversion at nucleotide position 389, 279 and 110 of the ACAT-1. The absence of the cutting site indicates the 389 bp A allele, while its presence indicates the 279 and 110 bp C allele. The band size obtained is similar to the research work carried out by Wu et al. [36].

The AA genotype is homozygote for the absence of the site (band at 389 bp), AC genotype is heterozygote for the presence and absence of the site (bands at 389, 279 and 110

bp), and CC genotype is homozygote for the presence of the site (bands at 279, 110 bp). A negative control was included in each set of the reaction.

2.4 Statistical Analyses

Data are presented as Mean ± SD; GraphPad prism computer software version 5.01 was used to compare lipid profiles, ApoA1, ApoB and lipoprotein ratios levels between genotypes for control and stroke subjects respectively. 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 Posthoc Tukey´s test was used for comparing significant difference between wild type and mutant genotypes for both separate subjects. One-way ANOVA Bonferroni's multiple comparison test was also used for comparing the significant difference between genotypes for both subjects. A P-value < 0.05 was considered statistically significant.

3. RESULTS

Plate 1 below shows the electrophoresis of PCR products before digestion while Plate 2 to 4 show the electrophoregram of PCR digested amplicon using *Rsa1* restriction enzyme resolved on 2% Agarose gel.

3.1 Genotypic and Allelic Frequencies

Two hundred subjects were genotyped. The ACAT-1 gene polymorphism was highly prevalent in the stroke subjects compared to the controls. For the control subjects: sixty four (64) were homozygous for the wild-type (AA) and 36 were carriers of the C allele (33 AC and 3 CC). The stroke subjects had 38 homozygous for the AA genotype and 62 were carriers of the C allele (57 AC and 5 CC). The frequencies of AA, AC and CC genotypes for the ACAT-1 were 64%, 33% and 3% for the control while 38%, 57% and 5% were for the stroke subjects (P<0.0001) respectively. The frequencies of A and C alleles were 80.50% and 19.50% for the control subjects while 66.50% and 33.50% were for the stroke subject´s respectively. These frequencies did not differ from those predicted from the Hardy-Weinberg equilibrium. The allele frequencies were consistent with Hardy–Weinberg equilibrium for both control and stroke subjects (P = 0.8050 for control and 0.6650 for stroke subjects). The prevalence of the ACAT-1 C allele was significantly higher in the stroke subjects compared to the control (33.50% Vs 19.50%).
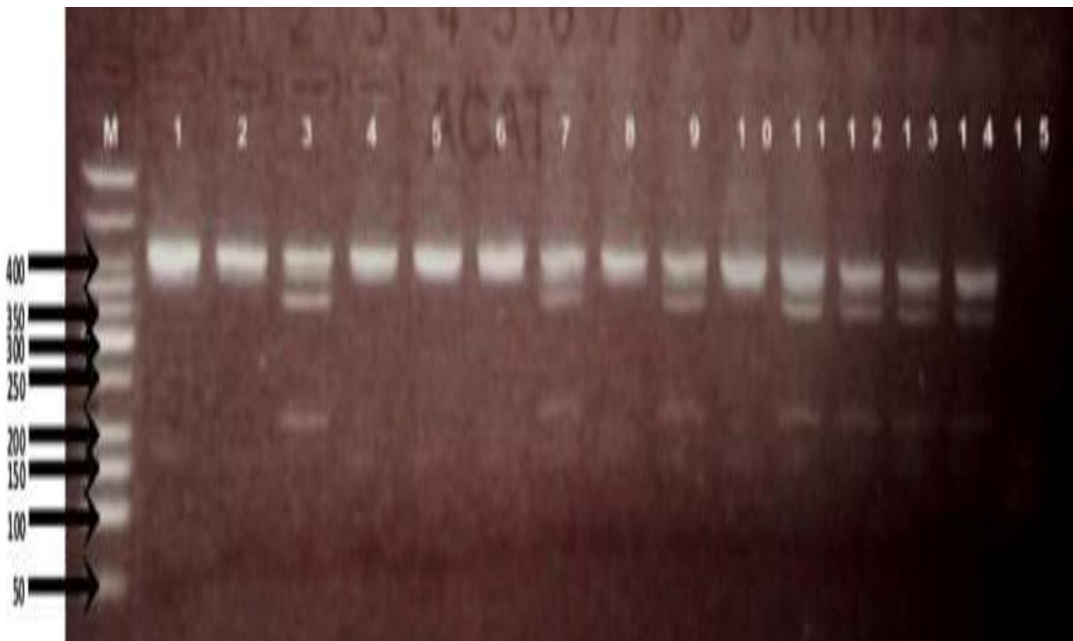

Plate 1. Electrophoresis of PCR products of the different samples; Lane M: 50-500 bp marker ladder; lanes: 1-14 DNA samples are 389 bp bands which are the target gene. Lane 15 is the non-template strand
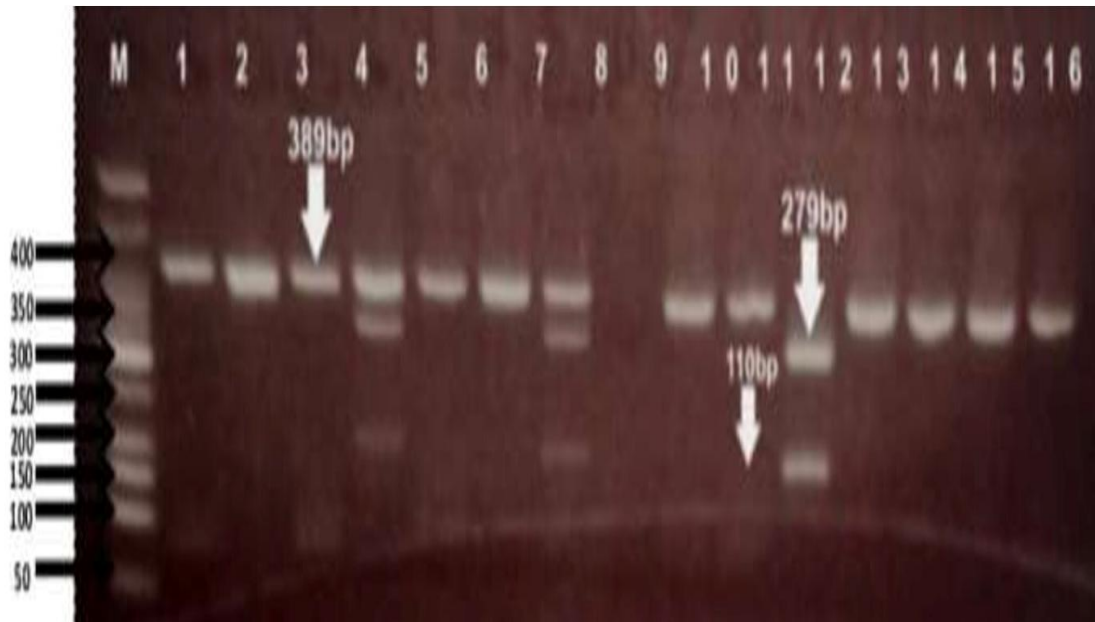
Plate 2. Electrophoregram of PCR digested amplicon using *Rsa1* restriction enzyme resolved on 2% Agarose gel. Lane M is 50 to 500 bp DNA marker ladder, lanes: 1-3,5, 6, 10, 11, 13-16 are AA genotypes (band at 389 bp), lanes: 4 and 7 are AC genotypes (bands at 389, 279 and 110 bp) and lane 12 is CC genotype (bands at 279 and 110 bp). Lanes 8 and 9 are non-template strands

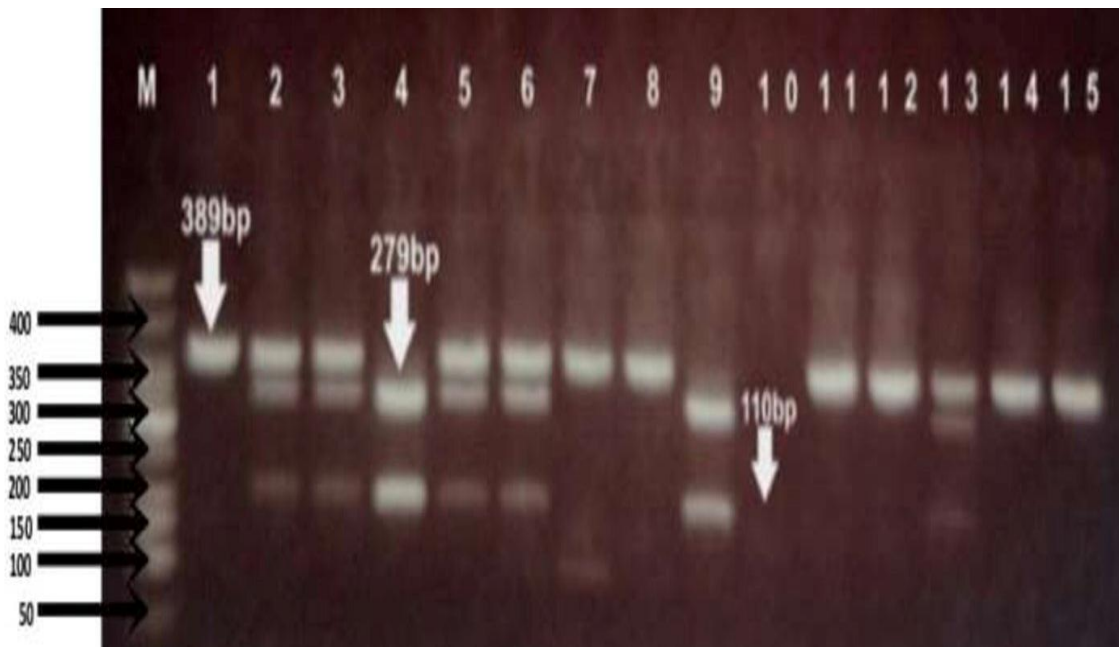**Plate 3. Digested ACAT-1 genes resolved on 2% Agarose gel showing different bands. Lane M is 50 to 500 bp DNA ladder. Lanes: 1,7,8,11,12,14 and 15 are homozygote wild type AA genotypes at band 389 bp. Lanes: 2,3,5,6 and 13 are heterozygote mutant AC genotypes (bands 389, 279 and 110 bp), Lanes 4 and 9 are homozygote mutant CC genotypes (bands 279 and 110 bp). Lane 10 is the non-template strand**
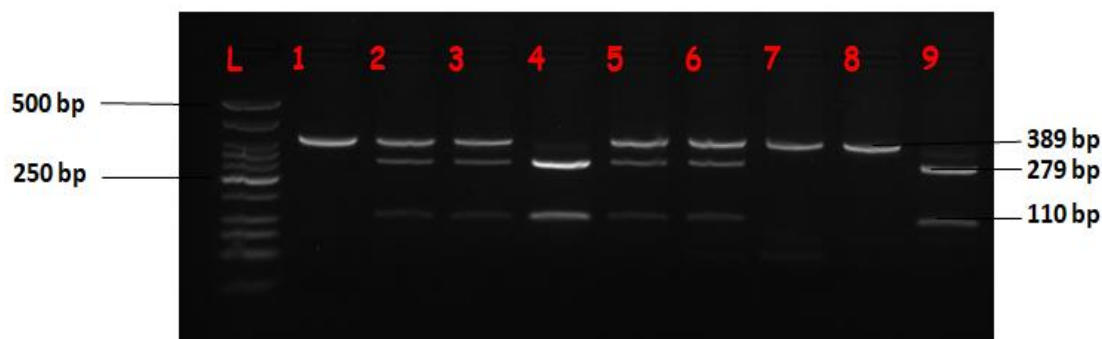
Plate 4. Electrophoresis of PCR digested amplicon resolved on 2% Agarose gel for DNA samples digested using *Rsa1* enzyme. Lane L (50 to 500 bp) is DNA ladder, lanes: Lanes 1, 7 and 8 are AA genotypes (band at 389 bp), lanes: 2,3,5 and 6 are AC genotype (bands at 389, 279 and 110 bp) and lanes: 4 and 9 are CC genotypes (bands at 279 and 110 bp).

Table 1. The number of observed and expected genotypes of the examined ACAT-1 gene SNP of control and stroke subjects according to Hardy-Weinberg equilibrium

SNP	Genotype/ Allele Frequency	Control subject		Stroke subject		P value; OR (95%CI)
-514C>T		Observed Frequency	Expected H-W Frequency	Observed Frequency	Expected H-W Frequency	
	AA	64(64%)	64.80	38 (38%)	44.2225	
	AC	33(33%)	31.39	57 (57%)	44.5550	
	CC	3(3%)	3.80	5 (5%)	11.2225	
P Value		0.8050		0.6650		
X^2 Value		0.2614		7.8018		
	A	80.50%		66.50%		0.0001
	C	19.50%		33.50%		0.0001

Table 2 shows Plasma lipid profiles and lipoprotein ratios for both control and stroke subjects with different genotypes. The subjects with AC and CC genotypes had higher serum TC levels than the subjects with AA genotype for both subjects.

Data are presented as Mean ± SD (n=100). TC, total 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 index; TC/HDL-C, total cholesterol/high-density lipoprotein-cholesterol; LDL-C/HDL-C, low-density lipoprotein-cholesterol/high-density lipoprotein-cholesterol; TG/HDL-C, triglyceride/high-density lipoprotein-cholesterol; AIP, atherogenic index of plasma; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B. One-way ANOVA Posthoc Tukey´s test was used for comparing significant difference between wild type and mutant genotypes for both separate subjects. a=highest, b= medium, c=lowest. Those genotypes that have the same letters are not statistically significant (P>0.05) while those that have different letters are statistically significant (P<0.05). Comparisons across the row were done using One-way ANOVA Bonferroni's multiple comparison tests. m=highest, n= medium, followed by o and p=lowest in that order. Those genotypes that have the same letters are not statistically significant (P>0.05) while those that have different letters are statistically significant (P<0.05). A P<0.05 was considered statistically significant.

Table 2. The effect of ACAT-1 gene genotypes on plasma lipid parameters for both control and ischemic stroke subjects

Parameters	Control subjects			Ischemic stroke subjects		
	Genotype			Genotype		
	AA (64)	AC (33)	CC (3)	AA (38)	AC(57)	CC(5)
TC (mg/dl)	164.41 ± 7.58^{bo}	170.17 ± 8.78^{ao}	177.07 ± 9.29^{ao}	209.36 ± 11.65^{cn}	218.02 ± 10.85^{bm}	231.30 ± 12.56^{am}
TG (mg/dl)	89.09 ± 5.19^{bo}	87.96 ± 4.41^{bo}	96.19 ± 5.67^{ao}	175.82 ± 16.03^{bn}	183.58 ± 13.34^{am}	194.69 ± 14.53^{am}
HDL-C (mg/dl)	104.07 ± 4.79^{amn}	106.71 ± 5.87^{am}	96.23 ± 5.04^{bn}	52.93 ± 5.90^{bp}	59.18 ± 4.63^{ao}	51.71 ± 3.89^{bp}
VLDL-C (mg/dl)	17.82 ± 0.90^{bo}	17.59 ± 0.83^{bo}	19.24 ± 0.98^{ao}	35.16 ± 2.86^{bn}	36.72 ± 2.32^{am}	38.94 ± 2.34^{am}
LDL-C (mg/dl)	42.52 ± 2.08^{cp}	45.87 ± 2.36^{bp}	61.60 ± 3.28^{ao}	121.27 ± 5.58^{bn}	122.12 ± 5.31^{bn}	140.65 ± 6.64^{am}
Non-HDL-C (mg/dl)	60.34 ± 3.10^{cp}	63.46 ± 3.16^{bp}	80.84 ± 3.87^{ao}	156.43 ± 6.73^{bn}	158.84 ± 6.28^{bn}	179.59 ± 5.82^{am}
TC/HDL-C	1.580 ± 0.021^{bo}	1.595 ± 0.053^{bo}	1.840 ± 0.062^{ao}	3.955 ± 0.710^{abm}	3.684 ± 0.615^{bn}	4.473 ± 0.663^{am}
LDL-C/HDL-C	0.409 ± 0.017^{co}	0.430 ± 0.022^{bo}	0.640 ± 0.032^{ao}	2.291 ± 0.548^{abm}	2.064 ± 0.403^{bn}	2.720 ± 0.596^{am}
HDL-C /LDL-C	2.448 ± 0.164^{am}	2.326 ± 0.176^{bn}	1.562 ± 0.198^{co}	0.437 ± 0.027^{bp}	0.485 ± 0.022^{ap}	0.368 ± 0.028^{cp}
TG /HDL-C	0.856 ± 0.050^{bo}	0.824 ± 0.050^{co}	0.999 ± 0.070^{ao}	3.322 ± 0.520^{abm}	3.102 ± 0.470^{bn}	3.765 ± 0.465^{am}
AIP	-0.0675 ± 0.002^{bop}	-0.0841 ± 0.003^{cp}	-0.0004 ± 0.001^{ao}	0.5214 ± 0.065^{abm}	0.4916 ± 0.059^{bn}	0.5758 ± 0.061^{am}
AC	0.5798 ± 0.030^{co}	0.5947 ± 0.032^{bo}	0.8401 ± 0.036^{ao}	2.955 ± 0.513^{abm}	2.6840 ± 0.586^{bn}	3.4730 ± 0.431^{am}
ApoA1 (g/L)	1.482 ± 0.35^{am}	1.324 ± 0.37^{amn}	1.314 ± 0.25^{amn}	1.201 ± 0.23^{an}	1.102 ± 0.22^{an}	1.109 ± 0.25^{amn}
ApoB (g/L)	0.726 ± 0.054^{an}	0.753 ± 0.065^{an}	0.797 ± 0.059^{amn}	0.779 ± 0.079^{bmn}	0.819 ± 0.063^{am}	0.859 ± 0.081^{am}

4. DISCUSSION

In the present study, ACAT-1 rs1044925 SNP was determined by PCR-RFLP. This polymorphism was present in 36% (AC=33% and CC=3%) of non-symptomatic control subjects and 62% (AC=57% and CC=5%) of patients with ischemic stroke. The C allele was significantly more frequent in the stroke subjects compared to the control (33.5% vs 19.5%). In this study, it was observed that there were significant differences in the genotypic and allelic frequencies of ACAT-1 rs1044925 SNP between normolipidemia control and ischemic stroke subjects (A allele = 80.5% vs 66.50%, P < 0.0001). The genotypic and allelic frequencies of ACAT-1 rs1044925 SNP in diverse populations are inconsistent. In a study carried out by Li et al. [28], they found no significant difference in the C allele frequency between normal controls (13.7%) and endogenous hypertriglyceridemia patients (15.3%).

Wu et al. [11] study shows that there was no significant difference in the genotypic and allelic frequencies of ACAT-1 rs1044925 SNP between the normolipidemic and hyperlipidemic subjects. It was observed in their study that the frequency of AA, AC and CC genotypes were 74.8%, 23.8% and 1.4% in normolipidemia, and 77.3%, 21.4% and 1.3% in hyperlipidemia (P > 0.05); respectively. The frequency of A and C alleles were 86.7% and 13.3% in normolipidemia, and 88.0% and 12.0% in hyperlipidemia (P > 0.05); respectively. Zhao et al. [35] reported that there was no difference in the minor allele frequency between normal controls (9.7%) and Alzheimer's disease patients (9.3%). In another study, the C allele frequency of ACAT-1 rs1044925 SNP was very high in the population of central and Southern Europe (35.4%) [30].

Studies have shown that dyslipidemia is a multifactorial which include hereditary and acquired risk factors [2, 36]. The effect of ACAT-1 rs1044925 SNP on plasma lipids and lipoprotein ratios showed that dyslipidemia was more prominent in the stroke subjects compared to the control. ACAT-1 rs1044925 C variant (CC genotype) had a significant (P<0.023-0.0001) higher TC, TG, VLDL-C, LDL-C, Non-HDL-C, CRI-1, CRI-II, TG/HDL-C, AIP and AC for the control subjects only, while their HDL-C and HDL-C/ LDL-C values were significantly lower when compared to AA genotype. This is an indication that the C variant of the rs1044925 SNP could not produce enough ACAT-1 protein

which leads to plasma dyslipidemia as observed in this study. For the stroke subjects, it was observed that ACAT-1 rs1044925 C variant (CC genotype) had significant (P<0.05) higher TC, TG, VLDL-C, LDL-C, Non-HDL-C and ApoB, while their HDL-C/ LDL-C values were significantly lower when compared to AA genotype. Li et al. [28] study shows that in Chinese population, control subjects with AA genotype had a higher serum mean concentrations of non-HDL-C and LDL-C when compared with those of C allele carriers (AC and CC genotype carriers), whereas hypertriglyceridemia subjects with AA genotype had a higher HDL-C level compared with those of C allele carriers. Yin et al. [37] study shows that the C allele carriers of *ACAT-1* rs1044925 were associated with an increased serum HDL-C level in the healthy controls and decreased risk of CAD and Ischemic stroke patients. Li et al. [28] found that serum HDL-C levels in patients with endogenous hypertriglyceridemia in China were lower in the C allele carriers than in C allele non-carriers.

Wu et al. [11] study shows that the C allele carriers of ACAT-1 rs1044925 SNP in male hyperlipidemic subjects had higher serum TC, HDL-C and ApoAI levels than the C allele non-carriers. They show that the variant had a higher TC levels attribute to the C variant of the rs1044925 SNP because it could not produce enough ACAT-1 protein and induce an increased cholesterol synthesis.

In the present study, it was observed that the AC variant of ACAT-1 rs1044925 SNP increase plasma HDL-C levels in the stroke subjects with hyperlipidemia, which may play a protective factor for atherosclerosis. In lipid-loaden macrophages, studies have shown clearly that ACAT-1 depletion in macrophages is proatherogenic [38,39]. To explain these reactions, the possible reasons might be as follows: Firstly, in the ACAT-1 deletion model, the ABCA1-mediated cholesterol efflux from the cell to protect macrophages from free cholesterol toxicity and this process may not be sufficient if ACAT-1 is lacking [29]. Defects in the pathways of the ABCA1-mediated cholesterol efflux, the efflux of the cholesterol will decrease rapidly and the free cholesterol will increase in the cell. Secondly, ACAT-1 is expressed in various cells, tissues, macrophages, adrenal glands and kidney. Not only from macrophages, the circulating HDL-C levels were regulated by cholesterol efflux from these various cells that

the ACAT-1 are expressed in, therefore, the circulating HDL-C levels might reflect the cholesterol efflux from others cells and tissues. In ACAT-1 deficiency macrophages, cholesterol synthesis was increased by 134% (p=0.001) in ACAT-1 macrophages compared to wild type macrophages, which suggests that ACAT-1 affects the regulation of cholesterol metabolism in macrophages [40]. Therefore, the possible mechanism by which ACAT-1 gene variants protect against atherosclerosis is that inhibiting ACAT-1 may directly or indirectly diminish foamy macrophage formation, thus further reducing the incidence of atherosclerosis. However, this protective effect against atherosclerosis depends on the cellular cholesterol efflux ability in reverse cholesterol transport, by which HDL is able to extract excess cholesterol from peripheral tissues and transfer this cholesterol to the liver for biliary excretion.

5. CONCLUSION

The present study shows that the genotypic and allelic frequencies of rs1044925 were significantly different between Ischemic stroke patients and controls. The C allele carriers of ACAT-1 rs1044925 SNP was responsible for increased dyslipidemia in lipid parameters for both subjects. The results of this study suggest that the polymorphism of rs1044925 in the ACAT-1 gene is associated with plasma lipid profiles and lipoprotein ratios in patients with ischemic stroke.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the author and producers of the products because I do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the author.

CONSENT

As per international standard or university standard, patient's written consent was collected and preserved by the author.

ETHICAL APPROVAL

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. Control and stroke subjects who were not willing to participate in the research were excluded from the study.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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