THE EFFECT OF GENETIC VARIANTS, ANTHROPOMETRY AND THE ENVIRONMENT ON LIPID PROFILE IN ADULTS IN NORTHERN GHANA

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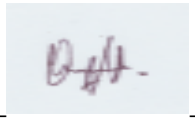
A Thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of PhD in Human Genetics

Johannesburg, 2019

Declaration

I, Godfred Agongo declare that this thesis my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy in Human Genetics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signed this on 24th day of October 2019 in Johannesburg

_____  _____

Dedication

To my wife and children and in loving memory of my grandmother Elizabeth Jampana Akendana (1934-2018)

Publications and presentations arising from this research project

Publication

- Agongo G, Nonterah EA, Debpuur C, Amenga-Etego L, Ali S, Oduro A, Crowther NJ, Ramsay M. (2018). The burden of dyslipidaemia and factors associated with lipid levels among adults in rural northern Ghana: An AWI-Gen sub-study. *PLoS ONE,* 13(11): e0206326.https://doi.org/10.1371/journal.

Submitted article

- Agongo G, Amenga-Etego L, Nonterah EA, Debpuur C, Chudhury A, Oduro AR, Crowther NJ, Ramsay M. Candidate gene analysis reveals strong association of *CETP* variants with high density lipoprotein cholesterol in Ghanaian adults: an AWI-Gen sub-study. *Frontiers in Genetics,* under review.

Poster presentations

- 2015 Wits Non-Communicable Disease Research and Leadership Program in Johannesburg, South Africa (20th November 2015)
    - Agongo G, Oduro RA, Crowther N, Ramsay M. The Effect of Genetic Variants, Anthropometry and the Environment on Lipid Profiles in Adults in Northern Ghana
- 2017 H3Africa Consortium meeting in Gaborone, Botswana (13th May, 2017)
    - Agongo G, Oduro AR, Nonterah EA, Amenga-Etego L, Debpuur C, Ali S, Crowther NJ, Ramsay M. Anthropometric, Demographic and Environmental Factors Influencing Lipid Levels in Rural Ghanaian Adults: the AWI-Gen study
- 2017 National Health Research Dissemination Symposium, Ghana (1st June, 2017)
    - Agongo G, Oduro AR, Nonterah EA, Amenga-Etego L, Debpuur C, Ali S, Crowther NJ, Ramsay M. Anthropometric, Demographic and Environmental Factors Influencing Lipid Levels in Rural Ghanaian Adults: the AWI-Gen study

Co-authored publications related to this study and published during the PhD research period

- Ramsay M, Crowther N, Tambo E, Agongo G, Baloyi V et al. (2016). H3Africa AWI-Gen Collaborative Centre: a resource to study the interplay between genomic and environmental risk factors for cardiometabolic diseases in four sub-Saharan African countries. *Global Health, Epidemiology and Genomics* (2016), 1, e20, page 1 of 13. doi:10.1017/gheg.2016.17.
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- Nonterah EA, Debpuur C, Agongo G, Amenga-Etego L, Crowther NJ, Ramsay M, Oduro AR. (2018). Socio-demographic and behavioural determinants of body mass index among an adult population in rural Northern Ghana: the AWI-Gen study. *Global Health Action*, 11: sup2, 1467588, doi: 10.1080/16549716.2018.1467588.
- Ali SA, Cassandra S, Agongo G, Alberts M, Amenga-Etego L et al (2018). Genomic and environmental risk factors for cardiometabolic diseases in Africa: methods used for Phase 1 of the AWI-Gen population cross-sectional study. *Global Health Action*, vol. 11, 1507133, doi: 10.1080/16549716.2018.1507133.
- Ramsay M, Crowther NJ, Agongo G, Ali SA, Asiki G et al. (2018). Regional and sex-specific variation in BMI distribution in four sub-Saharan African countries: The H3Africa AWI-Gen study. *Global Health Action*, 11: sup2, doi: 10.1080/16549716.2018.1556561.
- Nonterah EA, Boua PR, Klipstein-Grobusch K, Mickelsfield LK, Agongo G et al. (2019). Classical Cardiovascular Risk Factors and HIV are Associated with Carotid Intima-Media Thickness in Adults From Sub-Saharan Africa: Findings From H3Africa AWI-Gen Study. Journal of American Heart Association, 8(14):e11506J, doi: 10.1161/JAHA.118.011506.

Abstract

Genetic polymorphisms, environmental factors and gene-environment interactions are associated with variations in lipid levels. This evidence comes from studies in mainly Europeans and people of African descent but little or no data exists on the influence of these factors on lipid levels in indigenous black African populations. The aim of the current study was therefore to characterize the principal environmental and genetic factors that influence serum lipid levels in a rural, adult Ghanaian population and to determine if gene-environment interactions further modulate lipid levels. It was a cross-sectional survey that recruited 2016 men and women resident in the study area and aged 40-60 years.

Self-reported socio-demographic and behavioural data were collected using a structured questionnaire. Simple anthropometric measures were taken using standardised procedures and visceral and abdominal subcutaneous adipose tissue thickness were measured using ultrasound. Whole blood was collected for DNA extraction and serum was used for the measurement of total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and triglyceride (TG). Genotyping of candidate genes was performed using the H3Africa single nucleotide variant genotyping array. Multivariable linear regression analysis was used to test the association of environmental factors with lipid levels. The effect of genetic polymorphisms and gene-environment interaction on lipid levels was tested using regression models with Bonferroni-adjusted p values.

The prevalence of dyslipidaemia among the total population was as follows: low HDL-C: 60.30%, high LDL-C=5.55%, high TC=4.02% and high TG=2.12%. A greater proportion of women had low HDL-C compared to men (p=0.005). Subcutaneous abdominal fat was associated with TC (β=0.067, p=0.015) and TG (β=0.137, p<0.001) among women and LDL-C (β=0.139, p=0.006) and TC (β=0.071, p=0.048) among men. Body mass index was associated with TC (β=0.010, p=0.043) among men while waist circumference was associated with LDL-C (β=0.116, p<0.001) and TG (β=0.094, p<0.001) among women. Hip circumference was negatively associated (β=-0.053, p=0.043), while visceral fat was positively associated with TG (β=0.033, p=0.022) among women. Socioeconomic status, education, being unmarried and employment were associated with HDL-C (β=0.081, p=0.004), LDL-C (β=0.095, p=0.004) and TG (β=0.095, p=0.001) respectively, all among women, and TC (β=0.070, p=0.010) among men.

Tobacco smoking (β=0.066, p=0.024) among men and alcohol intake among women (β=0.084, p=0.001) were associated with HDL-C levels. For the genetic association analysis the lead variants for association with HDL-C were rs17231520 (β=0.139, p<0.0001) and rs34065661 (β=0.137, p<0.0001), both in *CETP*. The interaction between tobacco smoking and *ABCA1* (rs10124686) was associated with higher TC levels (p=0.041) while that between *CETP* (rs34620476) and tobacco smoking was associated with lower TG levels in men (p=0.020).

Associations of education, employment and adiposity with lipid levels suggest that future societal advances and increases in the prevalence of obesity may lead to associated adverse health consequences. Variants in *CETP* strongly modulate HDL-C levels in the study population. Total cholesterol and TG levels may be modestly influenced by gene-environment interactions of *ABCA1* and *CETP*, respectively, with smoking among men in the study population.

Acknowledgements

I first acknowledge the blessings of God that gave me the endurance to reach this far. I am very grateful to my primary supervisor, Prof Michèle Ramsay, for her mentorship, guidance and diverse support that made this PhD possible. I am equally grateful to Prof Nigel J Crowther for his careful supervision, particularly in the aspects of lipid biology and statistical analyses. I owe a debt of gratitude to Dr Abraham Rexford Oduro who has played a mentorship role in my career at the Navrongo Health Research Centre (NHRC) and provided diverse support towards my PhD studies.

I am thankful to Dr Cornelius Debpuur, Miss Mary Immaculate Anati, Dr Engelbert Nonterah, Dr Lucas Amenga-Etego, Mr Eric Fato, Miss Rita Afia and Mr Timothy Augose for the various roles they played in ensuring the success of the AWI-Gen phase 1 project in Navrongo. I appreciate the diverse support from Dr Stuart Ali, Prof Scott Hazelhurst, Mr Freedom Mukomana, Miss Cassandra Soo, Dr Ananyo Choudhury, Dr Liesl Hendry, Mr Yusuf Ismail, Miss Mmatshepo Taunyane, Mis Jocelyne Gayenga and Miss Mahtaab Hayat, all of the Sydney Brenner Institute for Molecular Bioscience at Wits University. I also appreciate the contribution of Prof Shane Norris, Yussif Gumah and the staff of the Developmental Pathway for Health Research Unit (DHPRU) laboratory in the Chris Hani Baragwanath hospital in Soweto where all lipid analysis were conducted. I acknowledge the sponsorship from the Navrongo Health Research Centre and the H3Africa AWI-Gen project.

All the authors who have contributed to my papers related to this thesis are acknowledged.

I owe a debt of gratitude to my wife Prudence Kupedimah and our children for their patience, love and support throughout my studies.

Table of Contents

List of tables

Chapter 1

Chapter 2

Chapter 3

List of figures

Abbreviations

AADM	African American diabetes mellitus
AAWI-Gen	Africa Wits INDEPTH partnership for Genomic research
ABCA1	ATP-binding cassette A1 gene
ABCG5/G8	ATP binding cassette subfamily G member 5/8 gene
ABOAII/CII	Apolipoprotein AII/CII
ADP	Adenosine diphosphate
A-LDL	Low density lipoprotein cholesterol subtype A
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APOAI	Apolipoprotein AI
APOB100	Apolipoprotein B100
APOCIII	Apolipoprotein CIII
APOD	Apolipoprotein D
APOE	Apolipoprotein E
ATP	Adenosine triphosphate
B-LDL	Low density lipoprotein cholesterol subtype B
BMI	Body mass index
CADD	Combined annotation dependent depletion
CE	Cholesteryl ester
CETP	Cholesteryl ester transport protein gene
CHD	Coronary heart disease
C-LDL	Low density lipoprotein cholesterol subtype C
CMD	Cardiometabolic disease
CV	Coefficient of variation
CVD	Cardiovascular disease

DAG	Diacylglycerol
DBP	Diastolic blood pressure
DGAT	Diacylglycerol:acylcoA acyltransferase
DNA	Deoxyribonucleic acid
EBI	European Bioinformatics Institute
eNOS	Endothelial nitric oxide synthase enzyme
eNOS	Endothelial nitric oxide synthase gene
ER	Endoplasmic recticulum
FA	Fatty acids
FABP	Fatty acid binding proteins
FH	Familial hypercholesterolaemia
GCKR	Glucokinase regulator
GPAQ	Global Physical Activity Questionnaire
GWAS	Genome wide association studies
GxE	Gene-environment interaction
H_2O	Water
H_2O_2	Hydrogen peroxide
H3Africa	Human Heredity and Health in Africa
HC	Hip circumference
HCL	Hydrogen chloride
HDAOS	N-(2-Hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium
HDL-C	High density lipoprotein cholesterol
HIV	Human immunodeficiency virus
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme-A
HWE	Hardy-Weinberg equilibrium
IL-6	Interleukin-6 gene

INDEPTH	International Network for the Demographic Evaluation of Populations and Their Health in Low- and Middle-Income Countries
LCAT	Lecithin-cholesterol ester transport protein gene
LD	Linkage disequilibrium
LDL-C	Low density lipoprotein cholesterol
LDLr	LDL receptor
LIPG	Endothelial lipase gene
LoFtool	Loss of function tool
LPL	Lipoprotein lipase
MAF	Minor allele frequency
MAG	Monoaylglycerol
MGAT	Monoacylglycerol:acylCoA acyltransferase
MMAB	Methylmalonic aciduria cb1B gene
MS	Metabolic syndrome
MTTP	Microsomal triglyceride transfer protein gene
MVK	Mevalonate kinase
MVPA	Moderate to vigorous-intensity physical activity
NCD	Non-communicable disease
NHDSS	Navrongo Health and Demographic Surveillance System
NHGRI	Nationa Health Genome Research Institute
NHRC	Navrongo Health Research Centre
NO	Nitric oxide
O_2	Oxygen
PBWT	Positional Burrows-Wheeler transform
PCSK9	Protein convertase subtilisin/kexin type 9 enzyme
PCSK9	Protein convertase subtilisin/kexin type 9 gene

PON1	Paraoxonase 1 gene
PON2	Paraoxonase 2
PON3	Paraoxonase 3
PPARɣ	Peroxisome proliferator-activated receptor gamma gene
QC	Quality control
RDB	RegulomeDB
REDCap	Research Electronic Data Capture
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SCARB1	Scavenger receptor class B member 1 gene
SES	Socioeconomic status
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
SORT1	Sortilin 1 gene
T2D	Type 2 diabetes
TAG	Triacylglycerol
TC	Total cholesterol
TG	Triglyceride
TNFα	Tumor necrosis factor alpha
VAT	Visceral adipose tissue
VIF	Variance inflation factor
VLDL	Very low density lipoprotein
WC	Waist circumference
WHR	Waist-to-hip ratio
WtHR	Waist-to-height ratio
Code	Nucleic acid

A	Adenine
C	Cytosine
G	Guanine
T	Thymine

The nucleic acid codes are listed above as outlined by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry and Molecular Biology (IUBMB).

CHAPTER 1: BACKGROUND AND LITERATURE REVIEW

1.1. Overview of lipids and cardiometabolic disease risk

Cardiometabolic disease (CMD) burden has become a major public health concern worldwide and is increasing in sub-Saharan Africa (SSA), accounting for a high proportion of age standardized deaths (WHO 2003)**.** In west Africa in particular CMD risk continues to experience an upward trend (Sossa *et al.*, 2013). These diseases, including type 2 diabetes (T2D) (Mbanya *et al.*, 2010; Katchunga *et al.*, 2016), hypertension (Ursua *et al.*, 2013), coronary artery disease (Won *et al.*, 2014) and metabolic syndrome (Chen *et al.*, 2012; Al-Bachir and Bakir, 2017) are associated with an increasing prevalence of obesity (Misra and Khurana, 2008). In the past decade this increased prevalence has been associated with a parallel increase in the prevalence of additional cardiometabolic complications such as nephropathy and nonalcoholic fatty liver disease (Lackner, 2011; Ellulu *et al.*, 2014). In Ghana in particular, a Demographic and Health Survey involving both urban and rural individuals from a random sample of 4251 demonstrated that the prevalence of obesity or overweight among adult (non-pregnant) women increased 2.5 fold in 10 years from 10% in 1993 to 25.3% in 2003 (Chu, 2011). Furthermore, it has been reported that cardiovascular disease (CVD) contributed to more than one-fifth (22.2%) of all deaths in autopsy cases within a five year period (2006-2010) in the national referral hospital (Korle-bu Teaching hospital) in Ghana (Sanuade *et al.*, 2014).

These obesity-related CMDs are known to also be associated with dyslipidaemias (Steyn *et al.*, 2005; Asiki *et al.*, 2015; Fakhrzadeh and Tabatabaei-Malazy, 2015). Notable among these are high levels for triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) and low levels of high density lipoprotein cholesterol (HDL-C). There is high prevalence of dyslipidaemia in rural and urban African populations. For instance, a study among rural Nigeria adults reported high TC=31.7%, high LDL-C=18.6%, high TC=31.7%, and low HDL-C=10.6% (Okaka *et al*., 2013). Similarly, a study in rural Togo showed that high TC was 64% and low HDL-C was 16.4% (Noubiap *et al*., 2018). A systematic review of dyslipidaemia prevalence in both rural and urban African adults reported that high TC=25.5%, high LDL-C=26.6%, high TG=17.0% and low HDL-C=37.4% (Ditorguéna *et al*., 2019). Dylipidaemia is reported to be associated with increased cardiovascular disease (CVD) risks (Gomes et al. 2009; Jurado *et al.*, 2009). These include overweight-obesity and metabolic syndrome (Stępień *et al.*, 2014; Núñez-Cortés *et al.*, 2015). High TG/HDL ratios (>3.08 for mens; >6.16 for femens) and

TC/HDL ratios (>0.85) in particular are known risk indicators of CVD and metabolic syndrome respectively in elderly populations (Samuel Acquah *et al*, 2011; Arthur *et al.*, 2012). A recent meta-analysis on 4010 individuals of European and Asian ancestry demonstrated an association between increased atherogenic index of plasma, HDL-C, LDL-C, TG and TC levels and T2D risk (Zhu *et al*., 2015).

Lifestyle and therapeutic interventions can reduce lipid levels and CVD risk. These include exercise, reduced dietary intake and mainly the use of lipid lowering drugs like fibrates and statins (Sever *et al.*, 2003; Pejic and Lee, 2006; Shao *et al*., 2011).

Lipid profiles show variations among groups of individuals based on geographical location, race or ethnicity and the socioeconomic status of the study population (Erem *et al.*, 2008; Dumitrescu *et al.*, 2011). On average, TG, TC and LDL-C have been shown to be lower in Africans than in whites or Asian-Indians and HDL-C levels higher in Africans (Ferris *et al.*, 2005; Bentley and Rotimi, 2012). East Asian populations tend to have lower LDL-C, HDL-C and TG levels compared with non-Asian groups (Karthikeyan *et al.*, 2009). In Ghana, a study on healthy urban adults indicated that Ghanaians have lower levels of TC and higher levels of HDL-C than their counterparts in Europe and North America (Nyarko *et al.*, 1997). Also, variations in lipid levels are demonstrated to be associated with environmental factors such as change in diet, nicotine exposure, alcohol consumption, reduced energy expenditure and socioeconomic status (Davis *et al.*, 2005; BeLue *et al.*, 2009; Dickie *et al.*, 2014). Although these environmental factors are known to influence lipid profiles, genetic factors clearly also play an important role (Bentley *et al.*, 2014).

Genome-wide association studies (GWAS) have shown that serum lipid levels are modulated by genetic variants in specific genes (*https://www.ebi.ac.uk/gwas*). Additionally, there is evidence on the effect of locus-specific variants of candidate genes on lipid levels in populations of both African and European descent. These variants include the ATP-binding cassette A1 (*ABCA1*) variant rs115763221, lecithin-cholesterol ester transport protein (*LCAT*) variant rs13306496 and lipoprotein lipase (*LPL*) variant rs201109344, with higher minor allele frequencies in African than European subjects (Bentley *et al.*, 2014). Despite the well documented evidence of genetic influences on serum lipid profiles the results of these studies are largely not from indigenous African populations. Results of studies show variations in the genetic determinants of lipid levels between populations of non-African descent and African Americans (Deo et al. 2009; Bentley *et*

al., 2014). This suggests that results of genetic studies from the indigenous African populations are likely to be different to those from non-African populations.

To date genetic and environmental factors influencing lipid profiles in African populations largely remain unknown. Studies on the effect of gene-environment interactions on lipid levels are mainly from non-Africans (Davis *et al.*, 2005; Zhang *et al.*, 2010; Aung *et al.*, 2013; Askari *et al.*, 2016). The paucity of data on both genetic and environmental determinants of lipid profiles in African populations makes it important to add to the existing data so that an accurate evaluation of the effect of environmental and genetic determinants on lipid profiles can be made. This is particularly important in rural African settings, including Ghana, where epidemiological transition is characterized by increasing prevalence of non-communicable diseases such as CVDs (Kabudula *et al*., 2017).This makes the present study in Ghana relevant as it will provide new insight into the influence of both genetic and environment factors on lipid levels in black African adults.

1.2. Synthesis, structure and function of lipids

1.2.1. Discovery of cholesterol and related molecules

The current knowledge of cholesterol and related molecules began in 1665 when Boule noted the milky appearance of lacteals and the subsequent entry of this emulsion into the bloodstream through the thoracic duct of animals after they were fed with a fatty meal (Olson, 1998). Later Francois Poulletier de la Salle isolated cholesterol in a solid form from bile and gallstones. Though he did not describe it in scientific terms, this solid isolation provided the foundation for the study of cholesterol structure. Eugene Chevreul rediscovered it in 1815 and called it "cholesterine", but it was not until 1833 that cholesterol was described in the blood by Boudet (Olson, 1998; Hoefner, 2008). Louis Rene Lecanu identified cholesterol in human blood in 1838 (Hoefner, 2008). The discovery of the lipoproteins was heralded by HDL when Michael Macheboeuf in 1929 isolated a stable water-soluble lipoprotein containing protein, lipid and phospholipid (Olson, 1998; Shah *et al.*, 2013). In 1964 Konrad Bloch and Feodor Lynen shared the Nobel prize for describing the pathway of cholesterol synthesis (Vance and Goldfine, 2002). In 1950 Oncley and colleagues discovered LDL. This was made possible through the isolation by ultracentrifugation of β-globulin which was later shown by chemical analysis to be LDL (Oncley and Gurof, 1950).

1.2.2. Lipid and lipoprotein synthesis

The liver and intestines are the sites of lipid and lipoprotein synthesis (Olga *et al*., 1967; Tytgat *et al*, 1971). It has been suggested that in the liver lipid and lipoproteins synthesis begins in the rough endoplasmic reticulum and continues in the smooth endoplasmic reticulum. Lipids and lipoproteins are remodeled in the Golgi apparatus before they are finally released into the circulation. Through these processes within the liver the lipids and lipoprotein particles undergo a change in chemical composition and size (Glaumann *et al.*, 1975). In the human intestines the specific site of lipoprotein synthesis and assembly is the intestinal crypt cells (Levy *et al.*, 2000).

1.2.2.1. HDL structure, synthesis and function

The HDL particles are spherical lipoprotein complexes and the smallest and densest of all the human lipoproteins. The molecule is composed of a core consisting of mainly triglyceride and cholesterol esters. The outer layer contains free cholesterol, phospholipids and various apolipoproteins including apolipoprotein A1 (APOAI), apolipoprotein AII (APOAII), apolipoprotein CII/CIII (APOCII/CIII) and apolipoprotein E (APOE) (Kontush *et al.*, 2015). The HDL particle is fractionated into two main sub-particles, HDL2 and HDL3. The former is the less dense and contains APOAI without APOAII while HDL3, the denser sub-particle contains both APOAI and APOAII (Kontush *et al.*, 2015).

The synthesis of HDL is preceded by small precursor particles generated mainly by the liver and intestines. These unstable HDL precursors acquire cholesterol through ATP-dependent ABCA1-mediated efflux of cholesterol and phospholipids from the hepatocytes or the intestinal cells (Rothblat and Phillips, 2010). These particles are then rapidly converted into mature HDL particles by the action of LCAT which generates cholesteryl esters from cholesterol transferred from VLDL, LDL and cell membranes. These cholesteryl esters due to their hydrophobic nature are assembled in the core of the mature HDL particle making it a lighter and larger spherical particle (Rye and Barter, 2014; Rached and Kontush, 2015).

The HDL particle performs a variety of cardioprotective functions including cholesterol efflux and reverse cholesterol transport, anti-oxidation, anti-inflammation, vasodilation and antithrombosis (Mcgrowder *et al.*, 2011; Ono, 2012; Hafiane and Genest, 2013). It is therefore an inverse predictor of CVD (Mcgrowder *et al.*, 2011; Luo *et al.*, 2014).

1.2.2.2. LDL structure, synthesis and function

The LDL particle is spherical with a size of 18-25nm and composed of a low density core and an outer denser coat. The core consists of esterified cholesterol and triacylglycerol (Alanazi *et al*., 2004) and it is divided into compartments (Orlovo *et al*, 1999). The outer coat consists of phospholipids that are surrounded by a single APOB100 protein (Prassl and Laggner, 2009) and free cholesterol that is inserted between the phospholipid fatty acid chains (Nikanjam *et al.*, 2007). There are subtypes of LDL which differ in density, size and chemical composition and these variations contribute to their clinical importance (Austin *et al*, 1994). The subtypes of LDL include A-LDL, B-LDL and C-LDL with secondary structures resulting from the main structural differences of APOB100 among the subtypes (Fernaandez-Higuero *et al*, 2014).

The synthesis of these lipids and lipoproteins involves a series of reactions that are catalyzed by enzymes. The formation of LDL results from the hydrolysis of TG from VLDL by lipoprotein lipase protein (LPL) to form cholesterol- rich VLDL remnants called intermediate low-density lipoprotein (IDL) *(*Ellsworth *et al*, 1986). The TG content is reduced through further hydrolysis of the remaining TG in the IDL particles by hepatic lipase. The LDL particles are derived from VLDL and IDL particles which undergo intramuscular processing leading to the removal of exchangeable apolipoproteins from the LDL particles and incorporated into other lipoproteins (Daniels *et al.*, 2009). The LDL particles contain mainly ApoB-100 and cholesterol esters (Feingold *et al.*, 1992; Veniant *et al.*, 1999).

The main function of LDL is the transport of cholesterol from the liver to tissues where it is incorporated into cell membranes (Ono, 2012). It is intimately associated with cardiovascular disease such as atherosclerosis, where it inhibits endothelial-dependent vasodilation. This is through the inhibition of endothelial nitric oxide synthase enzyme (eNOS) activity by LDL thereby decreasing the production of endothelial-derived nitric oxide (NO) (Laufs *et al.*, 1998; Zhu *et al.*, 2003). High LDL levels increase movement of endothelial nitric oxide synthase gene (*eNOS*) to membrane caveolae (Zhu *et al.*, 2003) where the increased association of eNOS with caveolin-1 (Cav-1) inhibits eNOS function (Garcia-Cardena *et al.*, 1997).

1.2.2.3. Cholesterol synthesis and function

Cholesterol occurs in the body bound to lipoproteins or integrated aggregates of lipids (Rader *et al.*, 2009). *De novo* cholesterol synthesis starts with the formation of acetoacetyl-CoA from acetyl-CoA which is catalyzed by acetoacetyl-CoA thiolase (Rusell, 1992; Daniels *et al.*, 2009).

Following this are multiple reactions that lead to the formation of cholesterol with each reaction being catalyzed by an enzyme. The product of these reactions include 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA), mevalonate, squalene and eventually cholesterol with each catalyzed by HMG-CoA synthase, HMG-CoA reductase and squalene synthase, respectively (Olivier and Krisans, 2000). These reaction pathways that lead to cholesterol synthesis occur in the cytosol and the membrane compartments of intestinal cells and hepatocytes (Rusell, 1992).

Cholesterol plays an important role in the human body because it is a component of cell membranes (Alberts *et al*, 1994; Girao *et al*, 1999) and at the level of the synapse in the central nervous system it plays an integral role in conducting nerve impulses (Barres and Smith, 2001). It is involved in bile acid productions by the liver and it is used by adrenal glands and gonads for steroid hormone production including androgens, estrogens, progesterones, glucocorticoids and mineralocorticoids (Hume and Boyd, 1978). It is also involved in vitamin D manufacture (Bouillon *et al*., 1995).

Though it plays vital roles in the human body, excess cholesterol build-up in arteries serving the heart is detrimental and can cause CVD and death (Stamler *et al*, 1986; Lloyd-Jones *et al.*, 2009).

1.2.2.4. Triglyceride structure, synthesis and function

Triglyceride is made up of a glycerol backbone to which 3 fatty acids are esterified. The length, position and orientation of the fatty acid carbon chain in the TG molecule determine its physical, chemical and functional properties (Small, 1991; Innis, 2011).

Plasma TG is derived from both dietary sources and *de novo* synthesis within the liver (Yen *et al.*, 2008). Dietary fat in the intestinal lumen is solubilized in bile salt and is readily lipolysed to free fatty acids (FA) and monoacylglycerol (MAG). These are hydrolyzed by pancreatic lipase which facilitates their diffusion into the enterocytes (Mu and Hoy, 2004). They are then transported to the endoplasmic reticulum by cluster determinant 36 (CD 36) (Abumrad and Davidson, 2012) where MAG is acylated with a fatty acyl-CoA and converted to diacylglycerol (DAG) by the enzyme Monoacylglycerol:acylCoA acyltransferase (MGAT). Further conversion leads to the formation of triacylglycerol (TAG) by the enzymes Diacylglycerol:acylcoA acyltransferase 1 and 2 (DGAT1 and DGAT2) (Pan and Hussain, 2012). Meanwhile the FA are bound by FA binding proteins (FABPs) and sent to microsomal compartments for re-

esterification (Adeli and Lewis, 2008). The MAG pathway is the main synthetic pathway in the enterocytes after feeding where large amounts of fatty acids and MAG are released from the digestion of dietary fats (Phan and Tso, 2001). This pathway is also active in adipose tissue (Polheim *et al.*, 1973), where it is needed for the storage of excess energy in the form of TAG (Shi and Cheng, 2009). About 20% of the TAG synthesis occurs through the glycerol phosphate pathway in the intestines (Coleman and Lee, 2004) and is stored in the cytosol and subsequently hydrolyzed and incorporated into chylomicrons. The glycerol phosphate pathway is the main pathway for TAG synthesis in the liver (Pan and Hussain, 2012).

Triglyceride is an important lipid in the human body. It is used by cells in the production of adenosine triphosphate (ATP). In cavities or areas in the body occupied by organs TG acts as a shock absorber or cushion to protect these organs. It is a component of the subcutaneous adipose tissue that protects the skin from heat (Toker, 2005). It is also the medium through which fat soluble vitamins are absorbed in the body and other lipids are transported (Shi and Cheng, 2009).

1.3. Studies linking lipid levels to CVD risk

Cardiometabolic disease is a major health problem that accounted for over 17 million deaths worldwide in 2015 mainly from CVDs (Wang *et al.*, 2016). In SSA nearly one million deaths occurred in 2013 due to CMD, constituting 38.3% of non-communicable disease (NCD) deaths and 11.3% of all deaths in the region (Mensah *et al.*, 2015). Many factors including dyslipidemia are associated with CMD risk (Bansal *et al.*, 2007; Mahalle *et al.*, 2014).

A number of longitudinal studies have demonstrated strong links between lipids and CVD risk. The Framingham Heart study was initiated by the United States of America (USA) Public Health Service in 1948 with the aim of studying the epidemiology and risk factors for CVD. In 1977 the Framingham offspring study, with a sample size of 5124 children and their spouses, observed that TG and lipoproteins were associated with heart disease (as reviewed in O'Donnell and Elosua, 2008). The Seven Countries Study which enrolled 12,763 men aged 40-59 years across seven countries (USA, Finland, Yugoslavia, Netherlands, Italy, Greece and Japan) (Feinleib, 1981) reported that the rate of heart attack and stroke were directly related to the levels of TC (Epstein, 1996). Also, data from a prospective study of 26,509 US women, who were followed for over 11 years, and the five study populations in the Cooperative Lipoprotein Phenotyping Study indicated an inverse association between HDL-C and coronary heart disease (CHD) and a direct association of TG, TC and LDL-C with CHD (Castelli *et al.*, 1977; Bansal *et al.*, 2007).

Similarly, a prospective cohort study of 7587 women and 6394 men aged 20 to 93 years from the general population of Copenhagen, Denmark who were recruited between 1976 and 1978 and followed till 2004, indicated that elevated non-fasting TG levels were associated with myocardial infarction, ischaemic heart disease and death (Nordestgaard *et al.*, 2007; Freiberg *et al.*, 2008).

Additionally, other clinical studies have documented the influence of lipids on CVD. For instance it has been observed in clinical trials that CVD correlates inversely with HDL-C (Barter *et al.*, 2007) and directly with LDL-C (Baigent *et al.*, 2005) though it has been suggested that merely increasing circulating HDL-C levels independent of LDL-C does not reduce CVD risk (Briel *et al.*, 2009). Also a systematic review of randomized control trials has shown that changes in TG levels predict CVD risk (Stauffer and Morrison 2013).

1.3.1. Dyslipidaemia and CMDs in African populations

There is considerable evidence from many clinical studies in African populations that dyslipidaemia is a prevalent condition, particularly in type 2 diabetic patients. Thus, a study of 248 T2D patients attending a hospital in an urban community in Ghana observed that 45% of the patients presented with high TC (>5.17mmol/l), 30.5% with low HDL-C (<1.03mmol/l), 26% with high TG (>1.69mmol/l) and 72.4% with high LDL-C (>2.58mmol/l) (Eghan and Acheampong, 2003). This high prevalence was confirmed by another study in Southern Ghana by Acquah et al (2011). In another study in Kenya, elevated levels of TC and TG that required treatment were observed in T2D patients with no obvious chronic complications (Otieno *et al.*, 2005). The results of a study in Sudan comprising healthy people and T2D patients pointed to the significant association of TG and HDL-C with advancing age, femen gender, obesity, physical inactivity and inadequate glycaemic control (Elnasri and Ahmed, 2008). This is further supported by results of a study in Kinshasa in the Democratic Republic of Congo which demonstrated that the mean level of TG was significantly higher and that of HDL-C significantly lower among other factors in T2D patients (Sungwacha *et al.*, 2013). Similarly hypertriglyceridaemia was strongly associated with T2D patients in Cameroon (Moor *et al*, 2017).

Furthermore, studies in Nigeria in arterial hypertensive and diabetic patients have reported dyslipidemia as a common presentation (Okafor *et al.*, 2008; Adamu *et al.*, 2013). In 2000 de novo cardiac cases in the Heart of Soweto Study, though there were varying gradients of lipid levels across the different ethnicities, low levels of HDL-C were widespread affecting 63% of the cohort (Sliwa *et al.*, 2012). In the analysis of dyslipidaemia in statin-treated patients as part

of the DYSlipidaemia International Study (DYSIS), it was found that diabetes mellitus, ethnicity, hypertension and the presence of coronary and cerebrovascular heart disease were strongly associated with high LDL-C levels. Low HDL-C level was significantly associated with diabetes mellitus and hypertriglyceridaemia was significantly associated with both diabetes mellitus and peripheral arterial disease (Raal *et al*, 2013).

In conclusion there is strong evidence from the studies conducted globally and including Africa, linking dyslipidaemia to CMD, but these are mainly in urban settings. It is therefore important to evaluate the factors affecting lipid levels in rural African adults so as to obtain comprehensive epidemiological data on dyslipidaemia and CMD. This will facilitate the implementation of measures and policies targeting these conditions in both men and women.

1.4. Treatment of dyslipidemia

The predisposition of an individual with dyslipidemia or hyperlipidemia to cardiovascular risk (Mahalle *et al.*, 2014) requires that measures are taken to prevent or treat dyslipidaemia. These measures include lifestyle changes and drug therapy. Lifestyle modifications such as aerobic exercises (Agarwal, 2012), healthy diet (Anand *et al.*, 2015), smoking cessation (Messner and Bernhard, 2014) and reduction in alcohol consumption (O'Keefe *et al*., 2014) have all be shown to improve lipid profiles and cardiac health. These therapeutic lifestyle changes are combined with statins for patients who do not respond to these changes (Scordo, 2014).

1.4.1. Lipid-lowering drugs

The American College of Cardiology (ACC)/American Heart Association (AHA) guidelines have recommended statins as first line therapy for patients with atherosclerotic cardiovascular disease (ASCVD) events. These guidelines identify four major groups for statin therapy. These groups include individuals: (1) with clinical ASCVD; (2) with primary elevations of LDL-C ≥4.9mmol/l; (3) 40 to 75 years old with diabetes and LDL-C 1.8-4.9mmol/l with no clinical ASCVD; (4) with no ASCVD or diabetes who are 40 to 75 years of age with LDL-C 1.8-4.9mmol/l. The mechanism of action of statins is based on partial and reversible reduction in the activity of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase which results in decreased cholesterol synthesis (Shao *et al*., 2011). A few reported side effects especially in the elderly are muscle-related symptoms (Nichols and Koro, 2007; Naci *et al*., 2013). In patients with familial

hypercholesterolemia (FH) statins are the recommended choice of treatment just as in cases with conventional hypercholesterolemia though the recommended doses for FH are slightly higher.

Other treatment approaches include fibrates, nicotinic acid, resins, ezetimibe in combination with statins, mipomersen which inhibits APOB100 synthesis and PCSK9 inhibitors which are novel (Robinson, 2013).

The enzymes that is encoded by *PCSK9* binds to the low density lipoprotein receptor (LDL-R) on the surface of the hepatocyte, resulting in the degradation of the LDL-R and higher plasma LDL-C (Surdo *et al*., 2011). Inhibitors of PCSK9 including alirocumab and evolocumab, which are monoclonal antibodies, bind free plasma PCSK9 and accelerate the degradation of the enzyme (Stein *et al*., 2012). This action results in less free PCSK9 being available in plasma to bind to LDL-R. This leads to a higher fraction of LDL-R recycling towards the hepatocyte surface resulting in the liver having the capacity to remove more LDL-C from the circulation and causing lower LDL-C plasma levels (Rosenson *et al.,* 2018). Other inhibitors of PCSK9 include antisense oligonucleotides, small interfering RNA, mimetic peptides and adnectins (Wang *et al.,* 2019).

Fibrates are used in patients with low HDL-C and/or high TG. They raise HDL-C levels due to increased HDL apoprotein production and reduced cholesteryl ester transfer from HDL to VLDL (Guerin *et al.*, 1996). Nicotinic acid (an essential water-soluble B-complex vitamin) inhibits the mobilization of free fatty acids from peripheral tissue which results in reduced hepatic synthesis of TG and secretion of VLDL-C and its conversion to LDL. Nicotinic acid improves HDL-C, LDL-C, TC and TG profile when it is used in higher pharmacologic dose than that required for its role as a vitamin (Kamanna and Kashyap, 2000; Shao *et al*., 2011). Its side effects when the dose is not properly managed include diarrhoea, conjunctivitis, and nasal stiffness among others (Knoops *et al.*, 2004). Bile sequestrants (resins), also used as adjuncts to statin therapy, bind to bile acids in the small intestines and cause the interruption of the interohepatic circulation of bile acids and increase the conversion of cholesterol to bile acid in the liver (Shao *et al*, 2011). Another class of lipid-lowering drugs for use together with statins in lowering LDL-C levels especially in patients with the metabolic syndrome and diabetes is ezetimibe. This drug slightly increases HDL-C and reduces TG levels through the inhibition of intestinal absorption of cholesterol (Simons *et al.*, 2004).

1.5. Environmental factors influencing lipid levels

Lipid levels are associated with environmental factors such as socioeconomic status (SES) (Vorster *et al.*, 2007), lifestyle factors including tobacco (Corella *et al.*, 2002; Yin *et al.*, 2012) and alcohol consumption (Ahaneku *et al.*, 2014), pesticide or chemical use (Ntzani *et al.*, 2013) and diet (Daoud *et al.*, 2014). Others are demographic factors such as gender and age (Dumitrescu *et al.*, 2011; Asiki *et al.*, 2015) and anthropometric factors including body fat mass and body fat distribution (Porter *et al.*, 2009; Ni *et al.*, 2015; Tang *et al.*, 2016). These factors and their influence on lipids vary across geographical, racial and ethnic boundaries (Davis *et al.*, 2005; BeLue *et al.*, 2008; Erem *et al.*, 2008).

1.5.1. Ethnic differences in lipid levels

Lipid levels are known to show ethnic variation (Donin *et al.*, 2010; Bentley and Rotimi, 2017a). Thus, TG, TC and LDL-C have been shown to be lower in Africans than in whites or Asian-Indians and lower in East Asians than in non-Asian groups (Ferris *et al.*, 2005; Karthikeyan *et al.*, 2009). Similarly low serum HDL,TG and LDL levels in African and populations of African-descent have been documented (Agyemang *et al.*, 2009; Deo *et al.*, 2009; Crowther and Norris, 2012).

1.5.2. Socio-demographic factors and lipid levels

There are conflicting findings from several studies on the influence of demographic factors on lipid levels. A study conducted in Rahat, Pakistan, indicated that dyslipidemia prevalence was observed to show significant differences among age groups but no difference was observed between men and women (Khan *et al.*, 2012). Another study also observed that serum lipid concentrations were significantly positively correlated (for TC, TG, LDL-C) or negatively correlated (for HDL-C) with age but contrary to the findings by Khan *et al*. (2012) indicated a significant difference in TC, HDL-C and LDL-C levels between men and women though TC was similar in both genders (Ni *et al.*, 2015). Similarly, while some studies have reported association of higher SES with higher LDL-C, TC, TG and lower HDL-C levels (Shohaimi *et al.*, 2014; Brown *et al*., 2016) other findings have shown contrary observations in urban settings (Gupta, Guptha, *et al.*, 2012). Furthermore, some studies have associated low education levels and unemployment with higher lipid levels (Gupta, Deedwania, *et al.*, 2012; Brown *et al*., 2016) while others have reported the contrary (Reddy *et al.*, 2012; Rodriguez *et al.*, 2014).

It is therefore important to include socio-demographic factors in models used in investigating factors associated with lipids so as to determine their effect on lipid levels particularly in rural settings where little or no data exists.

1.5.3. Lifestyle factors and lipid levels

Physical activity, nutrition, and substance use (tobacco, alcohol, chemicals and pesticides) have also been observed to be associated with variations in lipids. Studies have suggested that moderate alcohol intake increases HDL-C (Langer, Criqui and Reed, 1992; Gaziano *et al.*, 1993; Manttari *et al.*, 1997; Mukamal *et al.*, 2005) and decreases LDL-C (Castelli *et al.*, 1977; Langer *et al*., 1992) levels and thus enhances cardioprotection. Furthermore, a study in women has shown the inverse relation of alcohol drinking with LDL-C/HDL-C ratio (Wakabayashi, 2013b) but this inverse relation diminishes with heavy alcohol intake (Wakabayashi, 2013a). Contrastingly, there are reports of higher TG levels in alcohol drinkers (Langer *et al*,, 1992; Wiel, 2012) and this influences MS and hence CVD (Kannel and Vasan, 2009). To deepen the understanding on the exact influence of alcohol consumption on lipid levels assessment should be made on the quantities of alcohol consumed and the times these are consumed (Hojnacki *et al.*, 1991; Rakic *et al.*, 1998; Peasey *et al.*, 2005).

Other lifestyle factors affecting lipid levels include tobacco use (smoking or chewing) and pesticide or chemical exposure. There is empirical evidence that smoking causes elevated TG and reduced HDL-C levels though with lesser effect on LDL-C *(Craig et al., 1989; Imamura et al*., 2000; Campbell and Tishkoff, 2008). It has also been shown in patients with diabetes mellitus that smokers have higher lipid related indices than have non-smokers (Wakabayashi, 2014) and smoking cessation has been associated with improved lipid levels (Griffin *et al.*, 1994; Urahama *et al.*, 2008; Gepner *et al.*, 2011). Similarly, smokeless tobacco use is reported to influence lipid levels (Tuomilehto *et al.*, 1986; Dwyer *et al.*, 1988) and even after the cessation of its use there is no improvement in lipid profile (Allen *et al*., 1994).

There are conflicting reports on the influence of chemical exposure on lipid levels. While studies have reported a significant positive correlation of lipid profiles with pesticides (Lee *et al.*, 2011; Arrebola *et al*., 2014) other findings point to the contrary (Caba et at, 2015). The differences in amounts and types of pesticides used could account for these contradictions.

There are reports of association of favourable lipid profiles with physical exercise. A study of dialysis patients has shown an increase in HDL-C and a decrease in LDL-C levels with physical exercise for many days (Imamura *et al*., 2012). There is also evidence of the association between physical activity and improved lipid levels in apparently healthy adults (Brown *et al*., 2016). Forty-nine controlled trials representing up to 67 outcomes from 2,990 men (1,741 exercise and 1,249 control) showed statistically significant decrease in LDL-C, TC and TG and increase in HDL-C levels with exercise (Kelley and Kelley, 2006). Another meta-analysis involving 35 trials with a total of 1404 men and women indicated modestly increased HDL-C levels with exercise (Kodama *et al.*, 2007).

Yet another lifestyle factor affecting blood lipid balance is nutrition. Saturated fatty acids, cholesterol and excess caloric intake are known to cause the elevation of serum LDL-C (Grundy and Denke, 1990) whilst consumption of fruits and vegetables is associated with decreased LDL-C (Singh *et al*., 1992; Djousse *et al.*, 2004). Earlier studies on vegetarians had indicated a decrease in serum LDL-C and TC concentrations (Knuiman and West, 1982; Sacks *et al*., 1985). High-carbohydrate is associated with lower HDL-C concentrations than low-carbohydrate diet (Merchant *et al.*, 2007; McKeown *et al.*, 2009). A systematic review and meta-analysis of 11 studies with a total of 832 participants demonstrated lower levels of LDL-C, TC and TG with vegetarian diet(Wang *et al*., 2015). Results of another systematic review and meta-analysis of large randomized controlled trials of at least six month duration on the effect of varying quantities of carbohydrate and fat diets showed that carbohydrate restriction improved lipid markers compared with low-fat diets (Gjuladin-Hellon *et al.*, 2019).

1.5.4. Effect of anthropometry on lipid levels

Anthropometry, particularly fat mass and distribution, has been shown both in non-African and African populations to affect lipid levels. Thus, a study in Puerto Rico involving 858 adults reported that waist-to-height ratio (WtHR) was associated with the highest risk of a high TC/HDL-C ratio. Waist circumference (WC) had the highest risk of low HDL-C and high LDL-C levels whilst waist-to-hip ratio (WHR) was associated with the highest risk of high TG level (Palacios *et al.*, 2011). In another study involving Taiwanese adults comprising 21,038 men and 15,604 women BMI, WC and WHR were all significantly correlated with low HDL-C and high TG and TC levels. In comparison with BMI and WC, the WtHR was more strongly correlated with TC and TG in both men and women (W.-C. Li *et al.*, 2013). In another study in Iran BMI,

WC and WtHR had been found to be correlated with dyslipidaemia (Chehrei *et al.*, 2007). Several other studies have documented the influence of BMI and WC on lipid levels (Porter *et al.*, 2009; Ni *et al.*, 2015; Tang *et al*., 2016). Hip circunference (HC) is associated with favoruable lipid profiles with several studies demonstrating that a high HC was associated with lower TG, LDL-C, TG and higher HDL-C levels after adjusting for WC and BMI (Snijder *et al.*, 2004; Canoy *et al.*, 2006; Esmaillzadeh *et al.*, 2006; Ruige and Van Gaal, 2009).

Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) are each independently associated with higher TC, LDL-C, TG and lower HDL-C in both men and women (Porter *et al.*, 2009; Tang *et al*., 2016). However there have been reports of subtle differences in these associations between men and women. Thus, SAT is reported to be associated with higher TC in both men and women but was additionally associated with higher TG and lower HDL-C in women (Scheuer *et al.*, 2015). Further evidence from several studies have indicated independent association between VAT, and low HDL-C and high TG and between SAT, and low HDL-C and high TG concentration (Wildman *et al.*, 2011; Miazgowski *et al.*, 2014; Osawa *et al.*, 2014). Also, findings from the a Korean population and the Framingham offspring and the third generation cohort studies indicated that high VAT/SAT ratio was significantly correlated with lower HDL-C and higher TG (Kim *et al.*, 2011; Kaess *et al.*, 2012).

1.6. Environmental factors and lipid levels in Africans

Just as in non-Africans, studies in African populations have indicated that dyslipidemia is associated with environmental factors. These include alcohol consumption, physical activity and SES (Ebele *et al.*, 2009; Dickie *et al.*, 2014; Mamabolo *et al*., 2014; Asiki *et al.*, 2015). Additionally, findings from several studies have reported the influence of urbanization on increasing dyslipidaemia prevalence and cardiometabolic disease risk in Africans (Alberts *et al.*, 2005; Njelekela *et al.*, 2009; Ogunmola *et al.*, 2013). There is however a gradual increasing trend in lipid levels in African rural dwellers. For instance findings from cross-sectional studies in both rural Nigeria and Uganda reported a high prevalence of low HDL-C (43.1% and 71.3%, respectively) (Oladapo *et al.*, 2010; Asiki *et al.*, 2015). This calls for further investigation to ascertain the factors associated with serum lipid levels in rural African populations.

Lipid levels have been documented to be influenced by demographic factors in African populations including gender, age and ethnicity. Reports of gender differences in lipids in Africans are contradictory. While some findings show that women have more favourable lipid

levels than men (Borgo *et al.*, 2018), and vice versa (Asiki *et al.*, 2015), other studies report no gender differences in lipids (Adamu *et al.*, 2013; Ogunmola *et al.*, 2013). These gender differences are also influenced by age (Swiger *et al.*, 2014) though increasing age is associated with increased lipid levels in both genders (Oelofse *et al.*, 1996). The molecular mechanism behind this is not known but a number of reasons have been attributed to explain this effect (Auley and Mooney, 2015) including some of the major enzymes involved in lipid metabolism changing the rate of their activity with age (Niemi and Nikkila, 1957; Bey *et al.*, 2001). There are ethnic variations in lipid levels among Africans. A study carried out in South Africa indicated that black South African women had lower TC, LDL-C and higher HDL-C and TG compared to white South African women (Ellman *et al*.,, 2015). Similar observations in inter-ethnic differences in lipid levels among Africans have been made (Punyadeera *et al.*, 2001; Sliwa *et al.*, 2012). In addition, LDL and TG levels have been shown to be quite low in African subjects compared to non-Africans (Ferris *et al.*, 2005; Crowther and Norris, 2012). However, there is an information gap regarding lipid levels in intra-ethnic groups in Africa. Reports of several studies have shown unfavourable lipid levels with increasing anthropometric indices in African populations (Akpa *et al*., 2006; Crowther *et al*., 2006; Sliwa *et al.*, 2012; Mamabolo *et al*., 2014; Peer *et al.*, 2014), supporting data from outside Africa.

1.7. Effect of malaria and HIV infection on lipid levels in Africans

Infectious diseases have been shown to influence lipid levels (Visser *et al.*, 2013). This is important in west and central Africa where malaria is endemic (WHO, 2014) and in east and southern Africa where the highest prevalence of HIV is documented (WHO, 2014). In Nigeria while some findings have reported a positive correlation between malaria parasite status and cholesterol levels (Chukwuocha and Eke, 2011; Orimadegun and Orimadegun, 2015) others have shown negative or mixed associations (Jacob, 2014). A study to identify the most important risk factors for developing CVD in HIV-infected black South Africans, reported that high values of TG/HDL-C and TC/HDL-C and low HDL-C levels were observed in this population (van Rooyen *et al.*, 2014). Further reports on the combined effect of HIV infection and its treatment on lipid levels in both rural and urban Southern Africans have been documented(Gomo *et al.*, 2014; Clark *et al.*, 2015). These studies demonstrate that anti-retroviral therapy is associated with a more atherogenic lipid profile, as reported in studies from high income countries.

Lipids levels in Africans are therefore modulated by a complex interplay of environmental factors ranging from socio-demography, lifestyle, and anthropometry to infection. In order to maintain healthy lipid profiles in African populations monitoring and early interventions are required to restrict or minimize the effect of these factors on lipid levels

1.8. Genetic factors influencing lipid profiles

There is substantial evidence from candidate gene and genome-wide association studies (GWASs) of the influence of genetic variants on lipid level (Coram *et al.*, 2013; Bentley *et al.*, 2014; Kelishadi *et al.*, 2014). For example, variants in *PON1* (Durrington et al, 2001), *LCAT* (Hovingh *et al.*, 2005), cholesteryl ester transport protein gene (*CETP*) (Ridker *et al.*, 2009), and *LPL* (Pirim *et al.*, 2014) *(*genes involved in synthetic and metabolic pathways of lipids) are associated with serum lipid and lipoprotein levels. Candidate gene association studies are designed to test hypotheses, based on knowledge of the function of the gene products For example, exploring the functional impact of gene mutations on enzymes that play a role in lipid biosynthetic pathways. On the other hand GWASs are exploratory and may yield unanticipated results, with significant associations to genes whose functions are not known to be involved in lipids. Once associations are detected, hypotheses can then be developed in an attempt to explain mechanisms and to elaborate on biological plausibility.

The early literature on genetic associations with lipid levels includes many candidate gene studies. They were often performed in small studies, did not include adjustment for significant confounders like gender and age, and identified marginally significant associations that were often not replicated. Examples of such studies include the association of the microsomal triglyceride transfer protein (*MTTP*) and the *LPL* genes with lipid level variations. Thus, an investigation on participants of the Framingham Offspring study on the association of the common G493T polymorphism of the *MTTP* promoter with lipid level variations, observed no association of the mutation with variation in lipid levels in both men and women (Couture *et al.*, 2000). Similarly, findings of the ECTIM (Etude Cas-Témoin de I'Infant du Myocarde) study (Parra *et al.*, 1992) which tested the association between polymorphism in the promoter region of the *MTTP* gene and lipid levels showed that two polymorphisms at positions -400(A→T) and 164(T→C) were not associated with variations in lipid levels (Herrmann *et al.*, 1998). These findings are contradicted reports by other studies which showed that the *G493T* polymorphism of

the *MTTP* gene was associated with variations in lipid levels (Karpe *et al.*, 1998; Juo *et al.*, 2000).

Polymorphisms of the *LPL* gene and their associations with changes in lipid levels have been documented. Tests of association between three polymorphisms (*HindIII*, *BamIII* and *PvuII*) of *LPL* and circulating lipids indicated that only *HindIII* was significantly associated with low HDL-C but not TG (Heizmann *et al.*, 1991), contrary to an earlier report of significant association of the *PvuII* polymorphism with elevated TG (Chamberlain *et al.*, 1989). In another study it was also shown that the S447X mutation in the *LPL* gene did not show any significant association with lipids (Mattu *et al.*, 1994), casting doubt on the role of *LPL* in determining lipid levels.

In conclusion, these pre-GWAS investigations were generally contradictory in their findings, with small effect sizes and demonstrating no strong evidence for loci with large effects on lipid levels.

Recent advances in the field of genetic associations have come from large GWASs and are summarized in the GWAS catalogue (http://www.ebi.ac.uk/gwas/). These studies have been facilitated through the contributions of several projects that have provided information on genome architecture and global population variation. They include the HapMap project (The International HapMap Consortium; *et al.*, 2007), the 1000 Genomes Project (The 1000 Genomes Project Consortium, 2010) and Genome of the Netherlands Project (Boomsma *et al.*, 2014), and have contributed to the development of cost-effective chip-based arrays that are used to genotype thousands of individuals at several million genetic markers or single nucleotide polymorphisms (SNPs) across the human genome (McCarthy and Hirschhorn, 2008; Peiffer and Gunderson, 2009). Array-based SNP genotyping chips enriched for regions and genes previously associated with specific group of disorders have also been developed. These include the immunochip for autoimmune diseases and the cardiometabochip for cardiovascular, anthropometric and metabolic traits (Voight *et al.*, 2012).

The majority of studies on genetic markers influencing lipid traits have been performed in cohorts of European origin, though there is an increase in Asian studies with some studies in African Americans, but very few in Africa (Coram *et al.*, 2013; Willer, 2013; Bentley *et al.*, 2014; Dron and Hegele, 2016). Data on the genetic aetiology of dyslipidaemia in black Africans from GWASs is however scanty. In an effort to stem this gap the H3Africa Consortium (The

H3Africa Consortium, 2014) has gathered genomic data from the African continent and is building capacity for genomic science among African scientists. This genomic data, including whole genome sequences from 350 individuals from under-represented African populations, have been used to develop an Afrocentric chip-based SNP genotyping array that can be used in candidate gene and GWA studies in Africans (Mulder *et al.*, 2018). This will eventually assist in the identification of genetic markers associated with lipid level variations in Africans.

1.8.1. Genetic studies of lipid levels in African populations

Studies on genetic variation and lipid levels in African populations include candidate gene association studies and GWAS examples of which some are presented below.

Candidate gene association studies of lipid levels with polymorphisms in the *CEPT*, *LPL*, paraoxonase 1 (*PON1*), Scavenger receptor class B member 1 (*SCARB1*), Microsomal triglyceride transfer protein (*MTTP*), Apolipoprotein (*APO*), sortilin 1 (*SORT1*), Glucokinase regulator (*GCKR*) and Interleukin-6 (*IL-6*) genes in Africans have shown association of gene variants with lipid levels. Thus, it was shown by Rejeb *et al.* (2012) that the Q allele of the R451Q polymorphism of the *CETP* gene was associated with lower HDL-C and higher TC and apolipoprotein B (APOB) concentrations. The polymorphism was further shown to be associated with increased risk of significant coronary stenosis. Polymorphisms within *CETP* and *LPL* have been shown to be associated with lower LDL-C, TC and TG levels in black women of Xhosa ancestry but not in white women in South Africa (Ellman *et al*, 2015).

Findings from a study in Benin City, Nigeria, suggested that both common and rare variants of the *LPL* gene modulated lipid and lipoprotein levels (Pirim *et al.*, 2015). The influence of *LPL* polymorphisms on lipid levels is strengthened by the results of another study in Egyptian patients with acute myocardial infarction (AMI) which point to the association between *HINIII* and *PVUII* polymorphisms and TG levels, although these polymorphisms did not have any association with HDL-C, LDL-C and AMI (Bahrami *et al.*, 2015). A contrasting finding in Sudan however indicated that polymorphisms of *LPL* were not associated with lipid level variations when the genotypes at three *LPL* missense variants (D9N, N291S and S447X) were evaluated against lipid profiles (Abdel *et al.*, 2015).

The influence of *PON1* on lipid levels in African populations has been documented in studies in South Africa and Gabon, mainly involving the Q192R polymorphism. In South Africa in diabetic

patients the Q192R polymorphism was significantly associated with an increase in the concentration of PON1 and decreased activity in PONase which was more pronounced in diabetic patients than in non-diabetic individuals (Macharia *et al.*, 2014). This apparently influenced the lipid levels differently in the two groups (diabetic versus non-diabetic) since the *PON1* gene is known to affect both LDL and HDL levels through its ability to suppress the oxidative capacity of these lipoproteins (Mackness *et al*, 1991; Aviram *et al.*, 1998). This is in contrast to the findings of a study in diabetic patients in Gabon which indicated lower enzyme activities with the *PON1* genotypes in these patients than in non-diabetics (Abessolo *et al.*, 2014).

Other candidate genes whose variants have been found to affect lipid levels in African subjects include *SCARB1*, *MTTP*, and *IL-6* genes. The effect of genetic polymorphisms on lipid levels was shown in a study from Benin City, Nigeria by demonstrating the significant association of Scavenger receptor class B member 1 (*SCARB1*) variants with HDL-C (Niemsiri *et al.*, 2015). Also a study involving two unrelated Tunisian patients with severe deficiency of LDL and APOB found a mutation in the *MTTP* gene in these patients which probably contributes to that metabolic disorder (Najah *et al.*, 2013). It has been demonstrated in black and white South African women that obesity and serum lipids were influenced by the IVS4 +869 A>G polymorphisms of *IL-6* but this influence was modulated by dietary fat intake (Joffe *et al.*, 2014).

Apart from single candidate gene association studies demonstrating the impact of gene polymorphisms on lipids other studies involving multiple candidate genes in African populations have been undertaken. A study in an Algerian population reported a significant association between *SORT1* (rs629301) and both serum TC and LDL-C concentration, between *GCKR* (rs1260326) and TG and TC and between *CETP* (rs3764261) and HDL-C concentration (Meroufel *et al.*, 2015). Also other candidate genes and loci containing polymorphisms were involved in cholesterol metabolic pathways in individuals of the Maasai tribe resulting in their ability to mitigate the fat, cholesterol and lactose in their diet (Wagh *et al.*, 2012). In Durban, South Africa it was found that the genetic variations in several genes including *CETP* (Taq1B2B2), *APOE* (3/E3) and *LPL* (S447X) were associated with favourable lipid profiles in young Asian Indians (Ranjith *et al*., 2009). Hamid *et al.* (2015), on the other hand found no association between lipid profiles and the S447X *LPL* polymorphism.

There are conflicting reports regarding *APO* polymorphisms and variations in lipid levels in Africans. While some studies have indicated the influence of *APOD* (Desai *et al.*, 2002), *APOA5* (Ouatou *et al.*, 2014), and *APOE* (Masemola *et al*., 2007; Boulenouar *et al.*, 2013) another case-control study on obese and non-obese Egyptians observed no significant association between lipid level variations and *APOB* (XbaI) polymorphism (Bogari *et al.*, 2015).

Candidate gene studies for associations with lipid profiles in African populations are often conflicting and suggest either non-robust findings in studies with small sample sizes, or real differences in genetic-associations between cohorts in south and north Africa. Very few studies in sub-Saharan Africa have looked at the genetic correlates of lipid levels. Furthermore, these studies have not evaluated the effect of the gene-environment interactions on lipid levels. It is therefore important to investigate the influence of both genetic and environmental factors on lipid levels and to assess the gene-environment interaction effect on lipid levels. More specifically, there are very few studies on genetic variants and lipid levels in West African populations and therefore the current study is an important addition to understanding the effects of genetic variants in candidate genes on lipid levels in sub-Saharan African populations.

1.8.2. Selected candidate genes and lipid levels

There is evidence for several robust associations of gene variants with lipid levels and the present study sought to evaluate the effect of *LPL*, *CEPT*, *LCAT*, *ABCA1*, protein convertase subtilisin/kexin type 9 (*PCKS9*), *PON1*, mevalonate kinase (*MVK*) and methylmalonic aciduria cb1B (*MMAB*) gene variants on lipid profiles in a black African population from West Africa. These genes were selected based on evidence from GWAS studies (http://www.ebi.ac.uk/gwas). The number of studies in the NHGRI/EBI GWAS catalog (URL: https://www.ebi.ac.uk/gwas) showing the effect of almost all these genes on lipid levels as at November 2018 is illustrated in Table 1.1. Further, GWAS studies have shown that polymorphic variants of these genes are associated with lipid levels in populations of African descent (Fogarty *et al.*, 2010; Lettre *et al.*, 2011; Coram *et al.*, 2013; Moore *et al.*, 2015) and have confirmed the influence of SNPs in these genes on lipid profiles in African-Americans, populations of African ancestry or black Africans (Sirois *et al.*, 2008; Adeyemo *et al.*, 2012; Bentley *et al.*, 2014; Pirim *et al.*, 2016). The contribution of genetic variation in *ABCA1*, *LCAT*, *LPL*, *PON1* and *PCSK9* to lipid level variations has been documented in African-Americans (Hallman *et al.*, 2007; Bentley *et al.*, 2014). Also there are reports of association of *LPL* variants with lipid levels in West African

(Nigerians) (Pirim *et al.*, 2016) and *CETP* polymorphisms with North Africans (Rejeb *et al.*, 2012; Meroufel *et al.*, 2015).

Table 1.1: List of candidate genes and number of genome-wide significant associations ($p<5x10^{-8}$) on lipid levels in published studies in the NHGRI/EBI GWAS catalog (accessed in November, 2018)

Gene symbol	Gene name	Chr. No.	Number of hits in the GWAS catalogue				Reference
			LDL-C	HDL-C	TG	TC	
CETP	Choleseteryl ester transport protein	16	3	28			Blauw *et al.* (2018); Ridker *et al.* (2009); Willer *et al.* (2008)
LCAT	Lecithin-cholesterol acyltransferase	16		6			
ABCA1	ATP-binding cassette A1	9	1	1			Ridker *et al.* (2009); Willer *et al.* (2008)
LPL	Lipoprotein lipase	8	8	6	2		(Middelberg *et al.* (2011); Willer *et al.* (2008)
PCSK9	Protein convertase subtilisin/kexin type 9	1	1	1			(Theusch *et al.* (2014); Willer *et al.* (2008)
MMAB	Methylmalonic aciduria cb1B	12		1			Willer *et al.* (2008)
MVK	Mevalonate kinase	12		1			Willer *et al.* (2008)

1.8.3. Structure and functions of selected genes in lipid metabolic pathways

These selected genes (*CETP, LCAT, ABCA1, LPL, PON1, PCSK9, MVK and MMAB*) are structurally different and perform different functions in lipid metabolic pathways (Figure 1.1).

They encode proteins that are important in the regulation of lipid and lipoprotein levels in the blood.

1.8.3.1. *LPL* gene and protein

The *LPL* gene located on chromosome 8p22 is approximately 30kb long, has 10 exons (Goldberg, 1996) and encodes 448 amino acids. The 5'-untranslated region (5'UTR), the signal peptides and the first two amino acids are located in the first exon. The remaining 446 amino acids are located in the next eight exons whilst the 3'-untranslated region (3'UTR) is located in the 10th exon (Kirchgessner *et al.*, 1989). The gene is expressed in the parenchyma cells of the heart, the skeletal muscles and the white and brown adipose tissue (Jensen *et al.*, 1994). As part of its functions, the LPL enzyme hydrolyses TG in very low density lipoprotein (VLDL) and chylomicrons into free fatty acids and glycerol which are delivered to tissues for oxidation or storage (Garfinkel and Schotz, 1987). The catabolism of VLDL results in intermediate lipoprotein (IDL) which is eventually converted to LDL through TG depleted hydrolysis. The LPL-catalyzed hydrolysis of VLDL and chylomicron TGs results in the formation of mature HDL when apolipoproteins and surface phospholipids released from these TG-rich lipoproteins fuse with pre-β HDL (Patsch *et al*., 1978). High LPL activity is thus associated with lower TG and higher HDL-C whilst low *LPL* activity results in hypertriglyceridemia and atherosclerosis.

1.8.3.2. *CEPT* gene and protein

The CEPT protein is also important in lipid metabolism and lipid level variations. The gene codes for a hydrophobic glycoprotein that is excreted mainly from the liver and it is bound to HDL in the circulation (Tall, 1993). The human *CETP* gene is located on chromosome 6q21. It consists of 16 exons and 15 introns and is approximately 25kb long (Ordovas, 2000). It mediates transfer of TGs from VLDL-1 to HDL and/or LDL in exchange for cholesteryl ester (CE), resulting in larger, comparatively TG-enriched HDL and LDL particles (Rader *et al.*, 2009; Tall, 2010), and a decrease in HDL concentration (Carlquist and Anderson, 2007). The esterified cholesterol is moved through the LDL receptor and subsequently to the liver for excretion, an important step in the reverse cholesterol transport (RCT) pathway (Ono, 2012).

1.8.3.3. *ABCA1* gene and protein

Another important gene in the RCT pathway is the *ABCA1* gene. The gene product mediates the transport of cholesterol from cells to lipid-poor apolipoproteins secreted by the liver (Oram and

Heinecke, 2005; Rader *et al.*, 2009). The ABCA1 peptide also mediates nascent HDL biogenesis. A deficiency of *ABCA1* or loss of function of both alleles (Tangier disease) results in severe HDL deficiency and cholesterol accumulation (Rader *et al.*, 2009). The human *ABCA1* gene is located on chromosome 9q31.1 (Kolovou *et al.*, 2012) and contains 50 exons (Langmann *et al.*, 1999). The ABCA1 peptide is a 2261 amino acid transmembrane integral protein. It has two transmembrane proteins and two ABC domains. Each transmembrane protein has 12 membrane spanning domains. It belongs to the ATP binding cassette (ABC) gene family that consists of several sub-families that have various functions in transmembrane lipid and ion transport (Wang and Smith, 2014).

1.8.3.4. *LCAT* gene and protein

The *LCAT* gene also has an influence on human lipid and lipoprotein levels. This gene encodes a plasma enzyme which, principally at the HDL particle, esterifies free cholesterol, the molecules of which migrate to the inner core of the HDL particle. This process contributes to HDL maturation (Glomset, 1968). Mutations in *LCAT* thus contribute to a defective HDL biogenesis pathway (Fotakis *et al.*, 2015). Increased activity of LCAT therefore increases HDL-C levels and contributes to decreased atherosclerosis (Hovingh *et al.*, 2005). The gene plays an important role in the RCT pathway as it is key in the HDL-mediated removal of excess cholesterol from macrophages in the arterial wall and the subsequent delivery of this cholesterol to the liver for biliary excretion (Rader *et al.*, 2009). Identified in 1962 (Glomset, 1962), the human form of the gene is located on chromosome 16q21-22 and consists of 6 exons which are interspersed by 5 introns. The gene is 4.2bk long (Jonas, 2000).

1.8.3.5. *PCSK9* gene and protein

The *PCSK9* gene belongs to the protease K subfamily of subtilases and is a serine protease enzyme. The *PCSK9* gene is located on chromosome 1p32, contains 12 exons and 11 introns and is 22kb long (Seidah and Prat, 2007). The protease subfamily plays a role in processing of inactive precursor proteins to active products and is involved in the regulation of LDLr levels which are responsible for removing cholesterol-rich LDL particles from plasma (Benjannet *et al.*, 2004; Maxwell, Fisher and Breslow, 2005; Poirier *et al.*, 2008).

In performing its function of regulating LDLr levels, the PCSK9 enzyme binds to the LDLr in the liver (Ono, 2012). The enzyme is also expressed in the kidney and small intestines. It undergoes autocleavage in the endoplasmic reticulum (ER) after which the prodomain and

catalytic domain of the enzyme remain associated and the complex is secreted into the plasma. The circulating PCSK9 enzyme binds the LDLr, is internalized, and targets the receptor for lysosomal degradation, or the enzyme binds the LDLr in the Golgi and recycles the receptor to the membrane (Akram *et al.*, 2010). Since the LDL molecules, after being formed from VLDL, are cleared from the blood by LDLr-mediated endocytosis mainly in the liver, gain-of-function mutations of *PCSK9* promotes LDLr degradation. This leads to the build-up of LDL in the blood resulting in hypercholesterolemia (Benjannet *et al.*, 2004; Park *et al*., 2004).

1.8.3.6. *PON1* gene and protein

The *PON1* gene belongs to the *PON* family comprising *PON1*, *PON2* and *PON3* enzyme-encoding genes all of which are located close to each other on chromosome 7q21-22. The PON1 protein is 354 amino acids long and has a molecular mass of 43kDa (Primo-Parmo *et al.*, 1996). The human *PON1* is approximately 27kb long (Mackness *et al.*, 1996; Primo-Parmo *et al.*, 1996). The enzyme is synthesized in the liver and kidney and carried by HDL in serum. It is shown to protect LDL and HDL from peroxidation by degrading or hydrolyzing specific oxidized cholesteryl esters and phospholipids contained in oxidized lipoproteins (Barter *et al.*, 2004). This process is largely responsible for the antioxidation properties of the HDL particle which is cardioprotective (Litvinov *et al*., 2012).

1.8.3.7. *MMAB* and *MVK* genes and proteins

The *MMAB* gene encodes the mitochondrial enzyme cob(I)alamin adenosyltransferase that catalyzes adenosylcobalamin formation, a key cofactor in amino acid, lipid and cholesterol catabolism (Deodato *et al.*, 2006; Holleboom *et al.*, 2008). The gene is located on chromosome 12q24 and is 18.8kb long (Dobson *et al.*, 2002). Closely linked to the *MMAB* gene on the same chromosome location is the *MVK* gene which has 10 exons (Mandey *et al.*, 2006). This gene encodes the MVK enzyme that is necessary for the synthesis of isoprenoids which are essential intermediates in the biosynthesis of cholesterol (Goldstein and Brown, 1990).

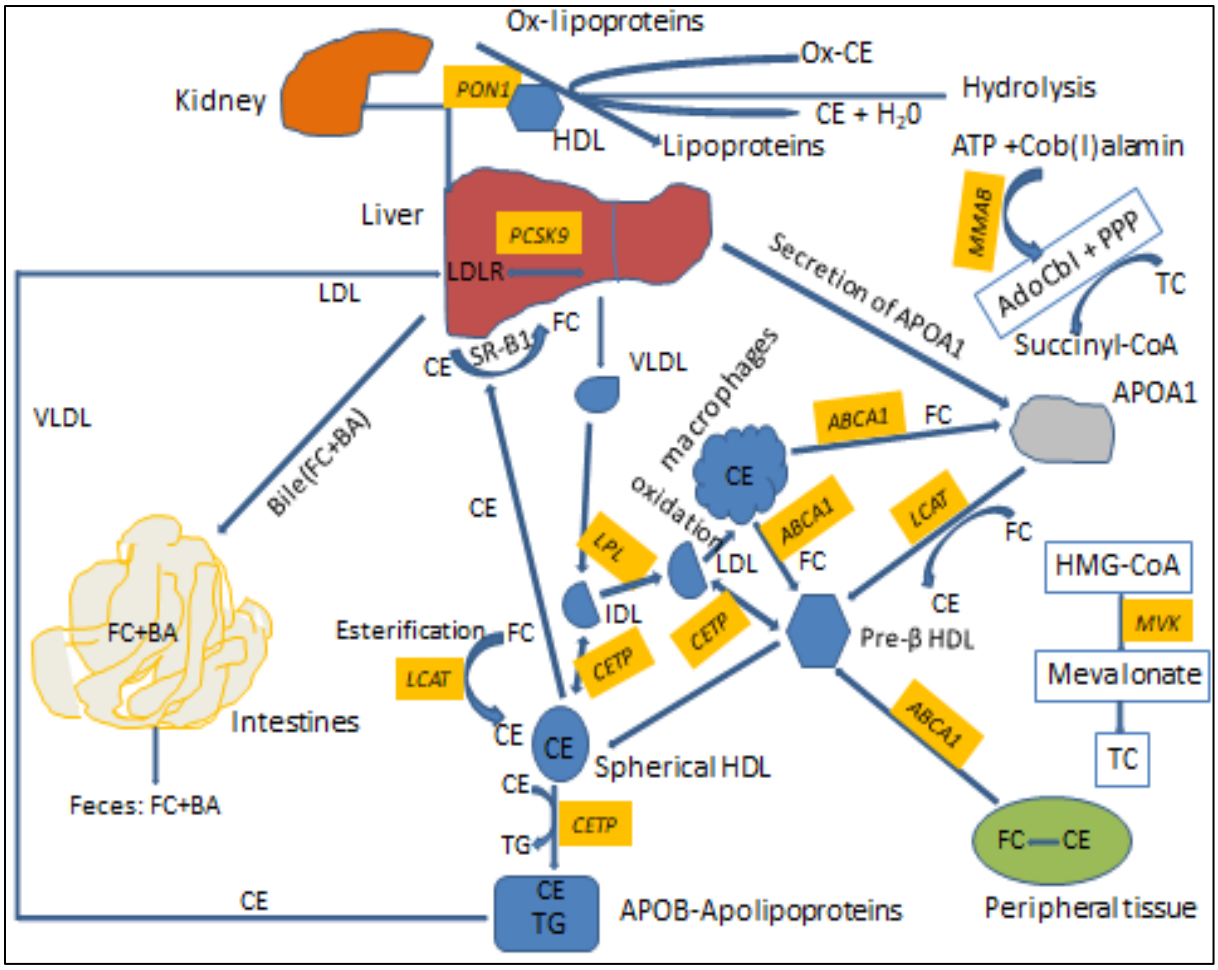

Figure 1.1: Diagrammatic representation of different lipid metabolic pathways and their associated genes. LDLR: low density lipoprotein receptor; SR-B1: scavenger receptor class B type 1; Ox-CE: oxygenated-cholesteryl ester; ABCG1: ATP-binding cassette G1; BA: bile acids; FC: free cholesterol; TC: total cholesterol; ATP: adenosine triphosphate; AdoCb1: adenosyl cobalamin; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme-A; APOA1: apolipoprotein A1. Selected candidate genes in yellow colour

1.9. Influence of the interplay between genetic and environmental factors on lipid levels

There are reports of statistically significant estimates of heritability for all plasma lipid traits in Caucasians ($p<0.0001$), Hispanics ($p<0.001$) and for HDL-C, LDL-C and TC ($p<0.001$) in African Americans (Malhotra and Wolford, 2005) and these estimates are shown to be over 50% in several studies (Mathias *et al.*, 2003; Pietiläinen *et al.*, 2009; Liu *et al.*, 2018). Some of the missing heritability in lipid traits may be accounted for by gene-environment interactions (GxE)

(Cole *et al*., 2015). Studies have shown that sociodemographic factors (age, gender, ethnicity, SES), body composition (BMI), alcohol intake, smoking, diet and exercise interact with genetic variants to influence lipid levels.

For example, a study in 3495 Chinese men in the presence of alcohol intake (1unit/day) GG carriers had increase in triglycerides, whereas AA/AG carriers of the aldehyde dehydrogenase 2 [*ALDH2*) (rs671)] variant had decrease in triglycerides (Tan *et al.*, 2012). Similarly it has been demonstrated in 765 non-drinkers and 567 drinkers in China that alcohol can modify the effect of *PSCK9* polymorphisms on serum TC and LDL-C. Compared to subjects with AG genotype, the subjects with AA genotypes had lower serum TC and LDL-C levels in drinkers than in non-drinkers (Aung *et al.*, 2013). An earlier study in Chinese adult men and women reported the effect of the interaction of three *APOA5* haplotypes (C-G-G, T-A-G and C-G-T) and alcohol consumption on serum lipid levels. The -1131T>C genotypes were positively associated with TG and, c.553G>T and c.457G>A genotypes positively associated with HDL-C levels in drinkers. In non-drinkers, -1131T>C genotypes were positively associated with TC, TG LDL-C levels; c.553G>T genotypes were positively associated with TC, TG, HDL-C and LDL-C while c.457G>A genotypes positively associated with TG and LDL-C levels (Yin *et al.*, 2011).

Smoking interaction with minor alleles of ATP binding cassette subfamily G member 5/8 (*ABCG5/G8*) multiple variants in Chinese men was significantly associated with reduced HDL-C (Junyent *et al.*, 2009). Another study involving a Spanish population of unrelated men and women indicated that *APOE* (S447X) and *LPL* (Sstl) variants significantly interacted with smoking in both men and women to increase HDL-C levels (Corella *et al.*, 2002). In a study involving 1123 European individuals TT alleles of endothelial lipase gene [*LIPG* (rs6507913)] variants in femens were associated with lower HDL-C levels in the presence of sedentary behavior (Smith *et al.*, 2009). The interactive effect of the G allele (AG and GG) of *LPL* rs326 and high-carbohydrate and low-fat diets on plasma lipids and apolipoproteins in healthy Chinese youth further supports the gene-environment interplay on lipid levels. Compared to the AA genotypes, men with the AG and AG genotypes of *LPL* rs326 had increased levels of HDL-C (Zhu *et al.*, 2014). This is in consonance with a study in black and white South African women which showed that dietary fat intake modulated the effect of *IL-6* variants (−174 G>C, IVS3 +281 G>T and IVS4 +869 A>G) on serum lipids. In white women who consume omega-3 intake, those with the IVS3 +281 T allele had lower TG levels. In black women, with increasing

total fat intake, triglycerides and TC:HDL-C ratio increased in those with the IVS4 +869 G allele (Joffe *et al.*, 2014).

A study in 493 men and 293 women in Nigeria has shown that the interaction of *APOH* polymorphisms with SES was associated with higher TC levels (Kamboh *et al.*, 1999). A sub-study of the third survey of the school-based surveillance system christened ‘’Childhood and Adolescent Surveillance and Prevention of Adult Non-Communicable disease (CASPIAN III)’’ showed that compared to the AA, AG/GG genotypes in rs1801177 in the *LPL* gene interacted with BMI and birth weight to significantly reduce HDL-C concentration (Askari *et al.*, 2016). This is in tune with a study in China, in twins which documented the association between adiposity (WC and BMI) and serum lipid profiles (LDL-C, TC, HDL-C and TG). The body composition measures were positively associated with all the lipid parameters, except HDL-C with which they were negatively associated. Shared genetic and twin-specific environmental factors contributed to these associations (Liao *et al.*, 2015). Another twin study indicated that increase in LDL-C, TG and TC were influenced mainly by genetic factors whereas decrease in HDL-C was influenced by both genetic and environmental factors (Zhang *et al.*, 2010).

It can therefore be concluded that little evidence-based information on the effect of gene-environment interaction on lipids in African populations exists. Most of the results are from Americans, Europeans, Asians and African-American populations.

The limited findings on gene-environment influence on lipids in Nigerians (Kamboh *et al.*, 1999) and South African women (Joffe *et al.*, 2014) do however support a role for gene-environment interactions as an important factor in influencing blood lipid levels in African populations. It is therefore important that further work is done with large sample sizes to evaluate the effect of the gene-environment interplay on blood lipid levels in black Africans.

1.10. Study rationale

Cardiovascular diseases are a major cause of mortality in sub-Saharan Africa with one of the main risk factors being lipid level abnormalities. There is well documented evidence of the influences of genetic and environmental factors on serum lipid profiles in non-indigenous African populations. Results of studies show differences in the genetic determinants of lipids between populations of non-African and African Americans. This suggests possible differences in genetic determinants between indigenous African and non-African populations. Few studies

on indigenous African populations have evaluated the influence of both genetic variants and environmental factors on lipid levels and how these interact to influence lipid levels. Furthermore these studies have mainly been conducted in urban settings.

The paucity of data on both genetic and environmental determinants of lipid profiles in African populations makes this study important. With a gradual increase in lipid levels, more specifically LDL-C and TC in rural African dwellers it is important to investigate the factors associated with this trend. This makes the current study in Ghana relevant as it will provide new insight on the influence of genetic and environmental factors and their interaction on lipid levels in black African adults, particularly in rural communities.

1.11. Study aim and objectives

Hypothesis

Environmental factors and genetic variations in the *CETP*, *LCAT*, *PON1*, *ABCA1*, *LPL*, *PCSK9*, *MMAB* and *MVK* genes are associated with blood lipid profiles in black Africans and interactions between these environmental and genetic factors further modulate lipid levels.

Aim

To investigate the genetic and environmental influences on blood lipid profiles in a West African adult population and to determine if gene-environment interactions occur.

Specific objectives

The aim will be achieved through the following specific objectives:

1. To determine the influence of demographic, anthropometric and behavioural factors on lipid profiles
2. To perform genetic-association studies of variants in eight genes (*CETP*, *LCAT*, *PON1*, *ABCA1*, *LPL*, *PCSK9*, *MMAB* and *MVK* genes) with lipid profiles (HDL-C, LDL-C, TG and TC) in an African study population
3. To determine the effect of gene-environment interaction on lipid profiles in the study population.

The outlay of the thesis is in the ‘divided block’ format. The investigation of each of the study objectives is covered by each respective results chapter below in a publication format and the methods used are discussed in each chapter.

CHAPTER 2: THE BURDEN OF DYSLIPIDAEMIA AND FACTORS ASSOCIATED WITH LIPID LEVELS AMONG ADULTS IN RURAL NORTHERN GHANA: AN AWI-GEN SUB-STUDY

2.1. Introduction

Dyslipidaemia is a primary risk factor for cardiometabolic diseases (CMDs) which caused over 17 million deaths globally in 2015, an increase of 12.5% from 2005 (Wang *et al.*, 2016). The contribution of dyslipidaemia to CMDs is evident from several longitudinal studies which have demonstrated the association of high levels of low density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG) and low levels of high density lipoprotein cholesterol (HDL-C) with cardiovascular disease (CVD) (Bansal *et al.*, 2007; Langsted *et al*., 2008; D'Agostino *et al.*, 2013; Reinikainen *et al.*, 2015). While dyslipidemia is progressively declining in developed and high-income countries, the same is not observed in middle- and low-income countries. Thus, it has been shown that TC levels decreased by 0.2mmol/l from the 1980s to 2008 in high-income countries, but declined by only 0.08-0.09mmol/l in middle- and low-income countries (Anand and Yusuf, 2011).

Serum lipid levels are reported to be influenced by anthropometric, demographic, environmental and genetic factors (Oladapo *et al.*, 2010; Dumitrescu *et al.*, 2011; Bentley *et al.*, 2014; Li *et al.*, 2016). Consequently, the burden of dyslipidaemia in sub-Saharan Africa has been attributed mainly to urbanization which is associated with environmental, behavioural and socio-cultural changes (BeLue *et al.*, 2009; Adediran *et al.*, 2012). In Ghana, previous studies have examined the burden of, and the factors associated with, dyslipidemia in mainly urban and semi-urban communities but few have evaluated the factors associated with serum lipid levels in rural settlements (Micah and Nkum, 2012; Asare-Anane *et al.*, 2013; Kodaman *et al.*, 2016; Vuvor *et al.*, 2016; Lokpo *et al.*, 2017). To date only one study has been conducted on lipid modulating factors in a rural settlement in Ghana but only the effect of anthropometric parameters was evaluated (Lokpo *et al.*, 2017). In rural communities in Northern Ghana where agriculture is the mainstay of the population (Binka *et al.*, 1999) pesticide use is common. Findings elsewhere

have associated pesticide use with increased lipid levels but this has not been evaluated to date in rural Ghana (Ntzani *et al.*, 2013; Arrebola *et al.*, 2014).

Just as urban settings in sub-Saharan Africa are experiencing a rising burden of dyslipidaemia and its adverse health consequences, rural areas are also facing the same challenge (Okaka and Eiya, 2013; Alsheikh-Ali *et al.*, 2014; Asiki *et al.*, 2015). Treatment and control of dyslipidaemia in resource-poor settings is expensive (Bovet *et al.*, 2006) and will require innovative approaches for prevention, diagnosis and treatment. Measuring the prevalence and understanding the factors associated with dyslipidaemia in rural settings will assist in channeling scarce resources toward its prevention. This study therefore determined the burden of dyslipidaemia and evaluated how anthropometric, demographic and environmental factors are associated with serum lipid levels among adults in rural Northern Ghanaian communities.

2.2. Methods

2.2.1. Study area

This study was carried out in the two Kassena-Nankana districts of north-eastern Ghana. The study area lies between latitude 10.30' and 11.10' north and longitude 1.1' west, and covers a total land area of 1675km^2, bordering northwards along the Ghana-Burkina Faso border (Figure 2.1). The area is characterized by Guinea Savannah vegetation dominated by semi-arid conditions and vast grassland integrated with short trees. The districts form the coverage area of the Navrongo Health and Demographic Surveillance System (NHDSS) which is implemented by the Navrongo Health Research Centre (NHRC) (Oduro *et al.*, 2012). The study area is typical of many rural areas in sub-Saharan Africa where agriculture is the mainstay of the local economy with about 90% of the people being subsistence farmers. Poverty levels are high due to insufficient agricultural production resulting from a short rainy season and prolonged dry season (Binka *et al.*, 1999). The major ethnic groups in the area are the Kassena and Nankana, with several minority groups including the Bulsas (Oduro *et al.*, 2012).

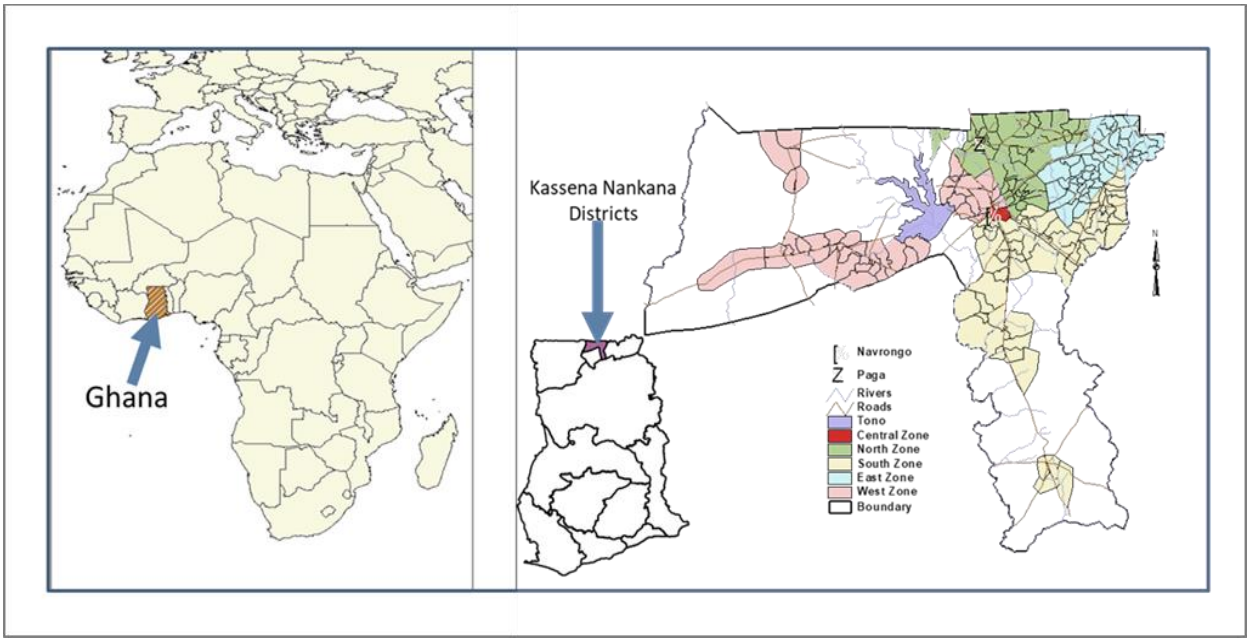

Figure 2.1: Maps showing Ghana in Africa, Upper east region in Ghana and the Navrongo HDSS covering the Kassena-Nankana districts of the Upper east region

2.2.2. Study design and population

This population based cross-sectional study was conducted as part of the Africa Wits-INDEPTH Partnership for Genomic studies (AWI-Gen) project under the broader Human Heredity and Health in Africa (H3Africa) Initiative (Michele Ramsay *et al.*, 2016). Participants were recruited from February to October 2015 in the west and east zones of the NHDSS area. These zones which are mainly Kassena and Nankana speaking communities respectively were purposefully selected for this study. A list of participants in the age range of 40 to 60 years was generated using the NHDSS database from each of the zones. A combined sample size of 2000 participants, plus 10% for non-response or refusal to participate, of roughly equal gender ratio and with fairly equal representation from the major ethnic groups, was randomly selected. Of this number, 1050 participants were sampled from the east and 1150 participants were sampled from the west zones. Individuals who were resident within the study area for at least 10 years were recruited into the study after witnessed informed consent was obtained. Pregnant women were excluded from the study (Ramsay *et al.*, 2016). A total of 2016 out of the 2200 sampled participants were recruited into the study. Participants who had no data for one or more of the investigated variables were excluded from the analytical data set for this paper. A sample size of 1839 comprising 993 women and 864 men were involved in these analyses.

2.2.3. Recruitment procedure

A day prior to participant enrolment a field worker visited households of sampled potential participants, explained the study to them individually, and invited each person to fast overnight and go to a recruitment site the next day. On the day of recruitment the potential participants at the recruitment site were sensitized and engaged on the project. They were taken through individual consent and those who agreed by way of informed thumbprint consent to participate in the project were taken through the recruitment process (see Figure 2.2).

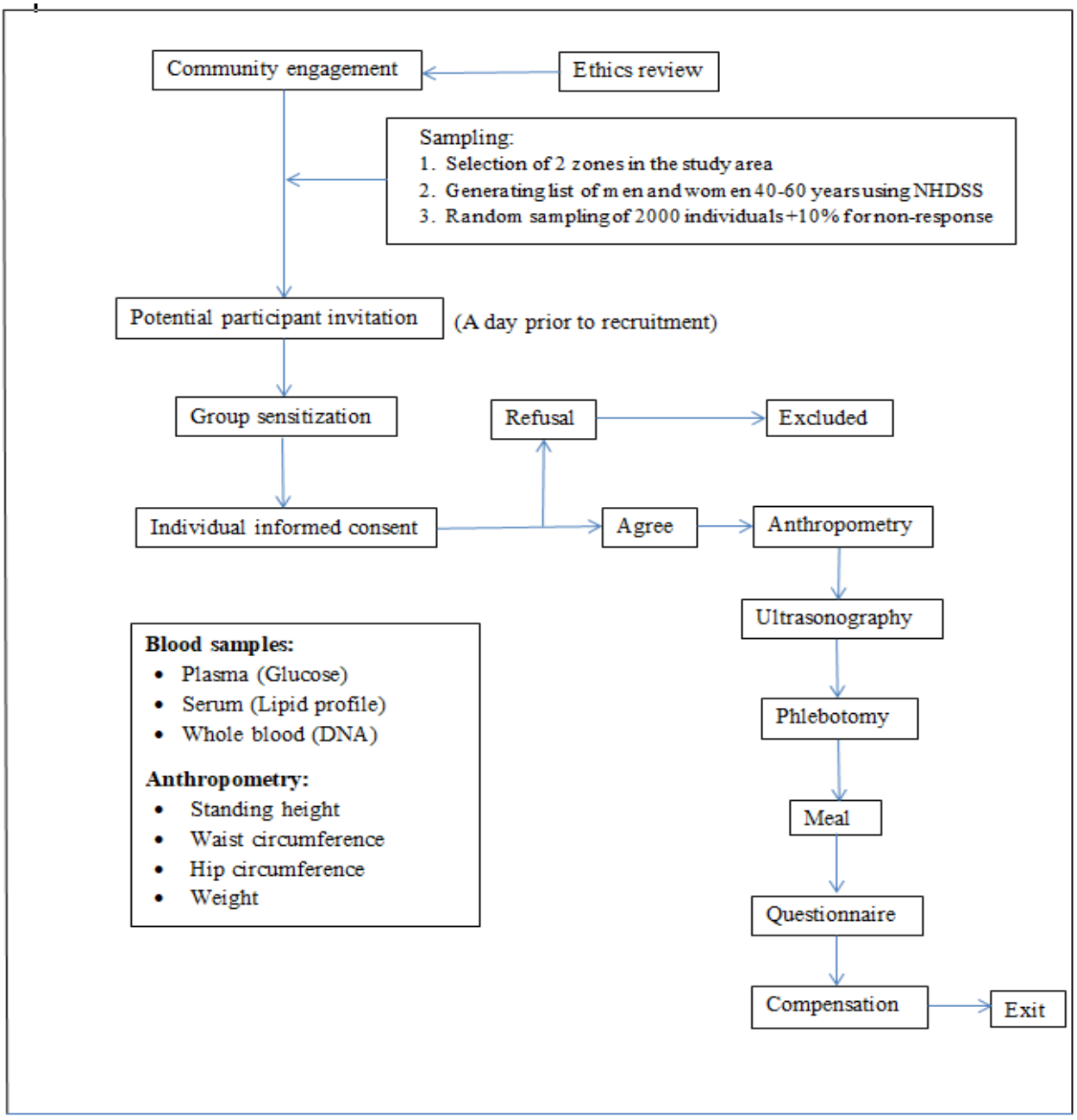

Figure 2.2: Recruitment procedure of study participants

2.2.4. Data collection

Data on socio-demographic, anthropometric and behavioural factors were captured on a paper questionnaire (Ramsay *et al.*, 2016) and uploaded into the Research Electronic Data Capture (REDCap) platform (Harris *et al.*, 2009). All participants were assigned a unique individual identification code on recruitment to ensure that both their identity, and data captured were anonymized. Data quality control included the checking of 10% of the entries for accurate data capture.

2.2.5. Anthropometric, blood pressure and body adipose tissue measurements

Standing height and weight of each participant were measured without shoes and in light clothes using a Harpenden stadiometer (Holtain, Crymych, Wales) fixed to the wall and a digital calibrated weighing scale, respectively. Waist and hip circumference were measured using a non-stretch measuring tape (Seca GmbH& Co. KG, Hamburg, Germany) according to the guidelines of the WHO 2008 report on waist and hip circumference (WHO, 2008). Body mass index (BMI), calculated as weight in kg/ height in m^2, was categorized as underweight: <18.5kg/m^2, normal weight:18.5- 24.9kg/m^2, overweight: 25.0- 29.9kg/m^2 and obese: ≥30kg/m^2 according to WHO recommendations (WHO, 1997).

Visceral and abdominal subcutaneous fat tissue thicknesses were measured twice using a LOGIQ e ultrasound system with a 2-5.5 MHz 4C-RS curved transducer (GE, Healthcare, CT, USA) and the mean taken. The visceral fat thickness was defined as the distance in centimeters (cm) from the peritoneum to the vertebral bodies and subcutaneous fat thickness as the depth in cm from the skin to the linea alba. In order to visualize the relevant anatomical structures the scan depth was set at 15 cm for visceral fat and 9 cm for subcutaneous fat. The site for both measurements was where the xyphoid line and waist circumference meet.

Blood pressure was measured using a digital sphygmomanometer (Omron M6, Omron, Kyoto, Japan). The participant was seated on a chair and the feet firmly rested on the floor. The measurement was taken from the left hand which rested on a desk with the antecubital fossa level with the heart and palm facing upwards. The measurements were repeated twice more, with two minutes between each interval. The mean of the last two measurements were used to calculate the systolic blood pressure (SBP) and diastolic pressure (DBP) of the participant. Hypertension

was defined as SBP>140mmHg and/or DBP>90mmHg and/or self-reported controlled treatment using hypertensive medication (Chobanian *et al*., 2003).

2.2.6. Lipid and biomarker analysis

Overnight fasting serum HDL-C, LDL-C, TG and TC were measured using an automated chemistry analyzer (Randox RX Daytona+, Crumlin, Northern Ireland) at the University of the Witwatersrand Developmental Pathway for Health Research Unit (DHPRU), Chris Hani Baragwanath Hospital, Soweto, South Africa.

The principle behind cholesterol assay involved enzymatic hydrolysis of cholesterol ester which resulted in the formation of cholesterol, the oxidation of which yielded hydrogen peroxide. This reacted with 4-aminoantipyrine in the presence of phenol and peroxidase to produce a quinone dye whose intensity is proportional to the TC concentration (Trinder, 1969). The reaction steps for the assay are as follows:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Chlesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$TC + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_20_2 + \text{4-Aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_20$$

The principle of the HDL-C and LDL-C assays consisted of two reaction steps (Siedel *et al*., 1983). The first was the elimination of chylomicrons, VLDL-C and LDL-C by cholesterol cholesterase, cholesterol oxidase and subsequently by catalase as follows:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Chlesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_20_2 \xrightarrow{\text{Catalase}} 2H_20 + O_{2.}$$

The second step was the specific measurement of HDL-C or LDL-C.

$$2H_20_2 + \text{4-Aminoantipyrine} + \text{HDAOS} \xrightarrow{\text{Peroxidase}} \text{Quinone imine dye} + 4H_20$$

where HDAOS is N-(2-hydroxy-3-sulfonyl)-3,5-dimethoxyaniline and 4-AA is 4-Aminoantipyrine.

The intensity of the quinone imine dye, measured at 600nm, was proportional to the HDL-C OR LDL-C concentration in the sample. Catalase was inhibited in the second reaction step by sodium azide.

The determination of TG concentration was based on the principle of its enzymatic hydrolysis with lipases resulting subsequently in the formation of a quinone dye whose intensity was proportional to the TG concentration (Rifai *et al*., 2001)

$$TG + H_2O \xrightarrow{\text{Lipases}} \text{Glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerolphosphate oxidase}} \text{Dihydroxyacetone + phosphate} + H_20_2$$

$$2H_20_2 + \text{4-Aminophenazone + 4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + HCl} + 4H_20.$$

A random selection of 150 samples was run in duplicates for glucose and all the lipids to ascertain the coefficients of variation (CVs) for these measurements. The CVs for glucose was 2.3% while the total CVs for the lipids were as follows: HDL-C<5%, LDL-C<6%, TC<4% and TG<10% respectively. Three control levels each were run for glucose and each of the lipid parameters. The CVs for the lipids are shown in Appendix D1- D4.

Dyslipidaemia was defined as follows: high TC (>5.0mmol/l), high LDL-C (>3.0mmol/l), high TG (>1.7mmol/l), low HDL-C (<1.0mml/l for men and <1.2mmol/l for women) (Graham *et al.*, 2007). Participants needing treatment for hyperlipidaemia was defined according to the ATP III guidelines ('Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol', 2002). High blood glucose was defined as fasting blood glucose ≥7.0mmol/l (American Diabetes Association, 2015).

2.2.7. Socio-demographic and lifestyle variables

Self-reported data on ethnicity, marital status, employment, education, alcohol and tobacco use, household amenities, physical activity and pesticide exposure were categorized using the definitions described below.

Ethnicity was defined by self-reported ethnic origin or ancestral lineage. Marital status: a participant who was staying with a partner for at least a year during the period of recruitment was considered currently married. Employment status: a person, who reported to be involved in any form of employment, whether formal or informal, was considered employed. Education: this was defined by self-reported attainment of formal education. Alcohol consumption: data on alcohol consumption was collected using the CAGE questionnaire (Mayfield, Mcleod and Hall, 1974) that was adapted and incorporated into the main AWI-Gen questionnaire. A person was defined as having a current problematic drinking pattern if they reported 'yes' to more than two of the following: they felt they should cut down on their drinking, felt bad or guilty about their drinking, people have annoyed them by criticizing their drinking, and ever had an alcoholic drink first thing in the morning to steady their nerves or get rid of a hangover. Current non-problematic drinking pattern was defined as the drinking of alcohol by a person who took alcohol but reported affirmative to less than three of the above listed attributes. Tobacco use was defined as present or past consumption of cigarettes, pipes or smokeless tobacco.

Socioeconomic status: this was assessed using the INDEPTH Health Equity tool which is an asset index generated by using principal component analysis to combine data on household possessions (http://indepth-network.org/resources/toolshttp://indepth-network.org/resources/indepth-health-equity-tool-measuring-socio-economic-status). The household assets were first broken down into categorical variables which were further converted into weights and principal components. The weights of the first principal component were used to develop an index from which scores of socioeconomic status of the households were derived. These scores were divided into quintiles with the first quintile being the poorest and the fifth the least poor. Physical activity was assessed using the Global Physical Activity Questionnaire (GPAQ) (Bull *et al*., 2009) which was incorporated into the main AWI-Gen questionnaire. Low-moderate to vigorous-intensity physical activity (MVPA) was calculated as minutes of physical activity per week (min/week). Low MVPA was defined as moderate to vigorous physical activity of two hours or less within a week. Pesticide exposure was defined as self-reporting of current work with pesticides or living close to a farm where pesticide was being used. Sleep duration was defined as the self-reported number of hours slept per night. Vegetable and fruit servings were estimated from self-reported quantities consumed per day using a serving size card. Malaria infection was defined as self-reported malaria fever within the past month. Menopausal status was categorized as follows: pre-menopausal status as having regular periods; menopausal

transition as having irregular periods within the past 12 months; post-menopausal status as having no periods within the past 12 months (Harlow *et al.*, 2012).

2.2.8. Ethical approval

The study was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M151050) (Appendix C1) and the Navrongo Institutional Review Board (ID No: NHRCIRB178) (Appendix C2) and was part of the H3Africa AWI-Gen project which was itself approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M121029) (Appendix C3). Community engagement was carried out prior to commencement of the study and signed or thump printed informed consent was obtained from each participant before being enrolled into the study.

2.2.9. Statistical analyses

All statistical analyses were performed using STATA 14.2 (StataCorp, College Station, Texas, 77845, US). All continuous variables were presented as means with standard deviations (SD) and compared among men and women using Student's t-test. Lipid levels were compared across age categories using one-way analysis of variance (ANOVA). Categorical variables were presented as percentages and compared between men and women using Pearson's χ^2 test. Awareness of dyslipidaemia status was calculated using the number of participants who reported that they had been told by a health professional that they had high cholesterol. Normality of lipids was checked using Kruskal Wallis test. Lipid levels were skewed and log-transformed to an approximate normal distribution (Appendix E) and gender stratified multiple linear regression analyses were used to determine associations of these lipid species with the selected variables measured in the study. Variables with a 20% significance level ($p<0.2$) at the univariable level were included in a multivariable linear regression analyses. Multi-collinearity among variables was assessed using the variance inflation factor (VIF). All variables in the multivariable analyses had VIF<5 and were therefore all included in the multivariable analyses. Residuals were approximately normally distributed and omitted variable bias was ruled out ($p>0.05$).

2.3. Results

2.3.1. Characteristics of study participants

A comparison of socio-demographic, behavioural, cardiometabolic and anthropometric variables across genders are presented in Table 2.1. The study population was 1839 participants and

consisted of 54% women. The mean age (±SD) of the study participants was 51 ± 6 years. The majority ethno-linguistic groups were Kassena (51.93%) and Nankana (43.12%) with the remainder constituting the minority ethnic groups (Bulsa, Dagaati, Sisaala, Mampruga, Frafra, Hausa, and Akan). The population was mainly illiterate (69.87%) and a small proportion had either secondary (9.03%) or tertiary education (1.90%) with men constituting a greater proportion ($p<0.001$). Over half (62.91%) of the participants reported having some form of employment and more men were employed ($p=0.035$) compared to women. Similarly, more men were least poor according to household attributes and were currently married compared to women ($p<0.001$ for both).

There was no difference between men and women in reported average fruit servings per day ($p=0.293$) but reported vegetable servings per day were significantly higher in women ($p=0.006$) and vendor meals per week significantly higher in men ($p<0.001$). The MVPA of the study population was 34.55 hours/week with men being more physically active than women ($p<0.001$). The mean sleep duration per night for men was significantly lower than that for women ($p<0.001$). Though not presented in the table, malaria infection in the past month was not significantly different between men and women (16.54% and 16.84% respectively; $p=0.868$), and the prevalence of self-reported HIV positive cases in the study population was 0.93%. The mean fasting blood glucose of the study population was 4.55 ±0.82 mmol/l with that of women being significantly higher than that of men ($p<0.001$). The mean values of SBP and DBP of the study population were 124.06 ± 21.61 mmHg and 77.13 ± 12.72 mmHg, respectively with no significant difference between men and women. All the anthropometric indices were higher in women than men ($p<0.001$) except visceral fat which was higher in men ($p<0.001$).

Table 2.1: Gender comparison of socio-demographic variables, food intake, exercise level, sleep duration, fasting blood glucose levels, blood pressure measurements and anthropometric variables

Variables	Men (n = 846, 46%)	Women (n =993, 54%)	Total (N = 1839)	p-value (men versus women)
Age (years)	50 ± 6.0	52 ± 6.0	51 ± 6.0	<0.001
Ethnicity				
Kassena	439 (51.89)	516 (51.96)	955 (51.93)	<0.001
Nankana	392 (46.34)	401 (40.38)	793 (43.12)	
Minority ethnic groups	15 (1.77)	76 (7.65)	91 (4.95)	
Educational status				

No formal education	517 (61.11)	768 (77.34)	1285 (69.87)	
Primary	192 (22.70)	161 (16.21)	353 (19.20)	<0.001
Secondary	111 (13.12)	55 (5.54)	166 (9.03)	
Tertiary	26 (3.07)	9 (0.91)	35 (1.90)	
Employment status				
Unemployed	292 (34.52)	390 (39.27)	682 (37.09)	0.035
Employed	554 (65.48)	603 (60.73)	1157 (62.91)	
Marital status				
Currently married	717 (84.75)	632 (63.65)	1349 (73.36)	<0.001
Currently unmarried	129 (15.25)	361 (36.35)	490 (26.64)	
Household SES categories				
Poorest	129 (15.25)	204 (20.54)	333 (18.11)	<0.001
Very poor	139 (16.43)	192 (19.34)	331 (18.00)	
Poor	154 (18.20)	194 (19.54)	348 (18.92)	
Less poor	203 (24.00)	227 (22.86)	430 (23.38)	
Least poor	221 (26.12)	176 (17.72)	397 (21.59)	
Fruit Intake (servings/day)	1.01 ± 1.63	1.10 ± 1.69	1.06 ± 1.63	0.293
Vegetable Intake (servings/day)	3.43 ± 1.46	3.24 ± 1.51	3.33 ± 1.49	0.006
Vendor meals (times/week)	1.17 ± 1.68	0.79 ± 1.31	0.97 ± 1.50	<0.001
MVPA (hours/week)	40.07 ± 28.78	29.84 ± 27.72	34.55 ± 28.66	<0.001
Sleeping (hours/night)	7.71 ± 1.34	8.28 ± 1.32	8.02 ± 1.36	<0.001
Fasting blood glucose (mmol/l)	4.47 ± 0.76	4.61 ± 0.86	4.55 ± 0.82	<0.001
SBP (mmHg)	124.97 ± 20.44	123.28 ± 22.55	124.06 ± 21.61	0.094
DBP (mmol/l)	77.03 ± 12.86	77.12 ± 12.59	77.13 ± 12.72	0.760
BMI (kg/m^2)	20.87 ± 3.15	22.28 ± 3.85	21.63 ± 3.61	<0.001
Hip circumference (cm)	84.1 ±7.9	89.3 ±9.9	86.9 ± 9.4	<0.001
Waist circumference (cm)	73.3 ±8.1	76.8 ±9.5	75.2 ±9.1	<0.001
Visceral fat (mm)	4.18 ± 1.21	3.54 ± 1.12	3.83 ± 1.20	<0.001
Subcutaneous fat (mm)	0.78 ± 0.38	1.15 ± 0.54	0.98 ± 0.51	<0.001

Data is given as mean ± SD or n (%)

2.3.2. Mean lipid levels stratified by gender and age category in the study population

The mean HDL-C, LDL-C, TC and TG were 1.14 ± 0.38 mmol/l, 1.73 ± 0.78 mmol/l, 3.23 ± 0.92 mmol/l and 0.64 ± 0.45 mmol/l respectively. There were no significant differences in measured lipid levels between men and women except for HDL-C level which was significantly higher in men than in women (p=0.001) (Figure 2.3).

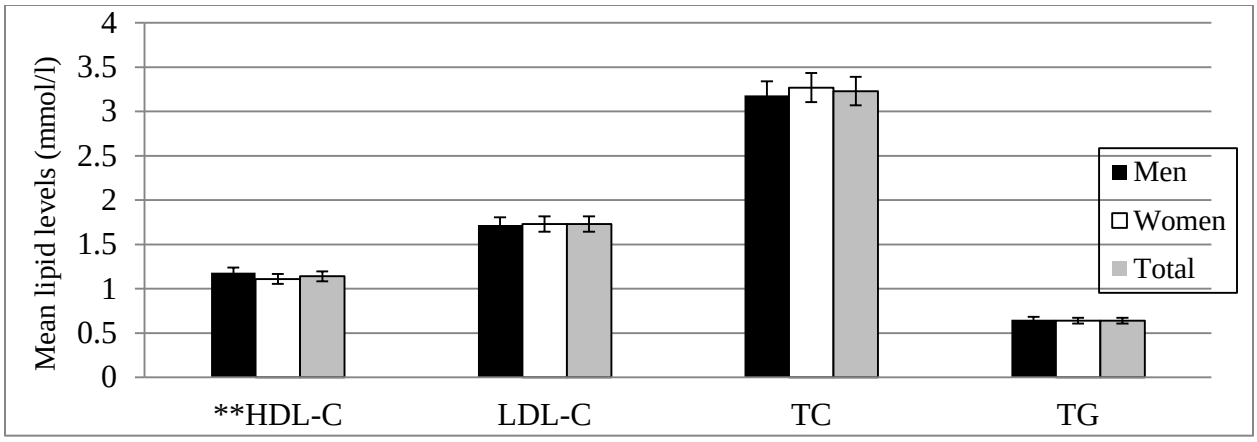

Figure 2.3: Mean lipid levels among men and women and in the total population; **p<0.005 men versus women

The mean lipid levels were stratified among men and women according to the following age categories: 40-44 years, 45-49 years, 50-54 years and 55-60 years (Table 2.2). Only in femens were correlations observed between age and lipids, in this case positive correlations of age with both TG (p=0.006) and TC (p=0.001). Significant gender differences were noted for HDL at age categories 45-49 and 55-60 years, with values higher in mens than femens. Mens had significantly lower TC and TG than femens in the age group 55-60 years.

Table 2.2: Mean lipid levels among men and women of the study population stratified by age category

Lipids	Gender	40-44 years	45-49 years	50-54 years	55-60 years	R-value (p-value) for age versus lipid[1]
HDL-C	Men	1.11	1.24**	1.15	1.19**	0.04 (0.13)
	Women	1.14	1.10	1.11	1.10	-0.0001 (0.99)
LDL-C	Men	1.65	1.82	1.67	1.72	0.02 (0.62)
	Women	1.66	1.73	1.66	1.80	0.05 (0.09)
TC	Men	3.16	3.29	3.13	3.10**	-0.02 (0.59)
	Women	3.05	3.22	3.29	3.37	0.12 (0.001)
TG	Men	0.62	0.69	0.68	0.60*	-0.02 (0.59)
	Women	0.61	0.62	0.62	0.67	0.09 (0.006)

Correlation performed using Pearson analysis; *p<0.05, **p<0.005 versus women

2.3.3. Prevalence of dyslipidaemia and other CVD risk factors in the study population stratified by gender

The prevalence of dyslipidaemia and other CVD risk factors among men and women is shown in Table 2.3. Smoking was far more common among men than women (p<0.001) but the use of smokeless tobacco did not differ between men and women (p=0.997). Alcohol consumption was very prevalent with higher levels in mens than femens (p<0.001). Pesticide exposure was more common among men than women (p<0.001). Women were less physically active compared to men (p<0.001). The prevalence of overweight and obesity in the study population was 10.82% and 2.66% respectively with women being more overweight and obese than men (p<0.001). The prevalence of high blood glucose was very low (0.54%) with no difference between men and women (p=0.309). Similarly, there was no difference in the prevalence of high blood pressure between men and women (p=0.464). The prevalence of hypercholesterolemia, hypertriglyceridaemia and elevated LDL-C in the total population was 4.02%, 2.12% and 5.55%, respectively. There were no significant gender differences for any of these sub-types of dyslipidaemia. However, the prevalence of low HDL-C concentration in the population was 60.30% and differed (p=0.005) between men (56.86%) and women (63.24%). Awareness of dyslipidemia in this population was low at 0.38% and did not differ between women and men (p=0.895). The proportion of the population needing treatment for hyperlipidaemia was also low (0.76%) with more men than women requiring treatment, but this difference just failed to reach statistical significance (p=0.055).

Table 2.3: Comparison across genders of the prevalence of smoking, alcohol intake, pesticide exposure, low physical activity, obesity, high blood sugar, high blood pressure, dyslipidaemia and required therapy for dyslipidaemia

Variable	Men (n=846, 46%)	Women (n=993, 54%)	Total (N=1839)	p-value
Behavioural factors				
Current smoker	185 (21.87)	14 (1.41)	199 (10.82)	<0.001
Former/current smokeless tobacco use	86 (10.17)	101 (10.17)	187 (10.17)	0.997
Former/current alcohol drinker	782 (92.43)	777 (78.25)	1559 (84.77)	<0.001
Pesticide exposure	509 (60.17)	497 (50.05)	1006 (54.70)	<0.001
Low physical activity	57 (6.74)	133 (13.39)	190 (10.33)	<0.001
Body weight				
Underweight	157 (18.56)	120 (12.08)	277 (15.06)	
Normal weight	631 (74.59)	683 (68.78)	1314 (71.45)	
Overweight	50 (5.91)	149 (15.01)	199 (10.82)	<0.001
Obese	8 (0.95)	41 (4.13)	49 (2.66)	

High fasting blood glucose	3 (0.35)	7 (0.70)	10 (0.54)	0.309
High blood pressure	190 (22.46)	209 (21.05)	399 (21.70)	0.464
Dyslipidaemia				
Low HDL-C	481 (56.86)	628 (63.24)	1109 (60.30)	0.005
High LDL-C	49 (5.79)	53 (5.34)	102 (5.55)	0.671
High TC	28 (3.31)	46 (4.63)	74 (4.02)	0.150
High TG	16 (1.89)	23 (2.32)	39 (2.12)	0.528
Self-reported dyslipidaemia	3 (0.35)	4 (0.40)	7 (0.38)	0.895
Needing therapy for dyslipidaemia	10 (1.18)	4 (0.40)	14 (0.76)	0.055

Data is given as n (%); low physical activity: ≤2 hours/week; underweight: <18.5kg/m^2, normal weight: 18.5-24.9kg/m^2, overweight: 25.0- 29.9kg/m^2; obese: ≥30kg/m^2; high blood pressure: SBP>140mmHg and/or DBP>90mmHg and/or self-reported controlled treatment using hypertensive medication; high TC: >5.0mmol/l; high LDL-C: >3.0mmol/l; high TG: >1.7mmol/l; low HDL-C: <1.0mml/l for men and <1.2mmol/l for women; high fasting blood glucose: ≥7.0mmol/l

2.3.4. Factors associated with lipid levels in mens and femens

The association of each of the study variables with LDL-C in both genders is illustrated in Table 2.4. In the univariate linear regression models formal education, high SES, BMI, waist circumference, hip circumference, visceral and subcutaneous fat were each associated with LDL-C among women. Age, formal education and waist circumference were associated with LDL-C in the multivariable regression model which explained only 8.2% (p<0.001) of the variance in LDL-C. In men the factors associated with LDL-C at the univariate level were formal education, high SES, vendor meals, sleep duration, BMI, waist circumference, hip circumference, visceral and subcutaneous fat. Only subcutaneous fat remained significant in the multivariable model which explained 5.1% (p<0.001) of the variance in LDL-C levels.

Table 2.4: Factors associated with LDL-C levels among men and women

Women				
	Univariate models		Multivariate model	
Variable	β-Coefficient (95%CI)	p-value	β-Coefficient (95%CI)	p-value
Age(years)	0.004 (-0.001, 0.009)	0.092	0.007 (0.002, 0.012)	0.004
Nankana ethnicity	-0.040 (-0.095, 0.016)	0.161	0.010 (-0.046, 0.065)	0.735
Some formal education[1]	0.131 (0.067, 0.196)	<0.001	0.095 (0.030, 0.160)	0.004
High SES[2]	0.135 (0.065, 0.206)	<0.001	0.026 (-0.049, 0.100)	0.500
Used smokeless tobacco	-0.077 (-0.167, 0.013)	0.092	-0.036 (-0.124, 0.053)	0.433
Vendor (meals/week)	0.018 (-0.003, 0.039)	0.085	0.013 (-0.007, 0.034)	0.191
Sleeping (hours/night)	-0.019 (-0.040, 0.001)	0.065	-0.010 (-0.030, 0.011)	0.359
BMI (kg/m^2)	-0.023 (0.016, 0.030)	<0.001	-0.009 (-0.023, 0.005)	0.201
Waist circumference (cm)	0.122 (0.094, 0.149)	<0.001	0.116 (0.060, 0.172)	<0.001
Hip circumference (cm)	0.089 (0.062, 0.116)	<0.001	0.012 (-0.032, 0.055)	0.596
Visceral fat (mm)	0.057 (0.033, 0.081)	<0.001	0.001 (-0.027, 0.028)	0.978

Subcutaneous fat (mm)	0.174 (0.125, 0.223)	<0.001	0.046 (-0.028, 0.119)	0.222
Men				
	Univariate models		Multivariate model	
Variable	β-Coefficient(95%CI)	p-value	β-Coefficient(95%CI)	p-value
Some formal education[1]	0.072 (0.006, 0.138)	0.034	0.022 (-0.045, 0.090)	0.514
Employed	0.056 (-0.012, 0.124)	0.109	-0.001 (-0.073, 0.073)	0.998
Currently unmarried	-0.061 (-0.151, 0.029)	0.184	-0.014 (-0.104, 0.075)	0.747
High SES[2]	0.148 (0.075, 0.221)	<0.001	0.044 (-0.037, 0.125)	0.285
Past or current smoker[3]	-0.045 (-0.113, 0.023)	0.197	-0.004 (-0.048, 0.040)	0.860
Past or current drinker[4]	-0.113 (-0.235, 0.010)	0.071	-0.028 (-0.157, 0.100)	0.663
Vegetable (servings/day)	0.020 (-0.002, 0.042)	0.081	0.021 (-0.003, 0.044)	0.087
Vendor (meals/week)	0.021 (0.001, 0.040)	0.036	0.008 (-0.012, 0.028)	0.419
Sleeping (hours/night)	-0.034 (-0.058, -0.010)	0.006	-0.023 (-0.047, 0.002)	0.067
BMI (kg/m^2)	0.023 (0.013, 0.033)	<0.001	0.001 (-0.012, 0.014)	0.896
Waist circumference (cm)	0.114 (0.074, 0.153)	<0.001	0.045 (-0.011, 0.101)	0.117
Hip circumference (cm)	0.111 (0.071, 0.151)	<0.001	0.032 (-0.025, 0.088)	0.270
Visceral fat (mm)	0.034 (0.007, 0.061)	0.013	0.001 (-0.028, 0.030)	0.968
Subcutaneous fat (mm)	0.243 (0.159, 0.326)	<0.001	0.139 (0,040, 0.238)	0.006

CI: Confidence Interval; all lipid values were logged; [1]education was coded as some formal education versus no education; [2]SES was coded as those with highest versus those with lowest SES; [3]smoking status was coded as those who are current or past smokers versus those who never smoked; [4]alcohol intake was coded as those who had ever drunk alcohol versus those who had never drunk.

The principal modulators of HDL-C levels among men and women are shown in Table 2.5. High SES, past or current drinking and sleep duration were associated with HDL-C among women in the univariate analyses but only high SES and past or current drinking remained significant in the multivariable model. Only 2.3% (p<0.001) of the variance in HDL-C among women was explained by this model. In men, Nankana ethnicity, past or current smoking, past or current drinking and waist circumference were associated with HDL-C levels in the univariate analysis. Only past or current smoking remained associated with HDL-C in the multivariable model and explained only 0.95% (p=0.062) of the variance in HDL-C levels.

Table 2.5: Factors associated with HDL-C levels among men and women

Women				
	Univariate models		Multivariate model	
Variable	β-Coefficient (95%CI)	p-value	β-Coefficient (95%CI)	p-value
High SES[1]	0.077 (0.022, 0.132)	0.006	0.081 (0.025, 0.136)	0.004
Past or current smoker[2]	0.099 (-0.018, 0.216)	0.096	0.087 (-0.030, 0.203)	0.144
Used smokeless tobacco	0.060 (-0.009, 0.129)	0.090	0.061 (-0.008, 0.130)	0.084
Past or current drinker[3]	0.086 (0.035, 0.136)	0.001	0.084 (0.033, 0.134)	0.001
Sleeping (hours/night)	-0.016 (-0.032, -0.001)	0.044	-0.013 (-0.029, 0.003)	0.110
Men				
	Univariate models		Multivariate model	
Variable	β-Coefficient (95%CI)	p-value	β-Coefficient (95%CI)	p-value

Age(years)	0.004 (-0.001, 0.008)	0.126	0.002 (-0.003, 0.007)	0.463
Nankana ethnicity	0.054 (0.003, 0.106)	0.039	0.041 (-0.018, 0.100)	0.170
Past or current smoker[2]	0.081 (0.028, 0.135)	0.003	0.066 (0.009, 0.124)	0.024
Past or current drinker[3]	0.118 (0.021, 0.214)	0.017	0.052 (-0.053, 0.156)	0.334
Pesticide exposure	-0.043 (-0.095, 0.010)	0.112	-0.005 (-0.064, 0.055)	0.872
MVPA (hours/week)	0.001 (-0.001, 0.002)	0.153	0.002 (-0.001, 0.001)	0.796
Malaria in past month	-0.054 (-0.125, 0.017)	0.133	-0.042 (-0.114, 0.029)	0.243
BMI (kg/m^2)	-0.007 (-0.015, 0.001)	0.107	0.001 (-0.011, 0.013)	0.876
Waist circumference (cm)	-0.033 (-0.065, -0.001)	0.043	-0.029 (-0.074, 0.017)	0.215
Hip circumference (cm)	-0.027 (-0.060, 0.005)	0.102	0.006 (-0.040, 0.052)	0.800

CI: Confidence Interval; [1]SES was coded as those with highest versus those with lowest SES; [2]smoking status was coded as those who are current or past smokers versus those who never smoked; [3]alcohol intake was coded as those who had ever drunk alcohol versus those who had never drunk.

Table 2.6 shows the factors associated with TC levels among men and women. In the univariate regression models age, Nankana ethnicity, high SES, BMI, waist circumference, hip circumference and subcutaneous fat were each associated with TC level among women. However only age, Nankana ethnicity and subcutaneous fat remained significant in the multivariable regression model and these explained 4.9% ($p<0.001$) of the variance in TC. Among men SES, fruit consumption and BMI, were associated with TC concentration in the univariate models. Employment and BMI were significant in the multivariate analysis. This model explained 3.0% ($p<0.001$) of the variance in TC among men.

Table 2.6: Factors associated with TC among men and women

Women				
	Univariate models		Multivariate model	
Variable	β-Coefficient (95%CI)	p-value	β-Coefficient (95%CI)	p-value
Age(years)	0.006 (0.003, 0.009)	0.001	0.007 (0.003, 0.010)	<0.001
Nankana ethnicity	-0.086 (-0.126, -0.046)	<0.001	-0.069 (-0.109, -0.029)	0.001
Currently unmarried	0.035 (-0.006, 0.076)	0.095	0.025 (-0.016, 0.066)	0.233
High SES[1]	0.055 (0.004, 0.107)	0.035	0.027 (-0.027, 0.081)	0.325
Sleeping (hours/night)	-0.012 (-0.026, 0.003)	0.129	-0.008 (-0.023, 0.007)	0.280
BMI (kg/m^2)	0.013 (0.006, 0.020)	0.001	-0.009 (-0.019, 0.001)	0.083
Waist circumference (cm)	0.042 (0021, 0.062)	<0.001	0.014 (-0.026, 0.053)	0.493
Hip circumference (cm)	0.039 (0.020, 0.059)	<0.001	0.030 (-0.001, 0.062)	0.061
Subcutaneous fat (mm)	0.089 (0.053, 0.125)	<0.001	0.067 (0.013, 0.120)	0.015
Men				
	Univariate models		Multivariate model	
Variable	β-Coefficient (95%CI)	p-value	β-Coefficient(95%CI)	p-value
Employed	0.074 (0.026, 0.122)	0.109	0.070 (0.021, 0.118)	0.005
High SES[1]	0.063 (0.011, 0.115)	0.018	0.011 (-0.046, 0.068)	0.710
Used smokeless tobacco	-0.050 (-0.126, 0.026)	0.197	-0.048 (-0.124, 0.027)	0.210
Pesticide exposure	0.042 (-0.005, 0.089)	0.079	0.018 (-0.030, 0.066)	0.463
Fruit (servings/day)	0.021 (0.007, 0.035)	0.004	0.016 (0.002, 0.030)	0.059

BMI (kg/m^2)	0.013 (0.006, 0.020)	0.001	0.010 (0.001, 0.019)	0.043

CI: Confidence Interval; [1]SES was coded as those with highest versus those with lowest SES

Table 2.7 shows the factors associated with TG after stratifying by gender. Age, Nankana ethnicity, education, being unmarried, high SES, waist and hip circumferences, visceral and subcutaneous fat were all associated with TG in the univariate analyses among women. In the multivariate linear regression analysis age, being unmarried, waist and hip circumferences, visceral and subcutaneous adipose tissue thickness were significant. This model explained 12.0% ($p<0.001$) of the variance in TG levels. The factors associated with TG among men were Nankana ethnicity, high SES, use of smokeless tobacco, fruit consumption, waist circumference and visceral and subcutaneous fat thickness in the univariate analyses. In the multivariate analysis only Nankana ethnicity had a significant association with TG levels. This model accounted for 3.3% ($p<0.001$) of the variance in TG levels among men.

Table 2.7: Factors associated with TG among men and women

Women				
	Univariate models		Multivariate model	
Variable	β-Coefficient (95%CI)	p-value	β-Coefficient (95%CI)	p-value
Age(years)	0.007 (0.002, 0.012)	0.006	0.008 (0.003, 0.012)	0.002
Nankana ethnicity	-0.075 (-0.131, -0.018)	0.010	-0.013 (-0.071, 0.045)	0.668
Some formal education[1]	0.097 (0.031, 0.164)	0.004	0.046 (-0.020, 0.113)	0.173
Currently unmarried	0.111 (0.53, 0.168)	<0.001	0.095 (0.037, 0.152)	0.001
High SES[2]	0.088 (0.015, 0.160)	0.018	-0.010 (-0.085, 0.066)	0.802
Vegetable (servings/day)	-0.017 (-0.034, 0.003)	0.096	-0.012 (-0.030, 0.006)	0.188
Malaria in past month	0.072 (-0.004, 0.148)	0.062	0.055 (-0.017, 0.127)	0.135
MVPA (hours/week)	-0.001 (-0.002, 0.001)	0.090	-0.003 (-0.001, 0.001)	1.000
Waist circumference (cm)	0.125 (0.097, 0.153)	<0.001	0.094 (0.043, 0.144)	<0.001
Hip circumference (cm)	0.068 (0.041, 0.096)	<0.001	-0.053 (-0.094, -0.011)	0.014
Visceral fat (mm)	0.083 (0.059, 0.107)	<0.001	0.033 (0.005, 0.062)	0.022
Subcutaneous fat (mm)	0.238 (0.188, 0.287)	<0.001	0.137 (0.065, 0.209)	<0.001
Men				
	Univariate models		Multivariate model	
Variable	β-Coefficient (95%CI)	p-value	β-Coefficient (95%CI)	p-value
Nankana ethnicity	-0.119 (-0.174, -0.059)	<0.001	-0.084 (-0.147, -0.022)	0.008
High SES[2]	0.110 (0.041, 0.178)	0.002	0.041 (-0.032, 0.115)	0.270
Used smokeless tobacco	-0.109 (-0.209, -0.009)	0.032	-0.078 (-0.177, 0.022)	0.125
Fruit (servings/day)	0.025 (0.006, 0.043)	0.008	0.016 (-0.002, 0.035)	0.085
Vendor (meals/week)	0.015 (-0.003, 0.033)	0.095	0.007 (-0.011, 0.025)	0.433
MVPA (hours/week)	-0.001 (-0.001, 0.001)	0.090	-0.003 (-0.001, 0.002)	0.347
Waist circumference (cm)	0.059 (0.022, 0.096)	0.002	0.028 (-0.016, 0.072)	0.207
Visceral fat (mm)	0.029 (0.004, 0.054)	0.023	0.003 (-0.024, 0.030)	0.844
Subcutaneous fat (mm)	0.152 (0.074, 0.231)	<0.001	0.076 (-0.016, 0.167)	0.105

CI: Confidence Interval; [1]education was coded as some formal education versus no education; [2]SES was coded as those with highest versus those with lowest SES

2.3.5. Factors associated with lipid levels in total population

The association of factors with lipid levels in the combined population is shown in Table 2.8 for LDL-C and HDL-C. All factors associated with HDL-C and LDL-C levels in either women or men or both show the same association in the combined population. The only exception is sleep duration which was associated with none of the lipid levels in the gender stratified analyses but was associated with LDL-C in the combined analyses. Interestingly, men gender was associated with higher HDL-C levels in the univariate analysis, but this association was lost in the multivariable model (Table 2.8). This suggests that one of the variables in the multivariable analysis was confounding the gender effect. To isolate this variable we performed a univariate regression analysis of HDL-C with only gender included as an independent variable. We then systematically added each variable from the multivariable analysis into the model, one at a time, until we observed a variable that attenuated the gender effect to non-significance. The variable that did this was smoking. Smoking was more common in mens than femens (see Table 2.8) and correlated positively and strongly with HDL-C levels in both the univariate and multivariate analyses (Table 2.8). The relationship between smoking and HDL-C was investigated further to determine if other variables were modifying this association. The main variable chosen was alcohol intake, as this correlated with HDL-C in the univariate and multivariate models and was very prevalent in the population (see Table 2.3). In a univariate regression model for HDL-C, smoking correlated positively with the lipid in alcohol drinkers (β=0.031, p<0.001, n=1559), but in non-drinkers, the relationship was negative but not significant (β=-0.014, p=0.69, n=280).

Table 2.8: Factors associated with LDL-C and HDL-C levels in the total population

	LDL-C			
Variable	Univariate models		Multivariate model	
	β-Coefficient (95%CI)	*P* value	β-Coefficient (95%CI)	P value
Age(years)	0.003 (-0.001, 0.007)	0.105	0.006 (0.002, 0.009)	0.002
Some formal education[1]	0.092 (0.047, 0.138)	<0.001	0.059 (0.013, 0.106)	0.012
Employed	0.032 (-0.011, 0.076)	0.145	0.007 (-0.036, 0.050)	0.750
High SES[2]	0.138 (0.087, 0.188)	<0.001	0.043 (-0.011, 0.036)	0.118
Used smokeless tobacco	-0.060 (-0.129, 0.009)	0.090	-0.032 (-0.100, 0.036)	0.354
Past or current smoker[3]	-0.032 (-0.077, 0.013)	0.166	0.006 (-0.027, 0.039)	0.714
Vendor (meals/week)	0.018 (-0.005, 0.032)	0.009	0.012 (-0.002, 0.025)	0.099
MVPA (hours/week)	-0.001 (-0.001, 0.002)	0.105	-0.003 (-0.001, 0.008)	0.603
Sleeping (hours/night)	-0.023 (-0.039, -0.008)	0.003	-0.016 (-0.032, -0.001)	0.044
BMI (kg/m^2)	0.023 (0.017, 0.028)	<0.001	-0.003 (-0.012, 0.007)	0.543

Waist circumference (cm)	0.118 (0.094, 0.139)	<0.001	0.074 (0.035, 0.112)	<0.001
Hip circumference (cm)	0.093 (0.071, 0.115)	<0.001	0.022 (-0.012, 0.056)	0.211
Visceral fat (cm)	0.040 (0.022, 0.057)	<0.001	0.003 (-0.016, 0.022)	0.760
Subcutaneous fat (cm)	0.177 (0.137, 0.217)	<0.001	0.069 (0.012, 0.125)	0.017
HDL-C				
	Univariate models		Multivariate model	
Variables	β-Coefficient (95%CI)	*P* value	β-Coefficient (95%CI)	P value
Men gender	0.047 (0.014, 0.080)	0.005	-0.028 (-0.074, 0.017)	0.224
Nankana ethnicity	0.037 (0.004, 0.070)	0.027	0.023 (-0.012, 0.057)	0.119
High SES[2]	0.037 (-0.003, 0.077)	0.068	0.057 (0.015, 0.100)	0.008
Past or current smoker[3]	0.080 (0.045, 0.116)	<0.001	0.077 (0.030, 0.125)	0.001
Past or current drinker[4]	0.028 (0.011, 0.045)	0.001	0.080 (0.032, 0.128)	0.001
MVPA (hours/week)	0.001 (-0.001, 0.002)	0.153	0.003 (-0.007, 0.001)	0.535
Sleeping (hours/night)	-0.010 (-0.022, 0.002)	0.096	-0.007 (-0.019, 0.006)	0.304
BMI (kg/m^2)	-0.005 (-0.009, -0.001)	0.047	-0.001 (-0.008, 0.007)	0.840
Waist circumference (cm)	-0.019 (-0.037, -0.001)	0.041	-0.015 (-0.044, 0.015)	0.341
Hip circumference (cm)	-0.016 (-0.034, 0.001)	0.065	0.003 (-0.024, 0.031)	0.805

CI: Confidence Interval; [1]education was coded as some formal education versus no education; [2]SES was coded as those with highest versus those with lowest SES; [3]smoking status was coded as those who are current or past smokers versus those who never smoked; [4]alcohol intake was coded as those who had ever drunk alcohol versus those who had never drunk; R^2=0.067 (p<0.001) for the LDL-C model and R^2=0.019 (p<0.001) for the HDL-C model

The factors associated with TC and TG in the combined population are shown in Table 2.9 and are the same as those in either men or women.

Table 2.9: Factors associated with TC and TG levels in the total population

TC				
	Unadjusted		Adjusted	
Variables	β-Coefficient (95%CI)	P value	β-Coefficient(95%CI)	P value
Men gender	-0.034 (-0.064, -0.004)	0.027	-0.009 (-0.051, 0.034)	0.679
Age(years)	0.003 (0.001, 0.006)	0.018	0.004 (0.002, 0.007)	0.002
Nankana ethnicity	-0.093 (-0.123, -0.063)	<0.001	-0.079 (-0.111, -0.046)	<0.001
Employed	0.030 (-0.001, 0.061)	0.062	0.035 (0.004, 0.066)	0.028
Currently unmarried	0.024 (-0.010, 0.058)	0.170	0.018 (-0.017, 0.053)	0.311
High SES[2]	0.055 (0.018, 0.091)	0.003	0.025 (-0.014, 0.063)	0.212
Past or current smoker[3]	-0.021 (-0.054, 0.011)	0.192	0.021 (-0.021, 0.064)	0.372
Pesticide exposure	0.026 (-0.004, 0.051)	0.090	0.003 (-0.029, 0.035)	0.862
BMI (kg/m^2)	0.010 (0.006, 0.014)	0.001	0.001 (-0.006, 0.007)	0.873
Waist circumference (cm)	0.038 (0.022, 0.055)	<0.001	-0.004 (-0.032, 0.024)	0.776
Hip circumference (cm)	0.038 (0.022, 0.054)	<0.001	0.014 (-0.011, 0.039)	0.278
Subcutaneous fat (cm)	0.094 (0.065, 0.124)	<0.001	0.063 (0.020, 0.105)	0.004
TG				
	Univariate models		Multivariate model	
Variables	β-Coefficient (95%CI)	P value	β-Coefficient (95%CI)	P value
Age(years)	0.003 (-0.001, 0.007)	0.105	0.004 (0.001, 0.008)	0.025
Nankana ethnicity	-0.095 (-0.136, -0.054)	<0.001	-0.047 (-0.092, -0.003)	0.036

Some formal education[1]	0.060 (0.015, 0.104)	0.009	0.022 (-0.024, 0.069)	0.343
Currently unmarried	0.064 (0.018, 0.111)	0.006	0.062 (0.015, 0.109)	0.010
High SES[2]	0.099 (0.049, 0.148)	<0.000	0.029 (-0.025, 0.082)	0.288
Used smokeless tobacco	-0.081 (-0.148, -0.013)	0.019	-0.051 (-0.119, 0.017)	0.114
MVPA (hours/week)	-0.001 (-0.003, -0.001)	0.026	-0.006 (-0.001, 0.001)	0.991
Sleeping (hours/night)	-0.011 (-0.026, 0.004)	0.142	-0.007 (-0.021, 0.009)	0.415
Vegetable (servings/day)	-0.014 (-0.028, -0.001)	0.046	-0.007 (-0.022, 0.007)	0.317
Vendor (meals/week)	0.012 (-0.002, 0.026)	0.084	0.007 (-0.008, 0.013)	0.362
Malaria in past month	0.058 (0.002, 0.114)	0.044	0.043 (-0.012, 0.098)	0.125
BMI (kg/m^2)	0.019 (0.014, 0.025)	<0.001	0.003 (-0.007, 0.013)	0.600
Waist circumference (cm)	0.100 (0.073, 0.118)	<0.001	0.075 (0.036, 0.114)	<0.001
Hip circumference (cm)	0.042 (0.020, 0.064)	<0.001	-0.074 (-0.107, -0.040)	<0.001
Visceral fat (cm)	0.053 (0.036, 0.070)	<0.001	0.028 (0.010, 0.047)	0.003
Subcutaneous fat (cm)	0.183 (0.143, 0.222)	<0.001	0.123 (0.067, 0.179)	<0.001

CI: Confidence Interval; [1]education was coded as some formal education versus no education; [2]SES was coded as those with highest versus those with lowest SES; [3]smoking status was coded as those who are current or past smokers versus those who never smoked; R^2=0.038 ($p<0.001$) for the TC model and R^2=0.073 ($p<0.001$) for the TG model

2.4. Discussion

The current study shows that the prevalence of dyslipidaemia, as defined by high serum levels of TC, LDL-C or TGs, was relatively low (<6.0% for all 3 lipid species) in this rural Ghanaian community, and the proportion of subjects that needed treatment for high cholesterol was very low at 0.76%. However the prevalence of low HDL-C levels was very high at just over 60.0%. The major determinants of serum lipid levels, identified through multivariable linear regression analysis, were varied and included socio-demographic, behavioural and anthropometric factors. Ethnic grouping also influenced lipid levels with women and men from the Nankana ethnic group having lower TC and TG respectively than participants from the other ethnic groups.

The burden of dyslipidaemia in this population, as described by high LDL-C, TC or TG levels, was lower than that observed in Ghanaian urban settings (Micah and Nkum, 2012; Kodaman *et al.*, 2016) and lower than that noted in urban environments in other sub-Saharan African countries (Adediran *et al.*, 2012; Khine and Marais, 2016). A previous study from Ghana comparing lipid profiles between urban and rural populations also demonstrated higher TC and LDL-C levels in the urban population, but higher TG levels in the rural cohort (Kodaman *et al.*, 2016). The higher prevalence of dyslipidaemia in urban settings may be due to reduced physical activity, westernization of diets and sedentary employment (Yusuf *et al.*, 2014; Qi *et al.*, 2015). Our study also showed that very few dyslipidaemic participants were aware of their status. This

may be due to the low background prevalence of hypercholesterolaemia and lack of access to appropriate medical facilities. A recent study in a rural South African population also showed a low level of awareness of dyslipidaemia (Reiger *et al.*, 2017).

Despite the low frequency of high TC, LDL-C and TG levels, we observed a very high prevalence of low HDL-C levels, as has also been observed in other studies from sub-Saharan Africa (Sumner *et al.*, 2011; Gradidge *et al.*, 2016). This raises the question of whether the current cut points used for diagnosing dyslipidaemia in African populations are appropriate, and whether the high prevalence of low HDL-C levels observed in this population suggest heightened CVD risk. One study has reported high rates of fatal CVD events in black African subjects with high HDL-C levels (Longo-Mbenza *et al.*, 2011), and it has been shown that HDL-C levels in black participants are higher than in white participants even though the occurrence of CVDs is increasing more in the black population (Dai *et al.*, 2009; Woudberg *et al*., 2016). Furthermore, CVD risk is greater in subjects with low HDL-C in combination with high LDL-C or high triglyceride when compared to those with isolated low HDL-C levels (Bartlett *et al*., 2016). This suggests that isolated HDL-C may not be a reliable indicator of CVD risk and supports the call for the shift of focus to HDL-C quality (HDL-C functionality and subclasses) rather than quantity (Briel *et al.*, 2009; *Woudberg et al*., 2016) and the use of HDL-C in combination with other lipid species for predicting CVD risk (Bartlett *et al*., 2016).

Reports of gender differences in lipid levels in African populations are inconsistent. While some findings show that men have more favourable lipid profiles than women (Asiki *et al.*, 2015) other studies report no gender differences in lipids (Adamu *et al.*, 2013; Ogunmola *et al.*, 2013). In this population men and women did not have significantly different lipid levels, with the exception of HDL-C which was lower in women. However, after adjusting for tobacco smoking within a multivariable linear regression model, the association between gender and HDL-C levels was no longer found to be significant. Furthermore, HDL-C levels correlated positively with smoking in mens and in the total population. This is contrary to the literature, which consistently describes a negative relationship between these variables (Campbell and Tishkoff, 2008; Wakabayashi, 2014; Abd *et al.*, 2016). These data therefore suggested that the higher HDL-C levels in mens were due to their higher frequency of tobacco smoking. Further analyses demonstrated that the positive relationship between smoking and HDL-C levels was only observed in alcohol drinkers, with the relationship being an inverse one (but not significant) in

those who did not drink. This combined effect of smoking and alcohol intake on HDL-C levels is a novel finding, and requires further investigation in a larger sample size to assess the interaction between these variables and to disentangle the relative effects of smoking and alcohol intake on serum HDL-C levels. However, it is important to note that only 14 (1.4%) of the women were current smokers, which could have confounded the analysis and likely explain the positive association with HDL-C in men. It is likely that these results were confounded by drinking which is supported by the subsequent findings related to drinking. This problem may be minimized in future studies by using more objective measures of both smoking and possible confounding variables, including alcohol intake.

Our study did not show significant association between HDL-C levels and age. It has been suggested that low HDL-C is associated with age after 60 years (Ge *et al.*, 2015). Similarly studies have reported decreased HDL-C levels with age in cohorts with upper age ranges >60 years (Adebonojo and Ogunnaike, 1989; Harman *et al.*, 2017). The lack of association of HDL-C with age in our study could possibly be due to the younger upper age range of our cohort. The positive association between age and LDL-C, TC and TG levels among women in this population reflects similar findings in previous studies (Goh *et al.*, 2007; Okęcka-Szymańska *et al.*, 2011). The molecular mechanisms involved in the increase of serum cholesterol and TG levels with aging is not fully understood, although a number of possible causes have been suggested (Auley and Mooney, 2015) including changes in hormone levels during menopause transition (Pardhe *et al.*, 2017).

Lipid levels are known to show ethnic variation (Donin *et al.*, 2010; Bentley and Rotimi, 2017). Our results show that the Nankana women have significantly lower TC and the Nankana men lower TG levels than their counterparts in the other ethno-linguistic groups analysed in this study. To our knowledge this is the first study in sub-Saharan Africa to assess differences in lipid levels between ethno-linguistic sub-groups within the same broad ethnic group (black Africans). This suggests possible genetic variability since the differences in lipid levels in these groups persist even after adjusting for anthropometric, behavioural and socio-demographic variables. However, these results must be confirmed in further studies that systematically compare lipid levels between these ethno-linguistic groups and adjust for a larger array of possible confounding factors.

The current study found a positive association of SES with HDL-C but a lack of association with LDL-C, TC and TG. This is contrary to several findings which indicate poorer lipid profiles with higher SES (Vorster *et al.*, 2007a; Shohaimi *et al.*, 2014; Brown *et al*., 2016). This may be due to the use of household assets in computing SES in our study rather than a combination of household assets, highest levels of education attained and employment status used in other studies (Shohaimi *et al.*, 2014; Brown *et al*., 2016).

There are mixed findings regarding the influence of employment status and education level on lipid levels. While some studies have associated low education levels and unemployment with higher lipid levels (Gupta, Deedwania, *et al.*, 2012; Brown *et al*., 2016) others have reported the contrary (Reddy *et al.*, 2012; Rodriguez *et al.*, 2014; Shohaimi *et al.*, 2014). The results of our study showed independent positive associations of formal education and employment with LDL-C among women and TC among men, respectively. The positive association of alcohol consumption with HDL-C levels corroborate the findings of other studies (Kechagias *et al.*, 2011; Ahaneku *et al.*, 2014). It has been suggested that alcohol consumption may raise HDL-C levels by elevating the transport rate of the major apolipoproteins, Apo-I and Apo-II (De Oliveira *et al.*, 2000).

Pesticide exposure among the study population was high due to agricultural activities in the study area but there was no association between pesticide exposure and lipid levels (Binka, *et al*., 1999). This finding contradicts results of other studies which show a positive association of LDL-C, TC, TG and negative association of HDL-C levels with pesticide or chemical exposure (Ntzani *et al.*, 2013; Arrebola *et al*., 2014). The lack of association of pesticide exposure with lipid levels in our study may be due to the evaluation of the association of the exposure as opposed to the blood concentration of pesticides with lipid levels. Further studies are required to evaluate the association of pesticide concentration on lipid levels in this population.

There is a lack of consistent evidence regarding the influence of sleep duration on lipid levels. While some studies show an association of unfavourable lipid levels with increased night time sleep duration other studies show the contrary (Wan *et al*., 2013; Abreu *et al.*, 2015). Though our study did not show association of sleep duration with any of the lipids in the gender stratified analysis, there was a negative association of sleep duration with LDL-C levels in the combined

analysis. This calls for further studies on rural African adults to ascertain the influence of sleep duration on lipid levels.

Though diet (vendor meals, vegetable and fruit intake) is known to influence lipid levels our study did not find any association of lipid levels with diet. The lack of association between lipids and diet in our study may also be due to a lack of detailed information on daily food intake. Despite the low level of obesity in this rural study, our results show that increased BMI was positively associated with TC among men and waist circumference was associated with both LDL-C and TG among women. Subcutaneous fat was associated with both TC and TG among women and both LDL-C and TC among men. The association of these markers of obesity with lipid levels are consistent with results of studies elsewhere (Porter *et al.*, 2009; Ni *et al.*, 2015; Tang *et al*., 2016). Whilst visceral fat was associated with only TG, subcutaneous adipose tissue was associated with increasing LDL-C, TC and TG in the study population. These findings, unlike studies elsewhere that have indicated visceral adipose tissue as a better marker of dyslipidaemia (Snijder *et al.*, 2004; Ruige and Van Gaal, 2009), suggest that subcutaneous adipose tissue is an important factor influencing lipid levels in this population. Furthermore TG was negatively associated with hip circumference and this is consistent with other findings (Snijder *et al.*, 2004). Hip circumference is a good measure of gluteofemoral fat which has been shown to protect against CVD risk factors such as insulin resistance, hypertension and dyslipidaemia (Snijder *et al.*, 2004; Ruige and Van Gaal, 2009).

This study has several strengths in terms of sample size, approach to biomarker analysis and the measurement of a large set of potential risk factors for dyslipidaemia. The sample of 1839 participants and the population-based study design is ideal for providing general baseline data. Many studies use derived LDL-C levels from the Friedewald equation, whereas this study used a direct measurement method and implemented stringent quality control processes (Porter *et al.*, 2009; Boshtam *et al.*, 2012). The study is anchored on the strength of the HDSS which provides an ideal platform for follow-up studies to establish the cause of dyslipidaemia among participants. Limitations are that participant age was often estimated based on an events calendar, and therefore may not be accurate, and lifestyle data were collected based on self-reported responses from the participants and could not be independently verified. The dietary data was limited and did not provide enough detail to truly establish whether food intake was

associated with lipid levels. Finally, this was a cross-sectional study that could only investigate factors associated with lipid levels but could not establish causality.

In conclusion a longitudinal study is required to investigate whether high prevalence of low HDL-C really is a CVD risk factor in this community. The association of Nankana ethnicity with a healthier lipid profile in older adults is interesting and suggests that further studies, including genetic analyses, are required to understand this association. The association of education and employment with higher lipid levels suggests that societal advances will lead to increasing lipid levels in future, and so this must be monitored over time to allow early interventions. Likewise, the association of anthropometric variables with high lipid levels in a population with a low level of obesity suggests that any increase in the prevalence of obesity in this community may lead to high TC and TG levels. Hence, interventions should be developed to restrict this. Also, the association of hip circumference with lower TG levels suggests an attenuation effect of this adipose tissue depot at least on TGs but again this requires a longitudinal study to determine the magnitude of this effect.

The current study investigated the effect of environmental factors on serum lipid levels, but it is also known that genetic factors modulate lipids (Bentley *et al.*, 2014). Particularly, it is important to note that only a very small proportion of the variance could be explained by the sociodemographic, lifestyle and anthropometric factors measured in this study. Notably, less than 2% of the variance in HDL-C could be explained by these factors in the total population. The next section of this study therefore investigated the effect of gene variants from selected candidate genes on serum lipid levels in this rural Ghanaian population.

2.5 Contribution of candidate to the study

I collected the data, designed the study, analysed the data and wrote the first and final drafts of the chapter for the thesis and the published manuscript. I am first author on the publication.

CHAPTER 3: CANDIDATE GENE ANALYSIS REVEALS STRONG ASSOCIATION OF *CETP* VARIANTS WITH HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN GHANAIAN ADULTS: AN AWI-GEN SUB-STUDY

3.1. Introduction

Cardiovascular disease (CVD) is a major health risk accounting for over 17 million deaths (about 30% of all deaths) globally each year. A major proportion (80%) of these deaths occurs in low and middle-income countries with the total number of annual deaths expected to reach 23.6 million by 2030 (Wang *et al.*, 2016). One major risk factor for CVD is dyslipidaemia which is a metabolic derangement that predisposes an individual to atherosclerosis (O'Donnell and Elosua, 2008; Reinikainen *et al.*, 2015). There is variation in the prevalence of dyslipidaemia across populations with the global adult prevalence of raised total cholesterol (TC) in 2008 estimated at 9.7% (WHO, 2011). Population and ethnic variations in lipid levels are attributed, in part, to genetic ancestry (Willer, 2013).

It has been documented that approximately 25% to 80% of the inter-individual variation in lipid phenotypes is heritable (Perusse *et al.*, 1997; Beekman *et al.*, 2002). Single nucleotide variants (SNVs) in the following genes have been shown to be associated with differences in lipid levels: a genome-wide association study (GWAS) on cholesteryl ester transport protein (*CETP*) in American women (Ridker *et al.*, 2009), a review of both GWAS and candidate gene studies on paraoxonase 1 (*PON1*) in Europeans and Asians (Durrington *et al*., 2001), candidate gene studies on lipoprotein lipase (*LPL*) in Europeans (Pirim *et al.*, 2014), ATP-binding cassette A1 (*ABCA1*) in Iranians (Kelishadi *et al.*, 2014), lecithin-cholesterol acyltransferase (*LCAT*) in Europeans (Hovingh *et al.*, 2005), protein convertase subtilisin/kexin type 9 (*PCSK9*) in black Americans and Europeans (Kotowski *et al.*, 2006), and methylmalonic aciduria cb1B (*MMAB*) (Sun *et al.*, 2016) and mevalonate kinase (*MVK*) both in Chinese (Miao *et al.*, 2017). Evidence from candidate gene and genome-wide association studies (GWASs) on the influence of genetic polymorphisms on lipid level variations comes primarily from studies in cohorts of European origin, and an increasing number in Asians and populations of African descent, but few in black Africans (Willer *et al*, 2013; Dron and Hegele 2016). Identifying the common genetic variants

associated with lipid levels in populations in Africa will assist in the detection of individuals at higher risk for dyslipidaemia.

The ability to identify gene loci that associate with serum lipid (high density lipoprotein cholesterol [HDL-C], low density lipoprotein cholesterol [LDL-C], triglycerides [TG] and total cholesterol [TC]) levels has been advanced by the use of genotyping arrays that were developed from several projects including the HapMap project (The International HapMap Consortium *et al*. 2007; Wu *et al*., 2013), the 1000 Genomes Project (The 1000 Genomes Project Consortium, 2010; Wood *et al.*, 2013) and the Genome of the Netherlands Project (Boomsma *et al.*, 2014; van Leeuwen *et al.*, 2015). These arrays are generally Eurocentric and under-represent common variants found in African populations. We therefore used the H3Africa Afrocentric array (Mulder *et al.*, 2018) to investigate the influence of SNVs at selected loci on lipid levels in our study population.

The aim of our study was to examine the genetic association of SNVs in the transcribed regions of eight genes previously associated with LDL-C, HDL-C, TC and TG and to perform a replication study for the associated SNVs in another African cohort. This is the first African study to use an Afrocentric gene array to perform and replicate a candidate gene analysis of serum lipid levels.

3.2. Methods

This is a candidate gene study that was conducted in the Kassena-Nankana districts of northern Ghana as part of the Africa Wits-INDEPTH Partnership for Genomic research (AWI-Gen) project (Ramsay *et al.*, 2016) under the broader Human Heredity and Health in Africa initiative (The H3Africa Consortium, 2014). A sample size of 2200 men and women aged 40-60 years and of roughly equal gender ratio, including 10% for non-response or refusal to participate, was randomly selected. Of this number, 2016 participants were recruited into the study (Agongo *et al.*, 2018).

Given this sample size, a power analysis was performed using Quanto software version 1.2.4 (Gauderman, 2002). Using information from a previous study that reported a mean HDL-C level in West Africans of 1.016±0.321mmol/l with an effect size of 0.388mmol/l for the rs328 variant in the *LPL* gene at ~5% minor allele frequency (MAF) (Bentley *et al.*, 2014), we determined that our study had >90% power to detect an effect size of at least 0.129mmol/l for lipid traits at

MAF>0.05 for a sample size of 1800 individuals at 5% nominal significance level. Only the *LPL* gene was chosen for the sample size determination in this study because it was the only gene from the selected genes that reported an association with one of the lipid traits in a previous study involving West Africans, which are similar to this study population.

This study was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M151050) (Appendix C1) and the Navrongo Health Research Centre Institutional Review Board (ID No: NHRCIRB178) (Appendix C2) and was part of the wider H3Africa AWI-Gen project that had prior approval from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M12109) (Appendix C3).

3.2.1. Demographic data, anthropometric measurements and lipid analyses

Since many of the participants did not know their exact birthdates, their ages were mainly estimated using information from the Navrongo Health and Demographic Surveillance System (Oduro *et al.*, 2012). Fasting whole blood samples were taken from participants for lipid measurements and DNA extraction for genetic analyses. Serum HDL-C, LDL-C, TC and TG were all measured directly using an automated chemistry analyzer and waist circumference was measured as described elsewhere (Ali *et al.*, 2018).

3.2.2. Selection of candidate genes

Genes were selected based on evidence from genome-wide association and candidate gene studies on lipid levels in peoples of African descent (Adeyemo *et al.*, 2012; Elbers *et al.*, 2012; Bentley *et al.*, 2014) and black Africans (Abessolo *et al.*, 2014; Niemsiri *et al.*, 2015). We analysed polymorphic markers in 8 candidate genes: *ABCA1*, *LCAT*, *LPL*, *PON1*, *CETP*, *PCSK9*, *MVK* and *MMAB* by selecting SNVs in the transcribed region of each gene (genome coordinates shown in Table 3.1).

Table 3.1: Number of variants tested within selected genes following quality control of data

Gene symbol	Chromosome position	Start transcript	End transcript	Number of SNVs
LPL	8	19,759,228	19,824,769	286
LCAT	16	67,973,653	67,978,034	6
CEPT	16	111,682,249	111,727,724	117
PON1	7	94,926,988	94,954,019	94
ABCA1	9	107,543,283	107,690,518	632
MVK	12	110,011,060	110,035,922	55

MMAB	12	109,991,542	110,011,679	84
PCSK9	1	55,505,221	55,530,525	118

3.2.3. DNA extraction and genotyping

Fasting blood samples were taken from participants after written informed consent for lipid measurements (Agongo *et al.*, 2018) and genetics analyses. DNA was extracted at the Sydney Brenner Institute for Molecular Bioscience (SBIMB), University of the Witwatersrand, South Africa using a modified protocol of the salting out method (Diego Chacon-Cortes, 2014). See Appendix F for DNA extraction protocol. Prior to genotyping the DNA samples were quantitated on the NanoDrop® DN-100 spectrophotometer (Thermo Fisher Scientific, MA, USA) and their integrity assessed (http://www.ctrnet.ca/operating-procedures). The concentration of DNA was calculated by the NanoDrop in ng/µl by checking 1µl of each sample. The ratio of the absorbances at 260/280nm indicated the estimated DNA purity. An acceptable purity range was 1.8-2.0 but a ratio below 1.5 was an indication of protein contamination.

Working samples to be used for integrity checks were stored at 4^0C. The integrity checks were done by running electrophoresis gels to ensure that structural integrity of the DNA samples were maintained after the extraction. Agarose gel (0.8%) was prepared by adding 0.8g agarose in 100ml TBE. The agarose was allowed to dissolve properly and cool to the touch before adding ethidium bromide. The mixture was put in a microwave and heated for 30 seconds, 50 seconds and then 30 seconds and shaken after each time. The gel was allowed to cool to the touch before it was poured. After inserting the combs the gel was allowed to dry before the combs were removed. A 1:8 diluted sample (i.e. 5µl deionsed H2O, 2µl of loading dye and 1µl of DNA) was loaded in each well. A 1kb molecular marker which acted as a positive DNA control and as a molecular ladder was used in each gel run. The gels were run at 100V for 30 minutes. The DNA band images were captured on the Gel Doc Ez as shown in Figure 3.1.

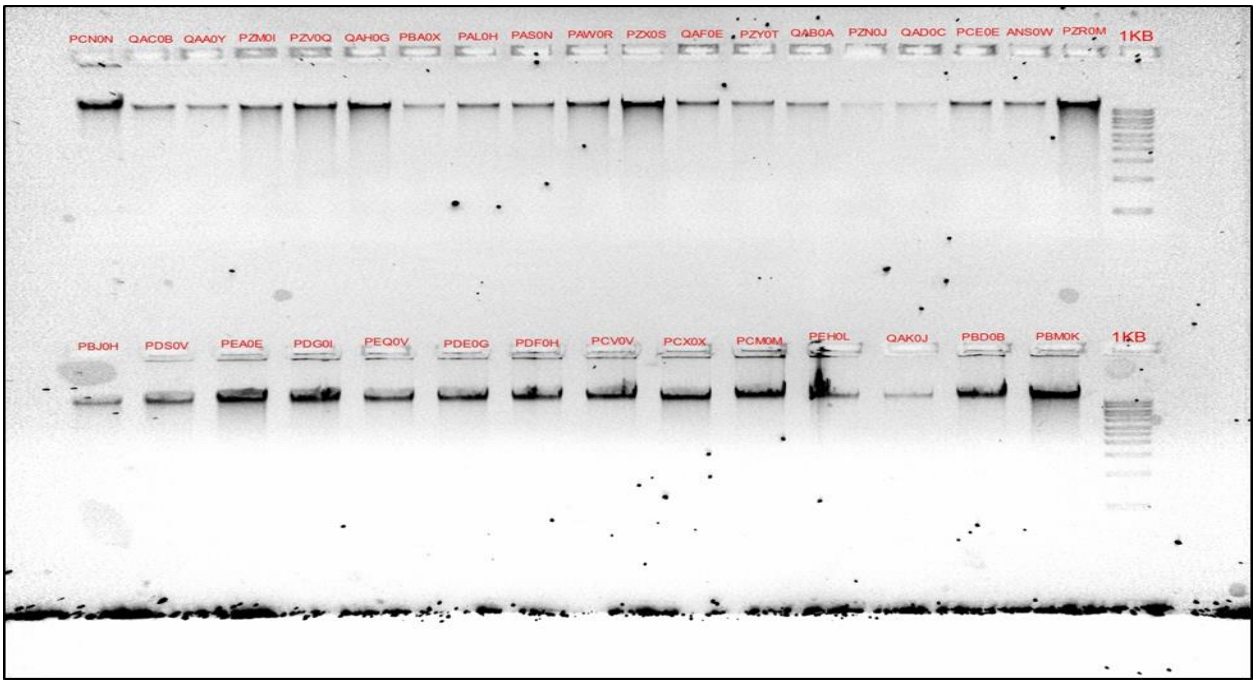

Figure 3.1: A gel image showing DNA bands and 1KB molecular marker; the labels represent the identification numbers of the DNA samples

Genotyping was performed using the H3Africa single nucleotide variant (SNV) array designed on the Illumina platform. The array is enriched for common variation in African populations and contains >2.2 million SNVs (Mulder *et al.*, 2018).

3.2.4. Imputation and quality control processes

The following pre-imputation quality control (QC) filters were applied to the genotyping data of the entire AWI-Gen dataset of 10,903 individuals from six different sites of which our study site is one. Individuals with a missing call rate>0.05 and SNVs with a missing call rate>0.05, MAF<0.01 and Hardy-Weinberg equilibrium (HWE) p-value<0.0001 were removed from the data set. Furthermore individuals with mismatch in recorded and genotyped sex, with relatedness, and/or with extreme heterozygosity deviations were excluded. SNVs that did not match the GRCh37 reference alleles or strands were also removed. The cleaned dataset (with 1,729,661 SNVs and 10,903 individuals) was, pre-phased with EAGLE2 and imputed using the Sanger Imputation Server with the African reference panel. The default Positional Burrows-Wheeler transform (PBWT) algorithm was used for imputation. At post imputation QC, poorly imputed SNVs (SNVs with IMPUTE2 information score<0.6), SNVs with MAF<0.01 and HWE p-value<0.00001 were excluded resulting in the final quality controlled imputed dataset

containing 10,903 individuals and 13,980,000 SNVs. The data for this study was extracted from the larger dataset. The final data set included 1855 participants from Ghana. Only SNVs with MAF>0.05 in the regions described in Table 1 were used for data analysis. The number of SNVs in the gene loci included the following: *ABCA1*=632, *CETP*=117, *LCAT*=6, *LPL*=286, *MMAB*=84, *MVK*=55, *PCSK9*=118 and *PON1*=94 (Figure 3.2).

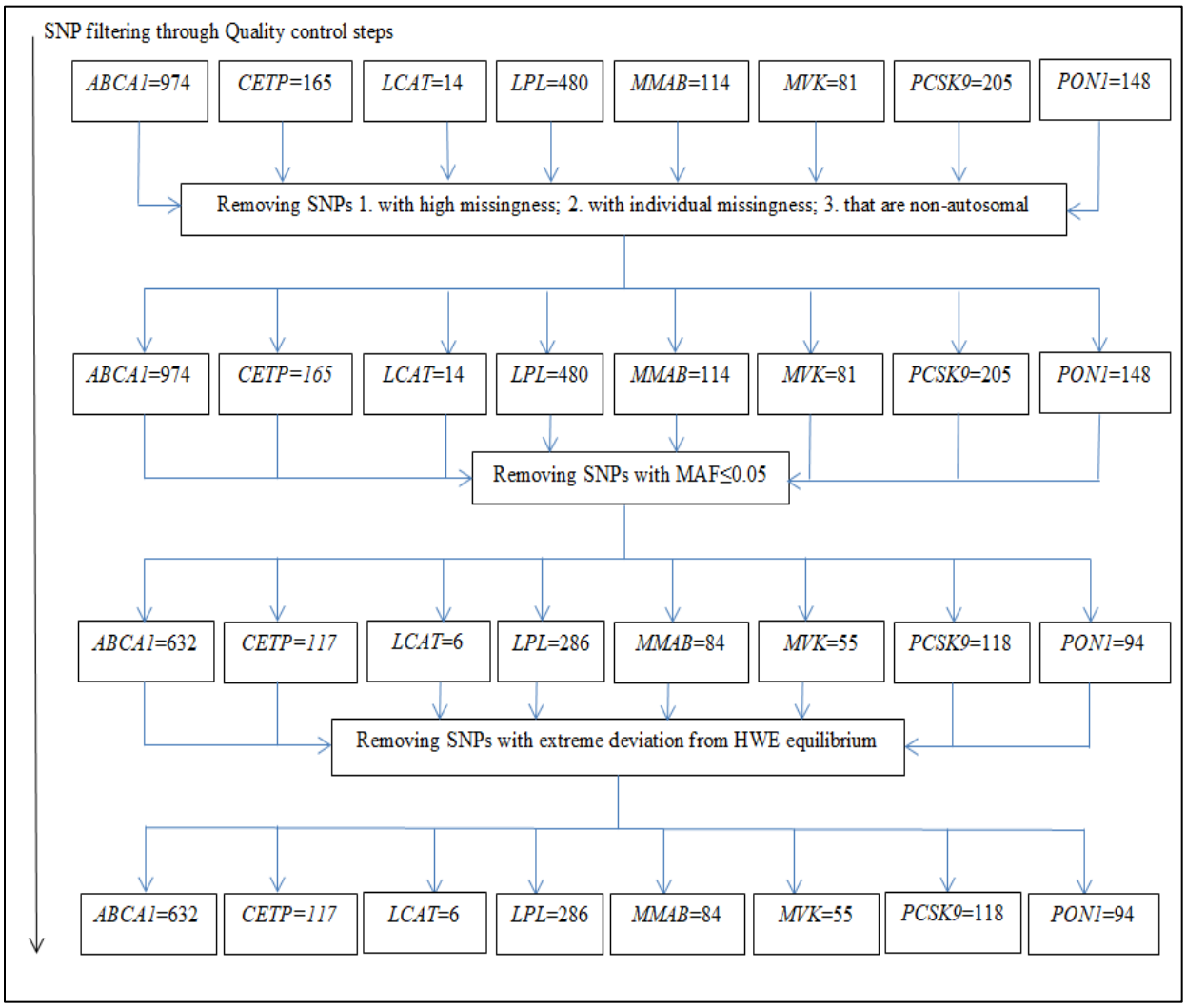

Figure 3.2: Quality control processes used for filtering genotyped SNPS for association analyses. Process starts from exclusion of high missingness to removal of SNPs with extreme heterozygosity rate

3.2.5. Data analysis

All continuous variables were presented as means with standard deviations (SD) and compared among men and women using Student's t-test. Lipid levels were log-transformed and presented

across genotypes of lead SNVs using analysis of covariance (ANCOVA) with adjustment for age, gender and waist circumference. PLINK version 1.90 was used for association analyses (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell *et al.*, 2007) using a threshold quality score >0.06 to call a genotype. Data on SNVs in the transcribed regions of selected genes were extracted from the H3Africa AWI-Gen genotyped data for further analyses. Pearson χ^2 test was used to assess deviation from HWE equilibrium by comparing observed to expected frequencies. Among the anthropometric, sociodemographic, lifestyle and environmental factors, age and waist circumference were the covariates most strongly associated with lipid traits in a previous study performed in the study population (Agongo *et al.*, 2018). Also, there were ethnic differences in TC and TG levels and gender differences in HDL-C levels in this population (Agongo *et al.*, 2018). Therefore, linear regression analyses were used to test for association between log-transformed lipid traits (Agongo *et al.*, 2018) and SNVs with adjustment for gender, age, waist circumference and ethnicity. Standardized β values and confidence intervals were calculated using the major allele as reference. All p-values were corrected for multiple testing using the Bonferroni method (Bland and Altman, 1995) and adjusted for covariates (age, gender, ethnicity and waist circumference). The Bonferroni correction for each lipid trait took into account the number of variants tested in each trait. LocusZoom plots were drawn using an online analysis tool at http://csg.sph.umich.edu/locuszoom by the University of Michigan, Department of Biostatistics, Centre for Statistical Genetics (Pruim *et al.*, 2010). All Bonferroni-adjusted p-values at 5% significance level after covariate adjustment were considered significant.

3.2.6. Functional analysis of significant SNVs

Functional variant analyses included localization of the variant within the gene region and combined annotation dependent depletion (CADD) scores to predict the damaging effect of the variant on protein structure and function. Another annotation was loss of function tool (LoFtool) score which predicted genic intolerance and consequent susceptibility to disease. A CADD score above 10 implied a deleterious effect and a low LoFtool score indicated damaging effect of the mutations on the gene. The RegulomeDB (RDB) score (Boyle *et al.*, 2012) was used to assess the regulatory potential of the variants. All of these were analysed using Variant Effect Predictor (McLaren *et al.*, 2016). The 1000 Genomes database (accessed using https://www.ncbi.nlm.nih.gov/dbvar) was used to compare the MAFs of the SNVs analysed in the current study with those observed in other populations.

3.2.7. Replication

All variants with p<0.05 in either the covariate-adjusted or unadjusted models (n=21) were selected for replication using the Africa America Diabetes Mellitus study (AADM), which has been previously described (Rotimi *et al.*, 2001). Briefly, AADM is a genetic epidemiology study of type 2 diabetes (T2D), enrolling participants from university medical centers in Nigeria (Enugu, Lagos and Ibadan), Ghana (Accra and Kumasi), and Kenya (Eldoret). Analyses were conducted using linear mixed models of the log transformations of serum lipids in up to 4317 participants with available data. The first 2 PCs of the genotypes were included in the model. All models also included a genetic relationship matrix to account for the random effect of relatedness, as related individuals were included in AADM, and were adjusted for T2D, as this is a case-control study. Models were run using EPACTS (http://genome.sph.umich.edu/wiki/EPACTS) with and without adjustment for additional covariates including age, gender and waist circumference. Replication was defined as an association in a consistent direction with Bonferroni-corrected p<0.0024 (i.e. 0.05/21).

3.3. Results

3.3.1. Characteristics of the study population

The demographic characteristics and lipid levels of the study population, stratified by gender, are shown in Table 3.2. Participants who had no genetic data were excluded from the analyses resulting in a sample size of 1855. The average age of the study participants was 51 years with women being significantly older than men (p<0.001). Waist circumference among women was significantly higher than that among men (p<0.001). There was no significant difference in LDL-C (p=0. 0.963), TC (p=0.066) and TG (p=0.939) levels between men and women but HDL-C levels were significantly lower (p<0.001) among women.

Table 3.2: Basic characteristics of the study population in northern Ghana

Variable	Men (n=866)	Women (n=989)	Total (n=1855)	p value
Age (years)	50 ± 6.0	52 ± 6.0	51 ± 6.0	<0.001
Waist circumference (cm)	73.4 ± 8.1	76.7 ± 9.5	75.2 ± 9.0	<0.001
HDL-C (mmol/l)	1.18 ± 0.41	1.11 ± 0.33	1.14 ± 0.42	<0.001
LDL-C (mmol/l)	1.74 ± 0.81	1.74 ± 0.74	1.74 ± 0.78	0.963
TC (mmol/l)	3.17 ± 0.92	3.25 ± 0.91	3.21 ± 0.92	0.066

TG (mmol/l)	0.64 ± 0.51	0.64 ± 0.35	0.64 ± 0.43	0.939

Data given as mean ± SD

3.3.2. Variants associated with lipid levels

We tested the association between variants within the transcribed regions of selected genes (*ABCA1*, *CETP*, *LCAT*, *LPL*, *MMAB*, *MVK*, *PCSK9* and *PON1*) and each lipid fraction as a continuous variable (HDL-C, LDL-C, TC and TG). There were associations between variants of *CETP*, *LCAT*, *PCSK9* and *PON1* and lipid levels at $p<0.05$ after adjustment for age, gender, ethnicity and waist circumference (Table 3.3). The lead associated SNVs were the following: rs17231520 and rs34065661 in *CETP* and rs1109166 in *LCAT* for HDL-C, rs11806638 in *PCSK9* for LDL-C and rs854558 in *PON1* for TC. For *CETP*, there were 11 SNVs that were significantly associated with HDL serum levels, with the strongest associations being observed at rs17231520 and rs34065661 (Table 3.3). All but one of these SNVs (rs3816117) showed a positive association between the minor allele (relative to the major allele) and HDL-C levels. One SNV within the *LCAT* gene (rs1109166) showed a significant negative association with HDL-C levels. With regards to LDL-C, 4 SNVs within the *PCSK9* gene were identified with significant associations with serum LDL-C levels. However, only one of these (rs11806638) remained significant ($p<0.05$) after adjustment for age, gender, ethnicity and waist circumference. All these variants demonstrated negative associations with LDL-C levels. Five SNVs in the *PON1* gene were significantly associated with serum TC levels, but only 3 (rs854558, rs854564 and rs854565) of these remained significant after adjustment for age, gender, ethnicity and waist circumference. All these SNVs were positively associated with TC serum levels. No significant gene associations were found for serum TG levels.

Table 3.3: Association of single nucleotide variants of selected candidate genes with lipid levels among the total study group (N=1855)

					Without covariates			With covariates[2]		
SNV(Gene)	A1/A2	Position[1]	MAF	pHWE	β (SE)	p_R value	p_B value	β (SE)	p_R value	p_B value
HDL-C										
rs17231520(*CETP*)	A/G	56995827	0.089	1.000	0.1386 (0.0209)	4.19e-11	4.90e-09	0.1387 (0.0209)	3.80e-11	4.44e-09
rs34065661(*CETP*)	G/C	56995935	0.090	1.000	0.1372 (0.0207)	4.47e-11	5.23e-09	0.1371 (0.0207)	4.30e-11	5.03e-09
rs711752(*CETP*)	A/G	56996211	0.246	0.573	0.0833 (0.0137)	1.35e-09	1.58e-07	0.0845 (0.0137)	7.45e-10	8.72e-08
rs708272(*CETP*)	A/G	56996288	0.245	0.531	0.0832 (0.0137)	1.42e-09	1.66e-07	0.0844 (0.0137)	7.82e-10	9.14e-08
rs891142(*CETP*)	T/C	57003977	0.098	1.000	0.0984 (0.0200)	9.29e-07	1.09e-04	0.0983 (0.0200)	9.55e-07	1.11e-04
rs891143(*CETP*)	T/C	57003980	0.097	0.692	0.0976 (0.0200)	1.16e-06	1.36e-04	0.0973 (0.0200)	1.24e-06	1.45e-04
rs4784740(*CETP*)	G/C	57001085	0.091	0.780	0.1008 (0.0208)	1.35e-06	1.58e-04	0.1009 (0.0208)	1.31e-06	1.53e-04
rs3816117(*CETP*)	T/C	56996158	0.378	0.459	-0.0539 (0.0124)	1.48e-05	1.73e-03	-0.0551 (0.0124)	9.17e-06	1.07e-03
rs158478(*CETP*)	C/A	57007734	0.300	0.294	0.0487 (0.0129)	1.59e-04	0.01858	0.0488 (0.0129)	1.49e-04	0.01740
rs891141(*CETP*)	G/T	57003723	0.168	0.115	0.0576 (0.0157)	2.46e-04	0.02875	0.0589 (0.0157)	1.76e-04	0.02053
rs289719(*CETP*)	T/C	57009941	0.440	0.963	0.0430 (0.0120)	3.62e-04	0.04236	0.0443 (0.0120)	2.30e-04	0.02685
rs1109166(*LCAT*)	T/C	67977382	0.219	0.946	-0.0406 (0.0145)	0.00510	0.03060	-0.0408 (0.0145)	0.00476	0.02853
LDL-C										
rs11806638(*PCSK9*)	A/C	55518160	0.396	0.961	-0.0601 (0.0152)	7.87e-05	9.29e-03	-0.0549 (0.0149)	2.27e-04	0.02683
rs11804420(*PCSK9*)	G/A	55520445	0.298	0.346	-0.0612 (0.0165)	2.02e-04	0.02389	-0.0513 (0.0161)	0.00147	0.1736
rs11800231(*PCSK9*)	A/G	55517940	0.215	0.149	-0.0682 (0.0184)	2.21e-04	0.02601	-0.0635 (0.0180)	4.25e-04	0.05017
rs45545732(*PCSK9*)	T/A	55519231	0.273	0.860	-0.0605 (0.0168)	3.08e-04	0.03634	-0.0521 (0.0164)	0.00147	0.17400
TC										
rs854558(*PON1*)	T/C	94945374	0.385	0.659	0.0435 (0.0110)	7.40e-05	6.95e-03	0.0404 (0.0109)	2.13e-04	0.02005
rs854564(*PON1*)	G/T	94948182	0.386	0.807	0.0412 (0.0110)	1.76e-04	0.01654	0.0383 (0.0109)	4.68e-04	0.04398
rs854565(*PON1*)	A/G	94948344	0.386	0.807	0.0412 (0.0110)	1.76e-04	0.01654	0.0383 (0.0109)	4.68e-04	0.04398
rs854566(*PON1*)	A/G	94948749	0.377	0.401	0.0405 (0.0110)	2.22e-04	0.02090	0.0374 (0.0109)	6.23e-04	0.05858
rs854567(*PON1*)	A/G	94948784	0.377	0.401	0.0405 (0.0110)	2.22e-04	0.02090	0.0374 (0.0109)	6.23e-04	0.05858

A1/A2: Minor/major allele; MAF: Minor allele frequency in the study population; pHWE: Hardy-Weinberg p value; N: total number of participants; SE: standard error; [1]Genomic position and chromosome in build 37; p_R: raw p value; p_B: Bonferroni-adjusted p value; [2] Covariates were gender, age, ethnicity and waist circumference

The LocusZoom plots for the significant associations are shown in Figures 3.3 and 3.4 and lead SNVs from *CETP*, *LCAT*, *PCSK9* and *PON1* are shown.

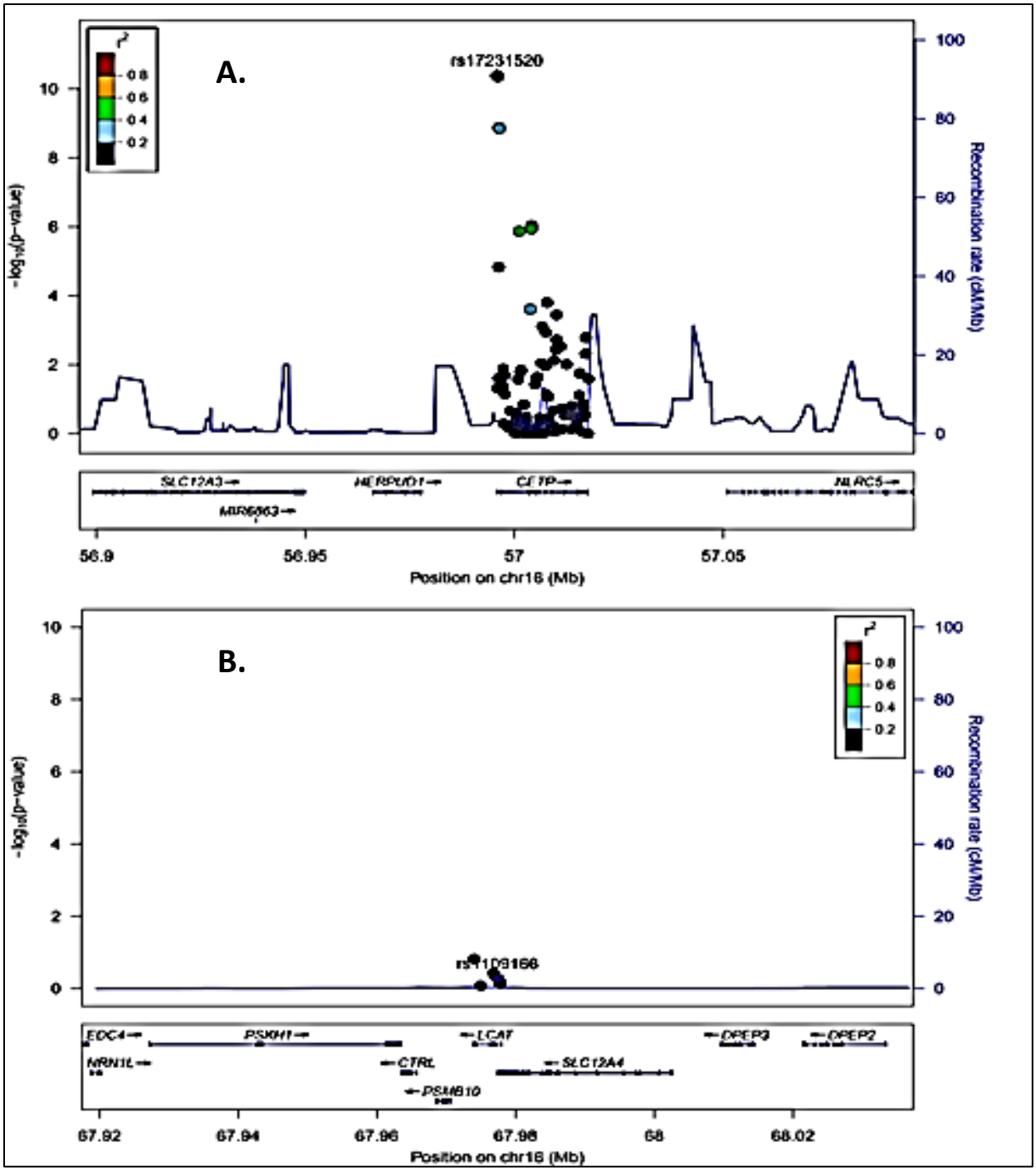

Figure 3.3: LocusZoom plots of: A. rs17231520(*CETP*) and B. rs1109166(*LCAT*) associated with higher and lower levels of HDL-C respectively after adjustment for gender, age, ethnicity and waist circumference

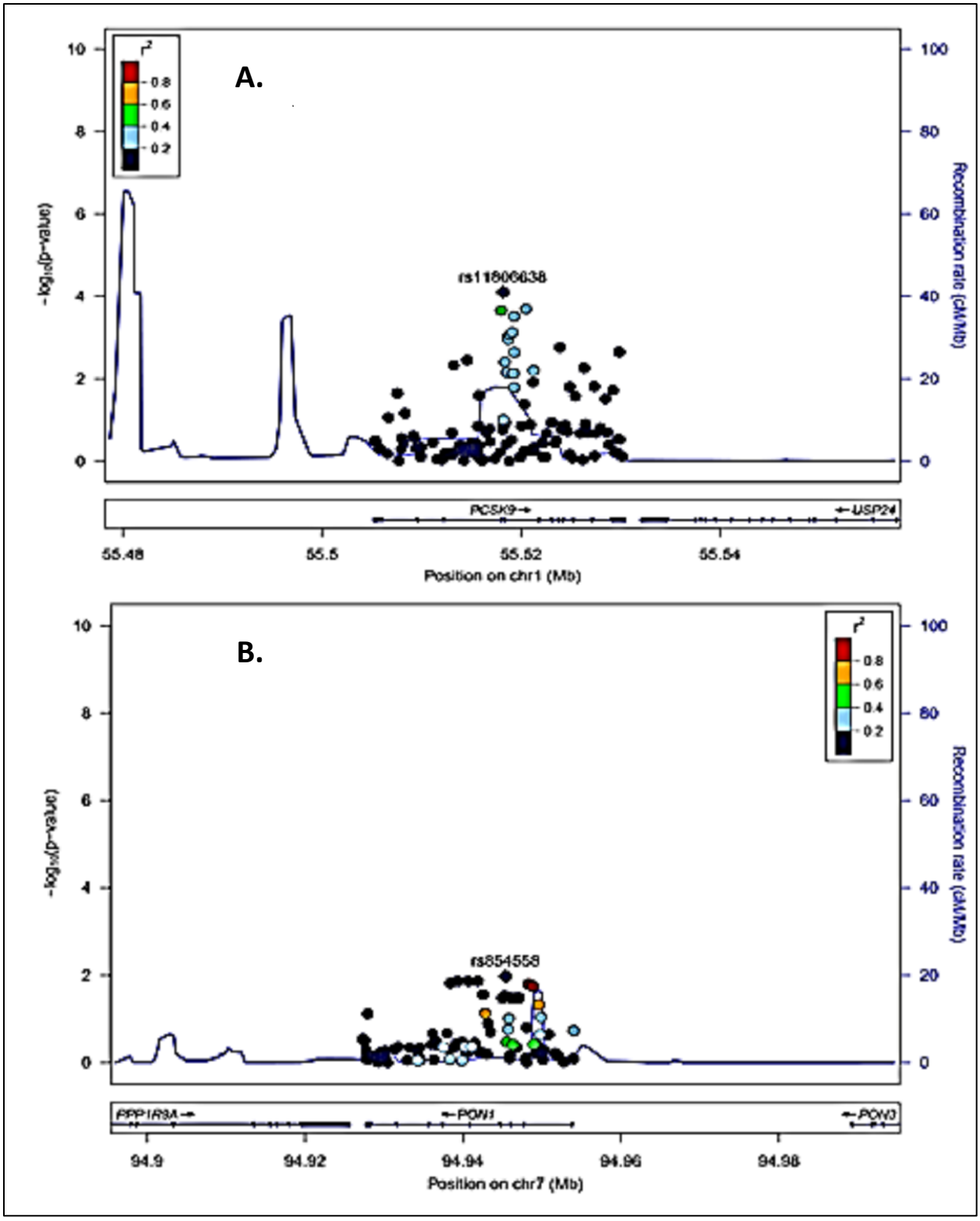

Figure 3.4: LocusZoom plots of: A. rs11806638(*PCSK9*) and B. rs854558(*PON1*) associated with lower levels of LDL-C and higher levels of TC respectively after adjustment for gender, age, ethnicity and waist circumference

Figure 3.5 shows the median serum lipid levels given for each genotype of each lead SNV. Both the rs17231520 and rs34065661 SNVs within *CETP* show an additive effect, with serum HDL-C levels increasing in the presence of the minor allele (A) of the rs17231520 and (G) of the rs34065661 variants . Similarly The SNV within *LCAT*, rs1109166, showed a dominant and negative effect of the minor allele (T) on serum HDL-C levels. The minor allele (A) at rs11806638 in the *PCSK9* gene demonstrated a negative effect on LDL-C levels, in a recessive manner. The serum levels of TC were positively associated with the minor allele (T) at rs854558 in the *PON1* gene, with the T allele acting in a dominant fashion.

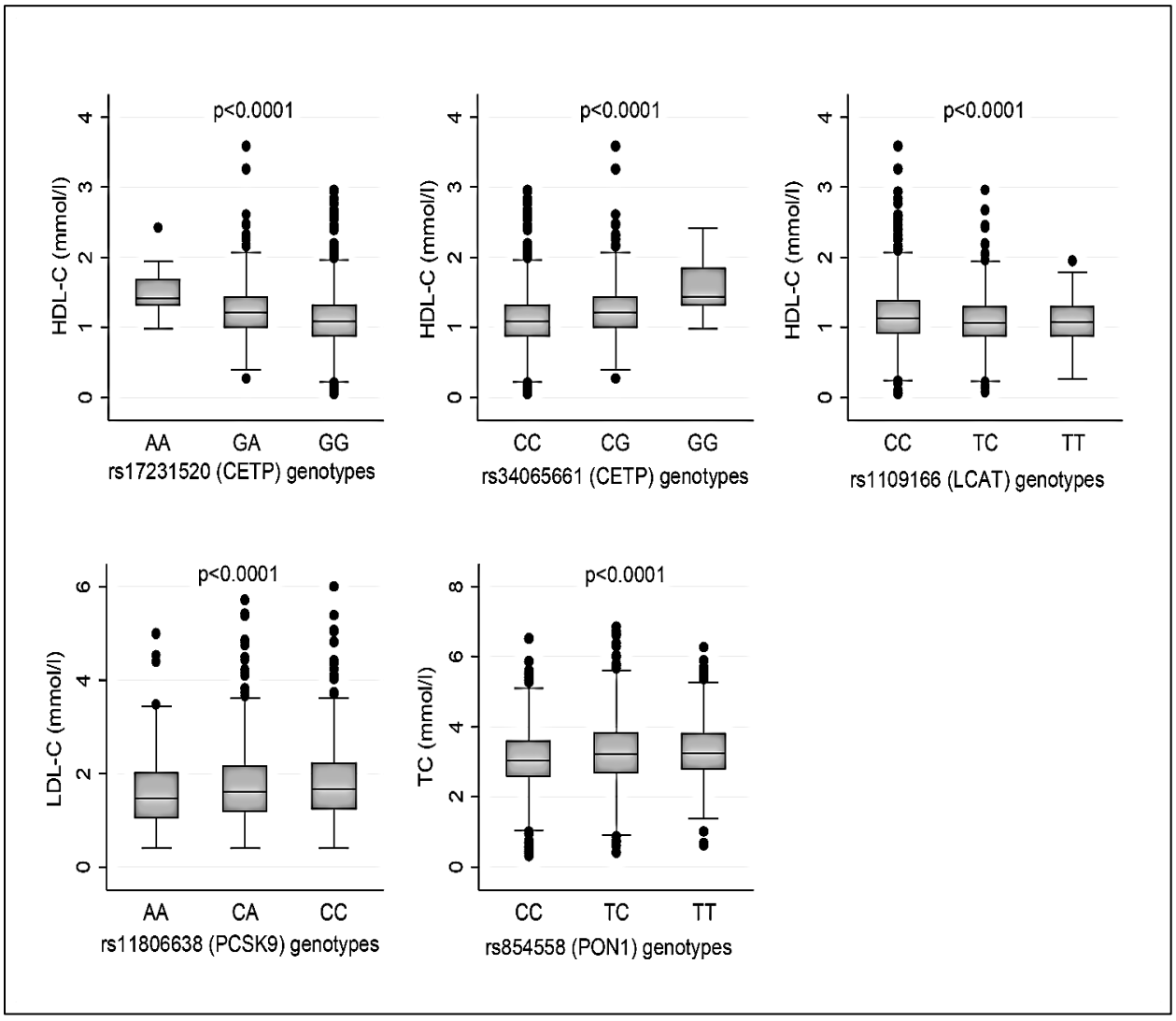

Figure 3.5: Median lipid levels for the genotypes of the lead SNVs among Ghanaian adults. The p value is adjusted for age, gender, ethnicity and waist circumference

3.3.3. Functional annotation of significantly associated variants

Results of functional analyses of the significantly associated variants are summarised in Table 3.4. The functional annotation shows that the leading associated variant (rs17231520: p=4.44e-09) in the *CETP* region was an upstream variant with an RDB score of 5 suggesting possible regulation of the rate of transcription. The second lead SNV in *CETP* (rs34065661: p=5.03e-09) was a missense variant which also had an RDB score of 5 but with a CADD score>10 which suggested a possible deleterious effect on gene structure and protein function. All other significantly associated variants were located in the introns of *CETP, LCAT, PCSK9* and *PON1* and had RDB scores of 4 or 5 which indicated possible effects on binding sites and regulation of transcription. The LoFtool score of all mutations in *PON1* was 0.787 suggesting benign susceptibility of these mutations to disease. The LoFtool scores of rs1109166 in *LCAT* and rs11806638 in *PCSK9* were 0.127 sand 0.467 respectively suggesting possibly damaging effects of the mutations on gene function.

The frequencies of the minor alleles for our lead SNVs were similar to what has been observed in 1000genomes African populations (Table 3.4). Notably, some of our associated variants, including rs17231520 and rs34065661 in *CETP*, are observed only in those with African ancestry.

Table 3.4: Functional annotations of lipid trait associated SNVs in Ghanaian adults and their minor allele frequencies in comparison to other population groups

Variant(Gene)	Localization	CADD score	RDB score	LoFtool score	Minor allele	Minor allele frequencies per population				
						Study population	Africa	Europe	East Asia	South Asia
rs17231520(*CETP*)	Upstream variant	4.38	5	0.970	A	0.089	0.082	0.000	0.000	0.000
rs34065661(*CETP*)	Missense variant	12.64	5	0.970	G	0.090	0.083	0.000	0.000	0.000
rs711752(*CETP*)	Intron variant	8.46	5	0.970	A	0.246	0.246	0.426	0.375	0.451
rs708272(*CETP*)	Intron variant	0.46	5	0.970	A	0.245	0.247	0.425	0.375	0.448
rs891142(*CETP*)	Intron variant	2.69	--	0.970	T	0.098	0.108	0.000	0.021	0.010
rs891143(*CETP*)	Intron variant	1.39	--	0.970	T	0.097	0.103	0.000	0.000	0.000
rs4784740(*CETP*)	Intron variant	0.86	6	0.970	G	0.091	0.096	0.000	0.000	0.000
rs3816117(*CETP*)	Intron variant	0.77	5	0.970	T	0.378	0.404	0.516	0.518	0.397
rs158478(*CETP*)	Intron variant	1.57	--	0.970	C	0.300	0.284	0.524	0.555	0.428
rs891141(*CETP*)	Intron variant	0.17	5	0.970	G	0.168	0.153	0.001	0.021	0.013
rs289719(*CETP*)	Intron variant	2.45	5	0.970	T	0.440	0.441	0.330	0.310	0.452
rs1109166(*LCAT*)	Intron variant	3.06	4	0.217	T	0.219	0.260	0.815	0.904	0.761
rs11806638(*PCSK9*)	Intron variant	3.86	5	0.467	A	0.396	0.356	0.064	0.027	0.064
rs854558(*PON1*)	Intron variant	1.92	--	0.787	T	0.385	0.340	0.714	0.702	0.614
rs854564(*PON1*)	Intron variant	3.01	5	0.787	G	0.386	0.341	0.286	0.299	0.387
rs854565(*PON1*)	Intron variant	2.23	--	0.787	A	0.386	0.341	0.286	0.298	0.387

CADD: combined annotation dependent depletion score predicts damaging effect of the variant on protein structure and function; LoFtool: loss of function tool score predicts gene intolerance and consequent susceptibility to disease; RDB: RegulomeDB score assesses the regulatory potential of the variant; the frequencies are for the 1000 Genomes data

3.3.4. Linkage disequilibrium among significant variants

Linkage disequilibrium (LD) among significantly associated SNVs was assessed using LDlink, a web-based tool (Machiela and Chanock, 2015). The value of r^2 in *CETP* ranged from 0.000 to 1.000 (Appendix G1) while that of *PON1* ranged from 0.993 to 1.000 (Appendix G2). In *CETP*, there was complete LD between two pairs of SNVs (rs711752 with rs708272 and rs17231520 with rs34065661) and strong LD between three pairs of SNVs (rs891142 with rs891143, rs4784740 with rs891142 and rs4784740 with rs891143) (Appendix H1). All the significant variants in *PON1* were in complete LD with each other (Appendix H2).

3.3.5. Replication results

Replication results of significant variants in our population using the AADM study are shown in Table 3.5. All the *CETP* variants in both the unadjusted and adjusted models were replicated with the two lead signals being rs34065661 (β=0.122, p=1.62e-07) and rs17231520 (β=0.120, p=2.68e-07) in the replication analyses. Similarly, all signals in *PCSK9* in the unadjusted model were replicated. However in the adjusted model the significant SNV (rs11806638) in our study was not replicated (study population: β=-0.055, p=0.02683; replication: β=-0.009, p=0.4535). None of the significant SNVs in *LCAT* and *PON1* were replicated.

Table 3.5: Replication results of lipid trait associated variants in East and West Africans (AADM study)

					Without covariates		With covariates[2]	
SNV(Gene)	A1/A2	Position[1]	N	MAF	β (SE)	p value	β (SE)	p value
HDL-C								
rs17231520(*CETP*)	**A/G**	**56995827**	**4317**	**0.039**	**0.2688 (0.0431)**	**5.04e-10**	**0.1202 (0.0233)**	**2.68e-07**
rs34065661(*CETP*)	**G/C**	**56995935**	**4317**	**0.039**	**0.2720 (0.0426)**	**1.94e-10**	**0.1218 (0.0232)**	**1.62e-07**
rs711752(*CETP*)	**A/G**	**56996211**	**4317**	**0.112**	**0.1574 (0.0267)**	**3.84e-09**	**0.0733 (0.0149)**	**8.50e-07**
rs708272(*CETP*)	**A/G**	**56996288**	**4317**	**0.112**	**0.1572 (0.0267)**	**3.94e-09**	**0.0734 (0.0149)**	**8.83e-07**
rs891142(*CETP*)	**T/C**	**57003977**	**4317**	**0.445**	**0.1609 (0.0381)**	**2.44e-05**	**0.0776 (0.0208)**	**0.00019**
rs891143(*CETP*)	**T/C**	**57003980**	**4317**	**0.455**	**0.1890 (0.0397)**	**2.05e-06**	**0.0975 (0.0218)**	**8.07e-06**
rs4784740(*CETP*)	**G/C**	**57001085**	**4317**	**0.041**	**0.2055 (0.0413)**	**6.89e-07**	**0.1055 (0.0227)**	**3.42e-06**
rs3816117(*CETP*)	**T/C**	**56996158**	**4317**	**0.285**	**-0.1134 (0.0228)**	**6.95e-07**	**-0.0480 (0.0125)**	**0.00012**
rs158478(*CETP*)	**C/A**	**57007734**	**4317**	**0.151**	**0.0327 (0.0246)**	**0.18320**	**0.0113 (0.0138)**	**0.41120**
rs891141(*CETP*)	**G/T**	**57003723**	**4317**	**0.427**	**0.1638 (0.0322)**	**3.82e-07**	**0.0902 (0.0176)**	**3.24e-07**
rs289719(*CETP*)	**T/C**	**57009941**	**4317**	**0.284**	**0.07487 (0.0223)**	**0.00081**	**0.0415 (0.0125)**	**0.00094**
rs1109166(*LCAT*)	T/C	67977382	4317	0.382	-0.0302 (0.0272)	0.26750	-0.0003 (0.0147)	0.98330
LDL-C								
rs11806638(*PCSK9*)	A/C	55518160	4287	0.189	-0.0826 (0.0238)	0.00051	-0.0089 (0.0147)	0.45350
rs11804420(*PCSK9*)	G/A	55520445	4287	0.128	-0.1151 (0.0265)	1.42e-05	-0.0279 (0.0118)	0.03157
rs11800231(*PCSK9*)	A/G	55517940	4287	0.099	-0.1035 (0.0292)	0.00039	-0.0258 (0.0130)	0.07349
rs45545732(*PCSK9*)	T/A	55519231	4287	0.115	-0.09416 (0.0274)	0.00060	-0.0257 (0.0134)	0.05602
TC								
rs854558(*PON1*)	T/C	94945374	4317	0.323	0.03918 (0.0237)	0.09874	0.0149 (0.0087)	0.08815
rs854564(*PON1*)	G/T	94948182	4317	0.322	0.03824 (0.0237)	0.10680	0.0150 (0.0087)	0.08526
rs854565(*PON1*)	A/G	94948344	4317	0.322	0.03677 (0.0237)	0.12050	0.0144 (0.0087)	0.09786
rs854566(*PON1*)	A/G	94948749	4317	0.329	0.03672 (0.0237)	0.12400	0.0153 (0.0088)	0.08096
rs854567(*PON1*)	A/G	94948784	4317	0.329	0.03827 (0.0237)	0.10890	0.0163 (0.0088)	0.06418

A1/A2: Minor/major allele; MAF: Minor allele frequency in AADM study; pHWE: Hardy-Weinberg p value; N: total number of participants; SE: Standard error; [1]Genomic position and chromosome in build 37; [2] Covariates were age, gender, waist circumference and T2D; results of replicated variants indicated in bold

3.4. Discussion

This study on adults in northern Ghana evaluated the associations between lipid levels and common nucleotide variants in the transcribed regions of eight candidate genes involved in lipid pathways. After adjustment for gender, age, ethnicity and waist circumference, SNVs in four genes (*CETP*, *LCAT*, *PCSK9* and *PON1*) were significantly associated with lipid levels. Variants in *CETP* and *LCAT* were significantly associated with HDL-C levels. One variant in *PCSK9* was significantly associated with LDL-C and another in *PON1* associated with TC levels.

Previous studies have suggested that African populations and populations of African descent tend to have higher HDL-C levels than Europeans suggesting a more favourable profile for CVD risk (Seedat *et al*., 1992; Chaturvedi *et al*., 1994; Johnson *et al*., 2004; Miljkovic-Gacic *et al*., 2006; D'Adamo *et al*., 2010). In this study higher HDL-C levels were strongly associated with an upstream variant, a missense variant and intronic variants in the *CETP* gene. The lead SNV (rs17231520) was in the upstream regulatory region and functional prediction suggests an impact on the rate of transcription. The high CADD score of the associated missense variant (rs34065661) is interesting as it suggests a deleterious effect on protein function. The two lead SNVs in *CETP* may be the causal variants for the region for high HDL-C in West Africans since they are functionally relevant, their effect sizes are larger than those of the others and they are common in African populations (>0.08), and were not observed in 1000 Genomes European and Asian populations. Additionally we identified several intronic SNVs in the *CETP* gene that were positively associated, except rs3816117, which was negatively associated, with HDL-C levels. Functional analysis suggests these intronic variants regulate gene transcription rate but their low CADD scores indicate that the effect of their regulatory activity on gene function is benign. The significantly associated variants in *CETP* probably impaired the ability of the gene product to accelerate the transfer of cholesteryl esters (CE) from cholesteryl ester-rich HDL-C formed by *LCAT* to other lipoprotein particles resulting in increased HDL-C levels (Brown *et al.*, 1989).

Most of the SNVs in the *CETP* gene replicate previous GWAS findings in peoples of African descent (Buyske *et al.*, 2012; Elbers *et al.*, 2012; Pirim *et al.*, 2016). Our replication analysis, using the African American diabetes mellitus (AADM) study involving over 4000 East and West Africans (Rotimi *et al.*, 2001), reveals that the directions of effects of the SNVs in *CETP* were consistent with

all those in our dataset. The minor allele frequencies of these variants in our population were similar to the allele frequencies in other Africans both in the 1000 Genomes data and the replication population but the effect sizes of the variants in our population were larger (Tables 3.3 and 3.5). The rs17231520 (A) and rs34065661 (G) alleles which are absent in European and Asian populations, were previously found to be positively associated with HDL-C levels among African Americans (Buyske *et al.*, 2012; Elbers *et al.*, 2012) and Black Africans (Pirim *et al.*, 2016). As in our study, the two variants were in strong LD in all these studies. In these studies the frequencies of the rs17231520 (A) and rs34065661 (G) alleles were similar to those in our populations but the effect sizes of these alleles were smaller in our study. Similarly our study replicated rs711752, rs708272, rs891141, rs891143 and rs289719 variants in *CETP* that were previously found to be associated with HDL-C in black Africans by Pirim et al. 2016. Though the MAFs of these variants in this African population were similar to those in our study, the effect sizes of the variants were generally larger in our population.

In our study the minor allele (T) of rs1109166 in *LCAT* was negatively associated with HDL-C levels. Since the T allele is the common non-African allele (0.76 to 0.90 in Europeans and Asians), its association with low HDL-C in Africans may not be functionally relevant, despite the fact that the low LoFtool score predicts a deleterious effect for this intronic variant (Le Hir et al., 2003; Chorev and Carmel, 2012). It may be tagging other functionally relevant variants through LD, which could interfere with the ability of *LCAT* to form CE and to promote cholesterol efflux from peripheral cells. Furthermore, this variant was not associated with HDL levels in the replication study. Our study is the first to report this association in an African population and requires further investigation. Meanwhile, a previous study on this population reported high prevalence of low HDL-C (>60%) (Agongo *et al.*, 2018). This suggests that in addition to the *LCAT* variant, there may be other variants, which may have been overlooked, that contribute more strongly to the low HDL-C levels in this population.

PCSK9 binds to the LDL receptor (LDLR) to form a complex which moves from the endosomal recycling pathway to the lysosome for degradation (Akram *et al.*, 2010). Since LDLR removes cholesterol-rich LDL particles from plasma (Poirier *et al.*, 2008), a loss of function or reduction in PCSK9 activity inhibits LDLR degradation and leads to the reduction of LDL-C in the blood (Park et al. 2004). In this study the rs11806638 (A) allele in *PCSK9* was negatively associated

with LDL-C level. Though we did not replicate this variant association in other black Africans from the AADM study (Rotimi *et al.*, 2001) the rs11806638 (A) allele was previously associated with lower LDL-C levels in African Americans (Musunuru *et al.*, 2012). Interestingly the allele frequency and effect size of the association in African Americans were similar to those in our population.

PON1 encodes an enzyme that exists within the HDL particle and protects HDL and LDL from peroxidation by degrading or hydrolyzing specific oxidized cholesteryl esters and phospholipids contained in oxidized lipoproteins (Barter *et al.*, 2004). This process contributes mainly to the antioxidation properties of the HDL particle which results in improved macrophage-mediated cholesterol efflux (Parthasarathy et al. 1990). Genetic variation in the gene is therefore associated with increased TC levels. Our results show positive associations of rs854558-T, rs854564-G, and rs854565-A alleles in *PON1* with TC in the study population. To the best of our knowledge this study is the first to report the association of these variants with TC levels, although other studies have shown associations of other SNVs in the *PON1* gene with serum lipid levels (Chang *et al.*, 2010; Bentley *et al.*, 2014; Luo *et al.*, 2018). The similar association observed in the lead variant and other signals (rs854564 and rs854565) in the gene with TC levels could be due to the complete LD between these signals and the lead variant. Though the frequency of the variant allele of rs854558 is substantial (0.385) in the study population, its effect size is small and it is unlikely to play a major role in hypercholesterolaemia and CVD risk.

Interestingly, there were no associations of variants of any of the selected genes with serum TG concentrations in the study population even though TG levels in African populations are typically lower than their European counterparts (Frank *et al*., 2014). The lack of association with TG levels could be due to the selection of only eight genes in the study and the probable exclusion of those that modulate TG levels in African populations.

In terms of limitations, the sample size was relatively small and therefore underpowered to detect variants with small effect sizes. However, power calculations suggested that there was sufficient power to replicate previously reported gene associations with lipids. Candidate genes were selected from studies involving people of African descent, but not resident in Africa, and thus genes chosen may not be appropriate for indigenous African populations. Furthermore, only eight genes were

included in the study and therefore additional genes that contribute more strongly to modulating lipid levels may have been overlooked. This is particularly so, when findings of a previous study in this population reported that over 60% of the study participants had low HDL-C levels (Agongo *et al.*, 2018), although results of the current study identified a strong positive association of HDL-C levels with *CETP* variants . Other genes with stronger modulating effects could be contributing to these low levels of HDL-C in this population. However, a GWAS for lipid levels is underway for the entire AWI-Gen dataset. The Bonferroni method to reduce false discovery rate assumes that pairwise tests are independent and this is considered by many to be overly conservative, as many SNVs from the same gene locus would be in linkage disequilibrium. Therefore results from sets of tests may not be independent (Lewis 2002). Another limitation of the study was that self-reported ancestry was not compared against genetic ancestry. Also naming of the locus using the gene and variants mapping to that gene may have excluded signals that do not map to the gene, but map nearby. We equally are mindful of the potential masking effect of comorbidity (hypertension, diabetes and stroke) in our study sample on the signals of association with lipid levels.

In conclusion the findings showed that several variants in *CETP*, *LCAT*, *PCSK9* and *PON1* in this Ghanaian population were significantly associated with HDL-C, LDL-C and TC with the strongest signal being that of rs117231520 (*CETP*) with serum HDL levels. Some of these variants replicate previously reported lipid trait GWAS loci in African Americans and strengthen the evidence of the influence of these signals on lipid levels in peoples of African descent. In addition to our study being the first of its kind to investigate association of genetic polymorphisms with lipid levels in Ghanaians it is also the first to show an association of rs854558, rs854564 and rs854565 in *PON1* with TC levels

The influence of genetic variants on lipid levels is modulated by environmental factors (Yin *et al.*, 2012; Zhang *et al.*, 2019). The next chapter of this study therefore investigated the effect of the interaction of selected environmental factors with gene variants from selected candidate genes on lipid levels.

3.5 Contribution of candidate to the study

I collected the data, designed the study, analysed the data and wrote the first and final drafts of the thesis chapter and the submitted manuscript on which I am the first author.

CHAPTER 4: INTERACTION BETWEEN *ABCA1* AND *CETP* SINGLE NUCLEOTIDE VARIANTS AND TOBACCO SMOKING INFLUENCES TOTAL CHOLESTEROL AND TRIGLYCERIDE LEVELS IN GHANAIAN ADULTS: NOVEL FINDINGS OF AN AWI-GEN SUB STUDY

4.1. Introduction

Cardiovascular disease (CVD) is associated with a cluster of risk factors among which is unfavourable blood lipids, including high levels of low density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglycerides (TGs) and low levels of high density lipoprotein cholesterol (HDL-C) (Lewington *et al.*, 2007; Sarwar *et al.*, 2007; Di Angelantonio *et al.*, 2009). Lipid levels vary among individuals and populations (Ferris *et al.*, 2005; Bentley and Rotimi, 2012) and these variations are due to the combined effect of genetic polymorphisms (Bentley *et al.*, 2014), environmental factors (Campbell and Tishkoff., 2008; Wakabayashi, 2014; Abd *et al.*, 2016) and their interactions (Yin *et al.*, 2012; Zhang *et al.*, 2019). A difference in the effect size of a lipid trait for a genetic variant in individuals with two different environmental exposures suggests the presence of a gene–environment interaction, rather than simply additive effects of the two exposures (Culverhouse and Suarez, 2007). The interactions between genetic risk factors and environmental phenotypes may explain some of the missing heritability of serum lipid traits.

Though studies on gene-environment (GxE) interactions are emerging on a genomic scale, including studies on lipid traits, there is a paucity of data on the influence of these interactions on lipid levels in black Africans. A recent review on the influence of GxE interaction on dyslipdaemia showed results of studies on mainly Europeans, Americans and Asians with none on sub-Saharan Africans (Cole *et al.*, 2015). GxE interactions reveal the susceptibility of individuals to negative or positive effects of environmental factors on lipid levels through the modulation of genetic factors. These investigations are therefore important in the identification of environmental factors that could counteract or aggravate genetic risk to dyslipidaemia in black Africans.

In the Kassena-Nankana districts of northern Ghana prevalence of alcohol consumption (84.77%), tobacco smoking (10.82%) and pesticide exposure (54.70%) are substantially high among men and women as opposed to obesity which is low (2.66%) (Agongo *et al.*, 2018). Though moderate alcohol

consumption is reportedly associated with favourable lipid profiles (Kechagias *et al.*, 2011; Ahaneku *et al.*, 2014) excess alcohol intake influences dyslipidaemia and is detrimental to cardiovascular health outcomes (Wakabayashi, 2013a). Similarly there are reports of association of unfavourable lipid levels with pesticide exposure (Ntzani *et al.*, 2013) and tobacco smoking (Corella *et al.*, 2002; Yin *et al.*, 2012). There is evidence of the modulating effect of alcohol consumption (Liu *et al.*, 2011; Tan *et al.*, 2012) and tobacco smoking (Lee *et al.*, 2004) on genetic factors to influence lipid levels in non-Africans but data on the effect of these gene-environment interactions on lipid levels in black Africans is lacking. Similarly there is no known study that has investigated the effect of pesticide exposure and gene interaction on lipid levels. This study therefore assessed the influence of the interaction between alcohol consumption, tobacco smoking and pesticide exposure on one hand and genetic variants for selected loci (*ABCA1*, *CETP*, *LCAT*, *LPL*, *MMAB*, *MVK*, *PCSK9* and *PON1*) on the other, on lipid levels in northern Ghanaian adults.

4.2. Methods

4.2.1. Study population

This GxE study was part of the wider H3Africa AWI-Gen study (Ramsay *et al.*, 2016) conducted among men and women aged 40-60 years in the two Kassena-Nakana districts of northern Ghana (Oduro *et al.*, 2012). A sample size of 2016 were recruited into the study (Agongo *et al.*, 2018). In calculating the sample for this study Qunato was used (Gauderman, 2002). Information of a study that reported a mean TC level in Chinese of 4.82±1.05mmol/l with an effect size of 0.406mmol/l for the E670G in the *PCSK9* gene at a ~5.0% minor allele frequency (MAF) was considered at 5% nominal significance level (Aung *et al.*, 2013). The Chinese study was used in the power calculation because there was no reported GxE study involving any of the selected genes in Africans and no reported GxE study involving any of the selected genes and lipid traits. Considering prevalence of alcohol consumption of 84.77% in the study population (Agongo *et al.*, 2018) and assuming an additive model the study had >90% power to detect an effect size of at least 0.013mmol/l for lipid traits at MAF>0.05 for a sample size of 1800 individuals.

4.4.2. Data collection

Self-reported socio-demographic variables including age, ethnicity and socio-economic status and environmental factors such as alcohol consumption, pesticide exposure and tobacco smoking were

collected using a structured questionnaire. These variables are defined elsewhere *(*Agongo *et al*., 2018). Whole blood was collected in EDTA as anticoagulent for DNA extraction and serum lipid analyses (Ali *et al.*, 2018). Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were all measured directly using an automated analyzer (Agongo *et al.*, 2018). Ethics approval was received from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M151050) (Appendix C1) and the Navrongo Health Research Centre Institutional Review Board (ID No: NHRCIRB178) (Appendix C2). The study was part of the wider H3Africa AWI-Gen project that had prior approval form the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M121029) (Appendix C3). Community engagement was carried out and signed or thumb-printed informed consent was obtained from each participant prior to the commencement of the study.

4.2.3. Gene selection

Eight candidate genes (*ABCA1, CETP, LCAT, LPL, MMAB, MVK, PCSK9* and *PON1*) were selected based on prior knowledge of their association with lipid levels in peoples of African descent (Adeyemo *et al.*, 2012; Elbers *et al.*, 2012; Bentley *et al.*, 2014) and black Africans (Abessolo *et al.*, 2014; Niemsiri *et al.*, 2015).

4.2.4. DNA extraction, genotyping and imputation

DNA was extracted manually using a modified salting out method (Diego Chacon-Cortes, 2014). Genotyping was performed using the H3Africa single nucleotide variant (SNV) array chip designed on the Illumina platform (https://www.illumina.com/services/sequencing-services.html). The array is enriched for common variants in African populations and contains over 2.3 million SNVs (Mulder *et al.*, 2018). Quality control was performed on the entire AWI-Gen dataset of 10,903 individuals by removing individuals with a missing call rate >0.05 and SNVs with a missing call rate >0.05. Variants with minor allele frequency (MAF) >0.001 and Hardy-Weinberg equilibrium p value <0.0001 were removed from the dataset. The cleaned dataset (with 1,729,661 SNVs and 10,903 individuals) was, pre-phased with EAGLE2 (Loh *et al.*, 2016) and imputed using the Sanger Imputation Server with the African reference panel. The default positional Burrows-Wheeler transform PBWT algorithm was used for imputation. At post imputation QC, poorly imputed SNVs (SNVs with IMPUTE2 information score <0.6), SNVs with MAF <0.01 and HWE p-value <0.00001

were excluded resulting in the final quality controlled imputed dataset. The data for this study was extracted from the final dataset with 13,980,000 SNVs, using the genome coordinates from each of the candidate genes (from the start to end of transcription, including intronic regions).

4.2.5. Data quality control process

Individuals in the AWI-Gen data set with a missing call rate>0.05 and SNVs with a missing call rate>0.05, MAF<0.01 and Hardy-Weinberg equilibrium (HWE) p-value<0.0001 were removed from the data set. Furthermore individuals with mismatch in recorded and genotyped sex, with relatedness, and/or with extreme heterozygosity deviations were excluded. SNVs that did not match the GRCh37 reference alleles or strands were also removed. The cleaned dataset (with 1,729,661 SNVs and 10,903 individuals) was, pre-phased with EAGLE2 and imputed using the Sanger Imputation Server with the African reference panel. The default Positional Burrows-Wheeler transform (PBWT) algorithm was used for imputation. At post imputation QC, poorly imputed SNVs (SNVs with IMPUTE2 information score<0.6), SNVs with MAF<0.01 and HWE p-value<0.00001 were excluded resulting in the final quality controlled imputed dataset containing 10,903 individuals and 13,980,000 SNVs. All SNVs with MAF <0.05 in the Ghanaian data were excluded from the study dataset and the final number of SNVs used from each gene for the analysis shown in Table 4.41. Participants with phenotype variables with missing data and data with zero values were excluded from the study data set resulting in a sample size of 1807 participants.

Table 4.1: Number of variants tested in selected candidate genes following quality control

Gene(Chromosome)	Transcribed region (start transcript-end transcript)	Number of SNVs
LPL(8)	19,759,228-19,824,769	286
LCAT(16)	67,973,653-67,978,034	6
CETP(16)	111,682,249-111,727,724	117
PON1(7)	94,926,988-94,954,019	94
ABCA1(9)	107,543,283-107,690,518	632
MVK(12)	110,011,060-110,035,922	55
MMAB(12)	109,991,542-110,011,679	84
PCSK9(1)	55,505,221-55,530,525	118

4.2.6. Statistical analyses

Gender was compared across environmental subgroups using Pearson χ^2 test. Age and lipid levels were presented as means with standard deviations (SD) using Student's t-test and were log-transformed and compared across environmental subgroups using analysis of covariance (ANCOVA). Gene-environment interaction (GxE) analyses were performed by testing the association of lipid levels (HDL-C, LDL-C, TC and TG) with SNVs of eight genes (*ABCA1*, *CETP*, *LCAT*, *LPL*, *MMAB*, *MVK*, *PCSK9*, *PON1*) under different environments (alcohol consumption, tobacco smoking and pesticide exposure). The significance levels were determined using the difference between two regression coefficients of two different environmental conditions (presence or absence) using the '-gxe' option in PLINK (Purcell *et al.*, 2007) and a threshold quality score >0.06 to call a genotype. The Bonferroni correction (Bland and Altman, 1995) for each lipid trait took into account the number of variants tested in that trait with all Bonferroni-adjusted p-values at 5% significance level being considered significant.

4.2.7. Functional analyses

Functional annotation of the significant SNVs was assessed using Variant Effect Predictor (McLaren *et al.*, 2016).

4.3. Results

4.3.1. Characteristics of the study participants

The basic characteristics of the study participants, stratified by different environmental subgroups, are presented in Table 4.2. Current alcohol consumption ($p<0.001$), current pesticide exposure ($p<0.001$) and current tobacco smoking ($p<0.001$) were significantly higher among men than women. There were no significant age differences between current alcohol consumers and non-consumers ($p=0.988$). However the average age of those who were currently exposed to pesticide was significantly higher than those without pesticide exposure ($p<0.001$) and average age of current smokers was marginally higher than nonsmokers ($p=0.043$). HDL-C levels were higher in current alcohol consumers ($p<0.001$), lower in those who were currently exposed to pesticides ($p=0.038$) and higher in current tobacco smokers than in non-alcohol consumers, those without current pesticide exposure and nonsmokers respectively. Those who were currently exposed to pesticides had higher

TC levels than those without pesticide exposure (p=0.019). No differences in TG levels existed among the different environmental subgroups.

Table 4.2: Characteristics of the study population stratified by different environment subgroups

Variable	Current alcohol consumption			Current pesticide exposure			Current tobacco smoking		
	Yes	No	p value	Yes	No	p value	Yes	No	p value
Gender*			<0.001			<0.001			<0.001
Women	366 (38.13)	594 (61.88)		464 (48.28)	497 (51.72)		5 (0.52)	956 (99.48)	
Men	554 (65.48)	292 (34.52)		495 (58.51)	351 (41.49)		191 (22.55)	656 (77.45)	
Age (years)	51.06 ± 5.7	51.06 ± 5.9	0.988	50.55 ± 5.82	51.68 ± 5.67	<0.001	51.84 ± 5.43	51.00 ± 5.81	0.043
HDL-C (mmol/l)	1.18 ± 0.39	1.10 ± 0.36	<0.001	1.12 ± 0.37	1.16 ± 0.39	0.038	1.19 ± 0.44	1.13 ± 0.37	0.044
LDL-C (mmol/l)	1.74 ± 0.79	1.73 ± 0.76	0.801	1.73 ± 0.77	1.74 ± 0.79	0.959	1.76 ± 0.83	1.73 ± 0.77	0.639
TC (mmol/l)	3.20 ± 0.92	3.22 ± 0.91	0.577	3.26 ± 0.94	3.16 ± 0.89	0.019	3.17 ± 0.90	3.21 ± 0.92	0.544
TG (mmol/l)	0.64 ± 0.35	0.64 ± 0.50	0.999	0.65 ± 0.52	0.63 ± 0.30	0.304	0.63 ± 0.34	0.64 ± 0.44	0.723

All variables were given as mean ± SD except *gender which was given as number (%).

4.3.2. GxE Interactions

Only men were used in the gene-tobacco smoking analysis due to the low prevalence of current tobacco smoking among women (0.52%). When the effect of the interaction between current smoking in men and *ABCA1* SNVs on TC levels was tested the interaction effect was statistically significant for rs10124686 (p=0.041). The interaction between tobacco smoking and *ABCA1* (rs10124686) was associated with lower levels of TC in men who were current non-smokers (β=-0.042) and associated with higher TC levels in men who were current smokers (β=0.276). The interaction between *CETP* (rs34620476) and tobacco smoking in men was significant (p=0.020) and was associated with higher TG levels in non-smokers (β=0.020) and lower TG levels in smokers (β=-0.160). No significant interactions were identified between alcohol consumption and pesticide exposure and any of the selected genes that influenced lipid levels in the study population (Table 4.3).

Table 4.3: Gene-tobacco smoking interaction association with TG and TC levels in Ghanaian men (n=846)

Gene(SNV)	Chr(Pos)	A1/A2	MAF	n_1	β_1 (S.E.)	n_2	β_2 (S.E.)	$p_{R_}$GxE value	$p_{B_}$GxE value
TC									
ABCA1(rs10124686)	**9(107553621)**	**A/G**	**0.072**	**1644**	**-0.042 (0.031)**	**198**	**0.276 (0.073)**	**6.47e-05**	**0.0409**
ABCA1(rs10115901)	9(107557574)	A/G	0.052	1644	-0.024 (0.036)	198	0.350 (0.089)	9.54e-05	0.0603
ABCA1(rs28690796)	9(107561452)	C/T	0.052	1644	-0.018 (0.036)	198	0.350 (0.089)	1.22e-04	0.0771
ABCA1(rs10115901)	9(107557574)	A/G	0.052	1644	-0.015 (0.036)	198	0.332 (0.088)	2.51e-04	0.1586
TG									
CETP(rs34620476)	**9 (56996649)**	**A/C**	**0.156**	**1655**	**0.020 (0.016)**	**199**	**-0.160 (0.045)**	**1.71e-04**	**0.0200**
CETP(rs708272)	9(56996288)	A/G	0.245	1655	0.038 (0.013)	199	-0.083 (0.039)	3.25e-03	0.3803
CETP(rs711752)	9(56996211)	A/G	0.246	1655	0.037 (0.013)	199	-0.083 (0.039)	3.50e-03	0.4098

A1/A2: minor/major allele; n_1: number of controls; n_2: number of cases; β_1: regression coefficient of controls; β_2: regression coefficient of cases; $p_{R_}$GxE: Bonferroni unadjusted gene-environment p value; $p_{B_}$GxE: Bonferroni adjusted gene-environment p value; S.E.: standard error; Bonferroni significant results in bold

The strongest signals that interacted with tobacco smoking to influence TC and TG levels are shown in Figure 4.1.

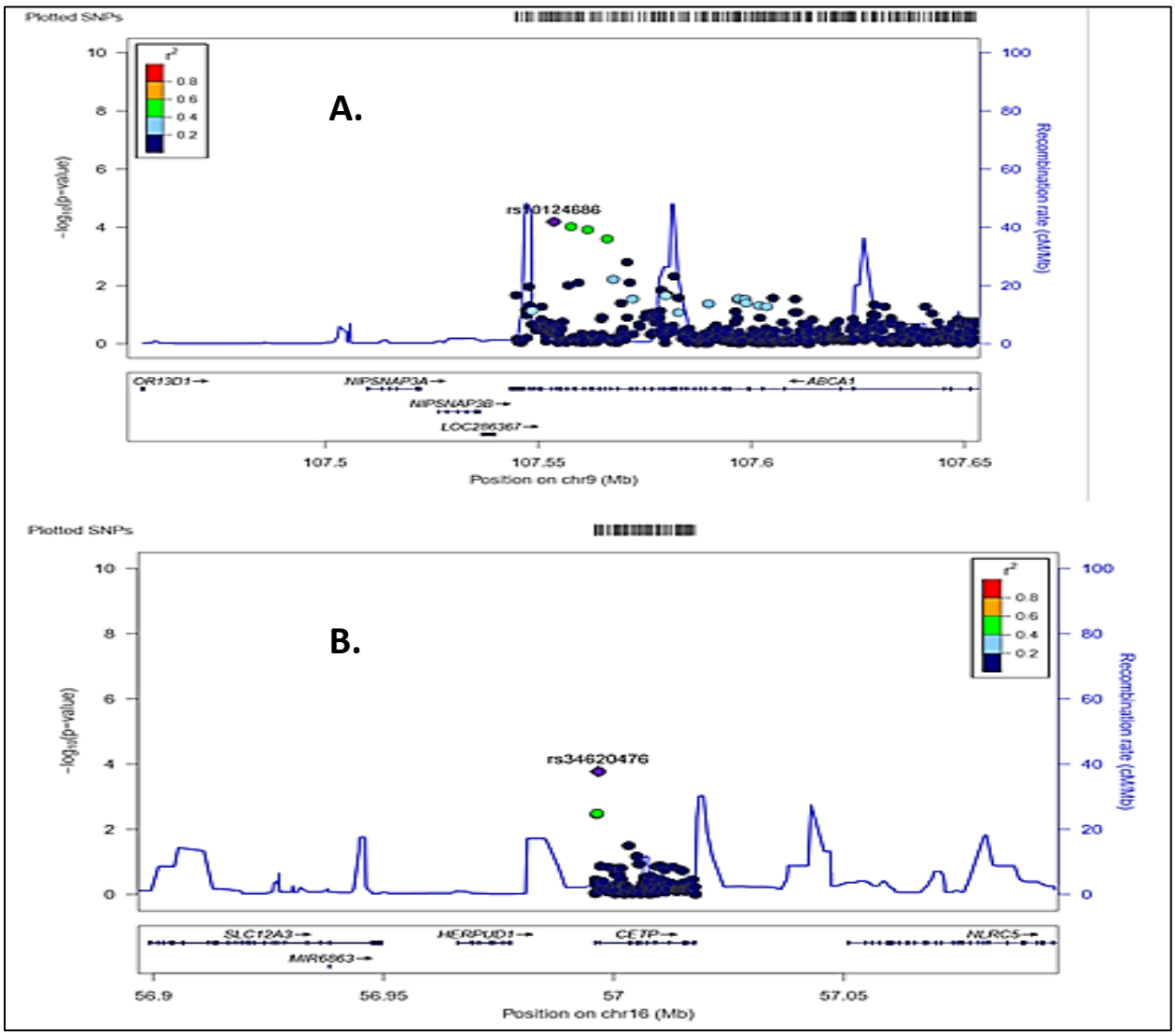

Figure 4.1: LocusZoom plots for gene-tobacco smoking interaction and lipid levels. A. *ABCA1*(rs10124686) interacts with tobacco smoking and is correlated with higher TC levels. B. *CETP*(rs34620476) interacts with tobacco smoking and is correlated with lower TG levels.

The distribution of the significantly associated SNVs among current smokers and non-smokers is shown in Table 4.4. There were only four individuals who were homozygous (AA) for the minor allele of *ABCA1*(rs10124686), 2 current smokers and 2 non-smokers. The prevalence of the homozygous state (AA) of the minor allele of *CETP*(rs34620476) was 21.74% among current tobacco smokers and 78.62% among non-smokers.

Table 4.4: Genotype distribution of significant SNVs associated with TC and TG among smoking and non-smoking Ghanaian men (n=846)

Genotypes	Smokers	Non-smokers	Total
ABCA1(rs10124686)			
AA	2 (50.00)	2 (50.00)	4 (100.00)
GA	34 (29.06)	83 (70.94)	117 (100.00)
GG	155 (21.38)	570 (78.62)	725 (100.00)
CETP(rs34620476)			
AA	5 (21.74)	18 (78.26)	23 (100.00)
CA	47 (23.62)	152 (76.38)	199 (100.00)
CC	139 (22.28)	485 (77.72)	624 (100.00

Data shown as n (%)

There is a recessive effect where the AA genotype of *ABCA1*(rs10124686) shows the highest increase in TC levels between smokers and non-smokers, but is based on only two individuals in each group. Similarly the *CETP*(rs34620476) variant shows a recessive mode of action where the AA genotype of the rs34620476 variant shows the lowest levels of TG between smokers and non-smokers (Figure 4.2).

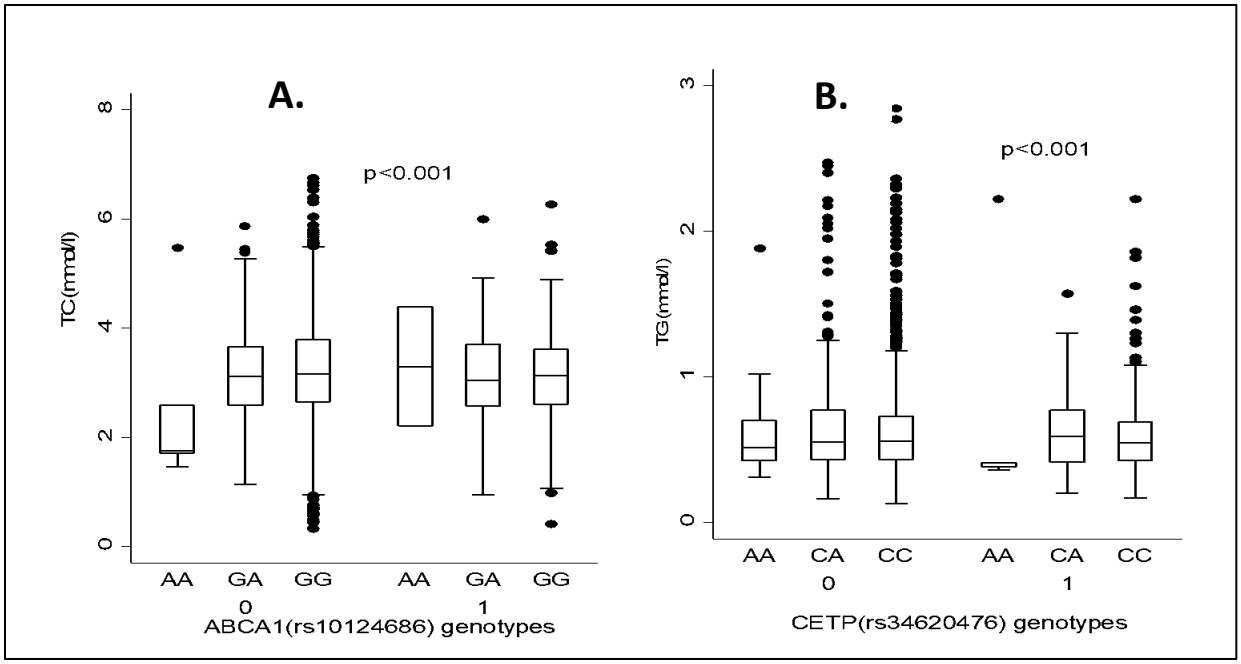

Figure 4.2: TC levels according to (A) *ABCA1*(rs10124686) genotypes and TG levels according to (B) *CETP*(rs34620476) genotypes in non-smokers (0) and smokers (1)

4.3.3. Functional analysis

Functional annotation using Variant Effect Predictor (McLaren *et al.*, 2016) revealed that rs10124686 in *ABCA1* and rs34620476 in *CETP* are intronic variants with LoFtool scores of 0.038 and 0.970 respectively. The low LoFtool score of rs10124686 in *ABCA1* indicated a probably damaging effect of the mutation on the gene structure and protein function. Each of the variants had RegulomeDB (RDB) scores of 5 suggesting a regulatory function of these variant in the rate of transcription. Comparing MAF of the variants in African populations to Europeans and Asians using Ensembl (Hubbard *et al.*, 2002) showed that the frequency of the A allele of *ABCA1* (rs10124686) in this population is comparable to that of other Africans (0.051) and higher than that in Europeans (0.013) and Asians (0.001). On the other hand just as in other Africans (0.154) the frequency of the A allele of *CETP* (rs34620476) in this population is lower than that in Europeans (0.425) and Asians (0.448) (Table 4.5).

Table 4.5: Functional annotations and MAFs of significant variants with TC and TG

Variant	Localization	LoFtool score	RDB score	Minor Allele	MAF in other populations		
					Africans	Europeans	Asians
ABCA1(rs10124686)	Intron	0.038	5	A	0.051	0.013	0.001
CETP(rs34620476)	Intron	0.970	5	A	0.154	0.425	0.448

4.4. Discussion

The study investigated the influence of gene-environment interaction of current alcohol consumption, pesticide exposure and tobacco smoking on lipid levels in a Ghanaian adult population. Only men were included in the smoking analysis because the proportion of current smoking femen participants was low (0.52%). The *ABCA1*(rs10124686) interaction with current tobacco smoking was significantly associated with higher TC levels, while the *CETP*(rs34620476) interaction with current tobacco smoking was significantly associated with lower TG levels in men.

Tobacco contains acrolein that attacks HDL-C and interferes with its cleansing ability resulting in the accumulation of total cholesterol in the blood stream (Goldenberg *et al.*, 2007). Tobacco smoking also contributes to accumulation of oxidized-LDL that leads to inflammation and higher risk of myocardial infarction and stroke (Yang *et al*., 2012). The *ABCA1* gene product mediates the transport of cholesterol from cells to lipid-poor apolipoproteins secreted by the liver (Oram and Heinecke, 2005; Rader *et al.*, 2009). The rs10124686 variant may lead to the deficiency of *ABCA1* and the interaction with tobacco smoking could be associated with cholesterol accumulation (Rader *et al.*, 2009). The A allele in *CETP*(rs34620476) impaired the ability of *CETP* to mediate the transfer of TG from very low density lipoprotein cholesterol (VLDL-C) to HDL-C and/or LDL-C in exchange for cholesteryl ester, resulting in larger TG-enriched HDL and LDL particles (Rader *et al.*, 2009; Tall, 2010). Inhibitors of CETP have been shown to increase serum HDL levels and reduce TG levels (Li *et al.*, 2013). Therefore, tobacco smoking may enhance the inhibitory effect of the A allele of *CETP* on CETP function, thus reducing TG levels.

There are reports on the influence of gene-smoking interaction on lipid levels (Lee *et al.*, 2004; Junyent *et al.*, 2009) but the significant association of TC and TG levels with *ABCA1*(rs10124686) and *CETP*(rs34620476)-tobacco smoking interaction, respectively, were not previously reported. Although these were statistically significant associations, the sample sizes were limited. Our study is the first to report these interactions in black African Ghanaians. Further studies on larger samples and more African populations are required to confirm these interactions.

In terms of the strength of this study, it is one of very few studies to evaluate the association of SNVs and gene-environment interaction with lipid levels in Africa thereby contributing knowledge on the influence of genetic factors on lipid levels in under-studied African populations. However the limitation of the study was that the sample size was relatively small and therefore underpowered to detect genetic variants with small effect sizes. Also, further GWA and GxE interaction studies in this Ghanaian population are required to confirm these findings. Another limitation is that naming of the locus using the gene and variants mapping to that gene may have excluded signals that do not map to the gene but nearby.

In conclusion the current study suggests that current smoking modulates the genetic influence on TC and TG levels in response to current tobacco smoking in men. This study is the first to show the influence of ABCA1 (rs10124686)-tobacco smoking interaction and CETP (rs34620476-tobacco smoking interaction with higher TC and lower TG levels respectively. Small group sizes can skew the results of any parametric statistical tests. Further studies with larger sample sizes are therefore required to confirm this.

4.5 Contribution of candidate to the study

I collected the data, designed the study, analysed the data and wrote the first and final drafts of the thesis chapter.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

5.1. General discussion

The study sought to determine the influence of demographic, anthropometric and environmental factors on lipid profiles and to perform genetic-association studies of variants in eight genes (*CETP*, *LCAT*, *PON1*, *ABCA1*, *LPL*, *PCSK9*, *MMAB* and *MVK* genes) with lipid profiles (HDL-C, LDL-C, TG and TC) in a rural African population and to determine the effect of gene-environment interactions on lipid profiles in this population. The results of the study show that the prevalence of hypercholesterolaemia, hypertriglyceridaemia and elevated LDL-C in the total population of 1839 (846 men and 993 women) was 4.02%, 2.12%, and 5.55% respectively and did not differ between the genders. The prevalence of low HDL-C levels was 60.30% and differed (p=0.005) between men (56.86%) and women (63.24%). Lipid level variations within the study population were associated with environmental factors, genetic polymorphisms and interactions between genetic and environmental factors.

Socio-demographic factors (age, gender, ethnicity, education and employment), behavioural factors (alcohol consumption and tobacco smoking) and anthropometric indices (BMI, visceral fat thickness, subcutaneous fat thickness, hip circumference and waist circumference) were associated with various lipid traits in the study population.

Men and women did not have significantly different lipid levels except for HDL-C which was lower in women. There are inconsistent reports of gender differences in lipid levels in Africans with some reporting more favourable lipid profiles in women (Borgo *et al.*, 2018), others in men (Asiki *et al.*, 2015) and yet others reporting no differences (Adamu *et al.*, 2013; Ogunmola *et al.*, 2013). Consistent with previous findings, age was significantly associated with higher LDL-C, TC and TG levels among women in this population (Goh *et al.*, 2007; Okęcka-Szymańska *et al.*, 2011). Several possible causes have been suggested for the positive association of age with lipid levels (Auley and Mooney, 2015) including hormonal level changes in menopause transition (Pardhe *et al.*, 2017). The association of higher educational status and employment with higher LDL-C and TC levels respectively in this population is consistent with previous findings in Africa (Adedoyin *et al.*, 2013) and elsewhere (Reddy *et al.*, 2012; Rodriguez *et al.*, 2014;

Shohaimi *et al.,* 2014) and confirms the observation that economic transition with its associated rising income levels in Africa is closely associated with gradual increase in CVD risk (Sliwa *et al.*, 2008; Stewart *et al.*, 2011; Adeboye *et al*., 2012). Though the prevalence of obesity was low in this population (2.66%) anthropometric (obesity-related) indices (waist circumference and body adipose fat) were strongly associated with higher lipid levels. Triglyceride was negatively associated with hip circumference and this is consistent with other findings (Snijder *et al.,* 2004). Hip circumference is a good measure of gluteofemoral fat which has been shown to protect against CVD risk factors such as insulin resistance, hypertension and dyslipidaemia (Snijder *et al.*, 2004; Ruige and Van Gaal, 2009).The association of anthropometry with higher lipid levels in the population is similar to previous findings in black Africans (Njelekela *et al.*, 2002; Crowther *et al*., 2006; Nwaiwu and Ibe, 2015). It is important to note that only a small proportion of the variance in lipid levels was explained by lifestyle and environmental factors. This is particularly so for HDL-C, for which only 1.9% of the variance is explained by these factors in this model. A greater proportion of the variance on lipid levels in this population may have been attributed to genetic variations.

The results of the study indicated that genetic factors also contribute to lipid level variation in this rural African population. Variants in *CETP*, *LCAT*, *PCSK9* and *PON1* (MAF>0.05) were associated with HDL-C, LDL-C and TC levels at $p<0.05$. The strongest associations were with rs17231520 (upstream variant) and rs34065661 (missense variant) in *CETP* with higher HDL-C levels. The lead SNV (rs17231520) was in the upstream regulatory region and functional prediction suggests an impact on the rate of transcription. Other intronic variants in the *CETP* region were also associated with HDL-C levels. The associated missense variant (rs34065661) had a high CADD score and this is interesting as it suggests a deleterious effect on protein function.

Replication analysis, using the African American Diabetes Mellitus (AADM) study involving over 4000 East and West Africans (Rotimi *et al.*, 2001), revealed that the directions of effects of the rs17231520, rs34065661, rs711752, rs708272, rs891142, rs891143, rs4784740, rs3816117, rs158478, rs891141and rs289719 variants in *CETP* were consistent with all those in this dataset. The minor allele frequencies of these variants in the study population were similar to the allele frequencies in other Africans both in the 1000 Genomes data set and replication population, but

the effect sizes of the variants in this Ghanaian population were larger. Additionally, most of the SNVs in the *CETP* gene replicate previous GWAS findings in peoples of African descent (Buyske *et al.*, 2012; Elbers *et al.*, 2012; Pirim *et al.*, 2016). Previous studies have suggested that African populations and populations of African descent tend to have higher HDL-C levels than Europeans suggesting a more favourable profile (Seedat *et al.*, 1992; Chaturvedi *et al*,, 1994; Johnson *et al.*, 2004; Miljkovic-Gacic *et al.*, 2006; D'Adamo *et al.*, 2010). The two lead SNVs in *CETP* may be the causal variants for the region for high HDL-C in Africans since they are functionally relevant, their effect sizes are larger than those of the others and they are common in African populations (>0.08), and were not observed in 1000 Genomes European and Asian populations.

Though *CETP* variants in this study population suggestively contribute to increasing HDL-C levels there may be other genetic variants that were not investigated, that may contribute more strongly in modulating HDL-C levels with net resultant low HDL-C levels. This may account for the observed low HDL-C levels previously reported in this population (Agongo *et al.*, 2018).

The study also revealed novel associations of rs11806638 (*PCSK9*) with lower LDL-C levels and rs854558 (*PON1*) with higher TC levels. The novel loss-of-function variant in *PCSK9* has clinical implications for this African population since this variant may be a therapeutic target for the management and control of CVD in this population. Further studies are however required in this population to confirm these associations.

The associations between gene-current tobacco smoking, gene-current alcohol consumption and gene-pesticide exposure interactions were assessed. The assessment of gene-tobacco smoking and lipid levels was only performed in men since a low proportion of women (0.52%) were current tobacco users. The results showed that an *ABCA1* (rs10124686) interaction with current tobacco smoking was significantly associated with higher TC levels while the *CETP* (rs34620476) interaction with current tobacco smoking was significantly associated with lower TG levels in men. Though previous reports have shown the influence of gene-smoking interaction on lipid levels (Lee *et al.*, 2004) this study is the first to report these particular interactions.

The outcome of the results of the current research therefore suggests that *CETP, LCAT, PCSK9, PON1* and *ABCA1* genes, involved in the lipid metabolic pathways, are associated with variations in lipid traits in this Ghanaian population with *CETP* and *ABCA1* exerting their influencing on lipid levels with the modulation of lifestyle factors (smoking and alcohol consumption).

5.2. Strengths and limitations of the study

This study has several strengths. The sample of over 1800 adult participants from the Kassena-Nankana district of Ghana and the population-based study design is ideal for providing general baseline data on environmental factors influencing lipid levels. Many studies use derived LDL-C levels from the Friedewald equation, whereas this used a direct measurement method and implemented stringent quality control processes (Porter *et al.*, 2009; Boshtam *et al.*, 2012). Another strength of the study is the measurement of a large set of potential environmental risk factors for dyslipidaemia. The study is anchored within a HDSS which provides an ideal platform for follow-up studies to establish the cause of dyslipidaemia among participants. Individuals were genotyped using an array enriched for gene variants that are common in African populations. It is one of very few studies to evaluate the association of SNVs and gene-environment interaction with lipid levels in Africa thereby contributing knowledge on the influence of genetic factors on lipid levels in under-studied African populations. Another strength of the study lies in its replication in the West and East African study cohort (Rotimi *et al.*, 2001) which supported the association of *CETP* variants with high HDL-C levels.

In terms of limitations, the participant age was often unknown and was estimated based on an events calendar, and therefore may not be accurate. Lifestyle data were collected based on self-reported responses from the participants and could not be independently verified. The dietary data was limited and did not provide enough detail to truly establish whether food intake was associated with lipid levels. This was a cross-sectional study that could only investigate environmental factors associated with lipid levels but could not establish causality. The sample size was relatively small and therefore underpowered to detect genetic variants with small effect sizes. However, power calculations suggested that there was sufficient power to replicate previously reported gene associations with lipids. Candidate genes were selected from studies involving people of African descent, but not resident in Africa, and thus genes chosen may not

be appropriate for indigenous African populations. Furthermore, only eight genes were included in the study and therefore additional genes that contribute more strongly to modulating lipid levels may have been overlooked. The Bonferroni method to reduce false discovery rate assumes that pairwise tests are independent and this is considered by many to be overly conservative, as many SNVs from the same gene locus would be in linkage disequilibrium. Therefore results from sets of tests may not be independent (Lewis, 2002). Replication analyses are required to confirm the association of the *ABCA1* (rs10124686)-tobacco smoking interaction and the *CETP* (rs34620476)-tobacco smoking interaction with higher TC and lower TG levels respectively. We equally are mindful of the potential masking effect of comorbidity (hypertension, diabetes, kidney disease and stroke) in our study sample on the signals of association with lipid levels. Further research is needed on gene-gene interaction and gene-environment interaction to fully elucidate the factors influencing lipid levels in this African population.

5.3. Conclusions

A longitudinal study is required to investigate whether high prevalence of low HDL-C really is a CVD risk factor in this community. The association of Nankana ethnicity with a healthier lipid profile in older adults is interesting and suggests that further studies, including genetic analyses, are required to understand this association. The association of education and employment with higher lipid levels suggests that societal advances will lead to increasing lipid levels in future, and so this must be monitored over time to allow early interventions. Likewise, the association of anthropometric variables with high lipid levels in a population with a low level of obesity suggests that any increase in the prevalence of obesity in this community may lead to higher TC and TG levels. Hence, interventions should be developed to restrict an obesogenic environment in the face of increased economic prosperity. Our findings showed that several variants in *CETP*, *LCAT*, *PCSK9* and *PON1* in this Ghanaian population were significantly associated with HDL-C, LDL-C and TC with the strongest signal being that of rs117231520 (*CETP*) with serum HDL levels. Some of these variants replicate previously reported lipid trait GWAS loci in African Americans and strengthen the evidence of the influence of these signals on lipid levels in peoples of African descent. In addition to our study being the first of its kind to investigate association of genetic polymorphisms with lipid levels in Ghanaians it is also the first to show an association of rs11806638 (*PCSK9*) with lower LDL-C levels and rs854558 (*PON1*) with higher TC levels. The

current study contributed to elucidating the genetic modification of TC and TG levels in response to current tobacco smoking in men. This study is the first to show the influence of *ABCA1* (rs10124686)-tobacco smoking interaction and *CETP* (rs34620476-tobacco smoking interaction with higher TC and lower TG levels respectively. However, further GWA and GxE interaction studies in this population are required to confirm these findings.

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7. APPENDICES

7.1. Appendix A: Plagiarism report and declaration

7.1.1. Appendix A1: Plagiarism report

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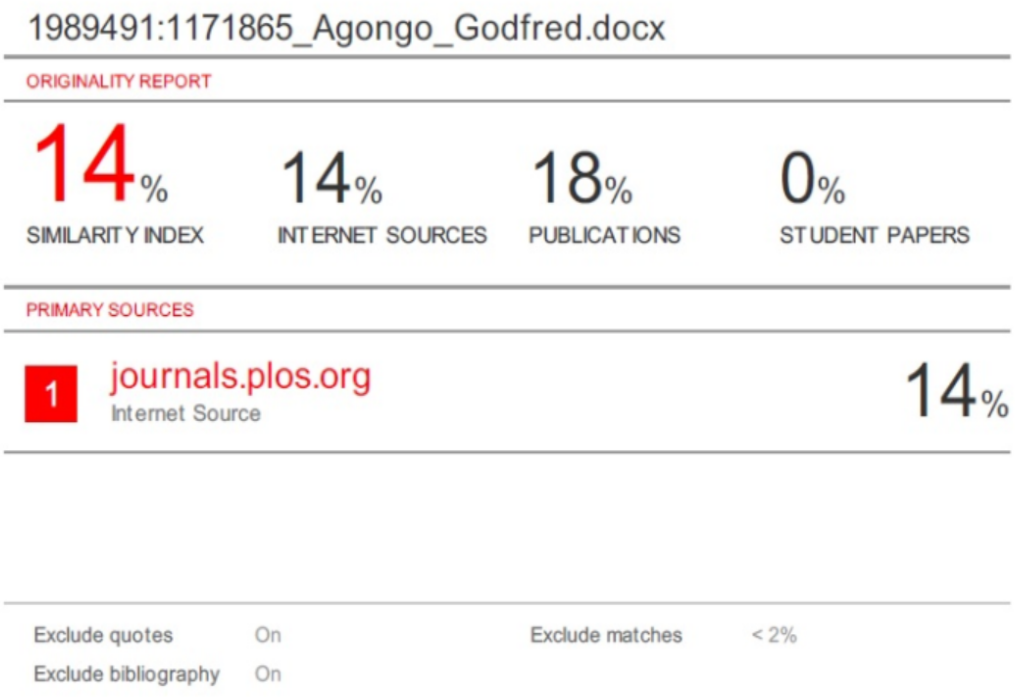

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ORIGINALITY REPORT

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PRIMARY SOURCES

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Exclude bibliography	On		

7.1.2. Appendix A2: Plagiarism declaration

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Godfred Agongo (Student number: 1171865) am a student registered for the degree of PhD in Human Genetics in the academic year 2015

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 29/03/2019

7.2. Appendix B: Co-author agreement document

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Godfred Agongo, student number [1171865], declare that this thesis is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis.

Signature of Student: ..... Date: 20/03/2019

Name of Primary Supervisor: Prof Michèle Ramsay

Signature of Primary Supervisor ..... Date: 28/3/2019

Name of 1st Co-supervisor: Nigel J Crowther

Signature of 1st Co-supervisor..... Date: 28/3/2019

Name of 2nd Co-supervisor: Abraham Rexford Oduro

Signature of 2nd Co-supervisor..... Date: 20/03/2019

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of his/her Thesis/Dissertation/Research Report. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article 1: Title: The burden of dyslipidaemia and factors associated with lipid levels among adults in rural northern Ghana: An AWI-Gen sub-study

Journal name, year, volume and page numbers: PLoS ONE, 13(11), doi.e0206326

Authors	Name	Signature	Date
2nd Author	Engelbert Adamwaha Nonterah		20-03-2019
3rd Author	Cornelius Debpuur		20-03-2019
4th Author	Lucas Amenga-Etego		20-03-2019
5th Author	Stuart Ali		28-MAR-2019
6th Author	Abraham Oduro		20-03-2019

6th Author	Nigel J. Crowther		28	9	2019
7th Author	Michèle Ramsay		25 - 4 - 2019		

Article 2: Title: Candidate gene analysis reveals strong association of *CETP* variants with high density lipoprotein cholesterol in Ghanaian adults: an AWI-Gen sub-study

Journal name, year, volume and page numbers: Frontiers in Genetics, Under review

Authors	Name	Signature	Date
2nd Author	Lucas Amenga-Etego		20-03-2019
3rd Author	Engelbert A Nonterah		20-03-2019
4th Author	Cornelius Debpuur		20-03-2019
5th Author	Stuart Ali		25-MAR-2019
6th Author	Amy R Bentley		17-03-2019
7th Author	Abraham Oduro		20-03-2019
8th Author	Charles Rotimi		19/03/19
9th Author	Nigel J. Crowther		28/3/2019
10th Author	Michèle Ramsay		28-3-2019

7.3. Appendix C: Ethics approval

7.3.1. Appendix C1: Wits Human Research Committee ethics approval

R14/49 Mr Godfred Agongo

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M151050

NAME: (Principal Investigator)	Mr Godfred Agongo
DEPARTMENT:	School of Pathology Division of Human Genetics Sydney Brenner Institute for Molecular Bioscience
PROJECT TITLE:	The Effect of Genetic Variants, Anthropometry and the Enviroment on Lipid Profiles in Adults in Northern Ghana
DATE CONSIDERED:	Adhoc
DECISION: CONDITIONS:	Approved unconditionally
SUPERVISOR:	Prof Michele Ramsay, Prof Nigel Crowther and Dr Abraham Oduro
APPROVED BY:	pp Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	23/11/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

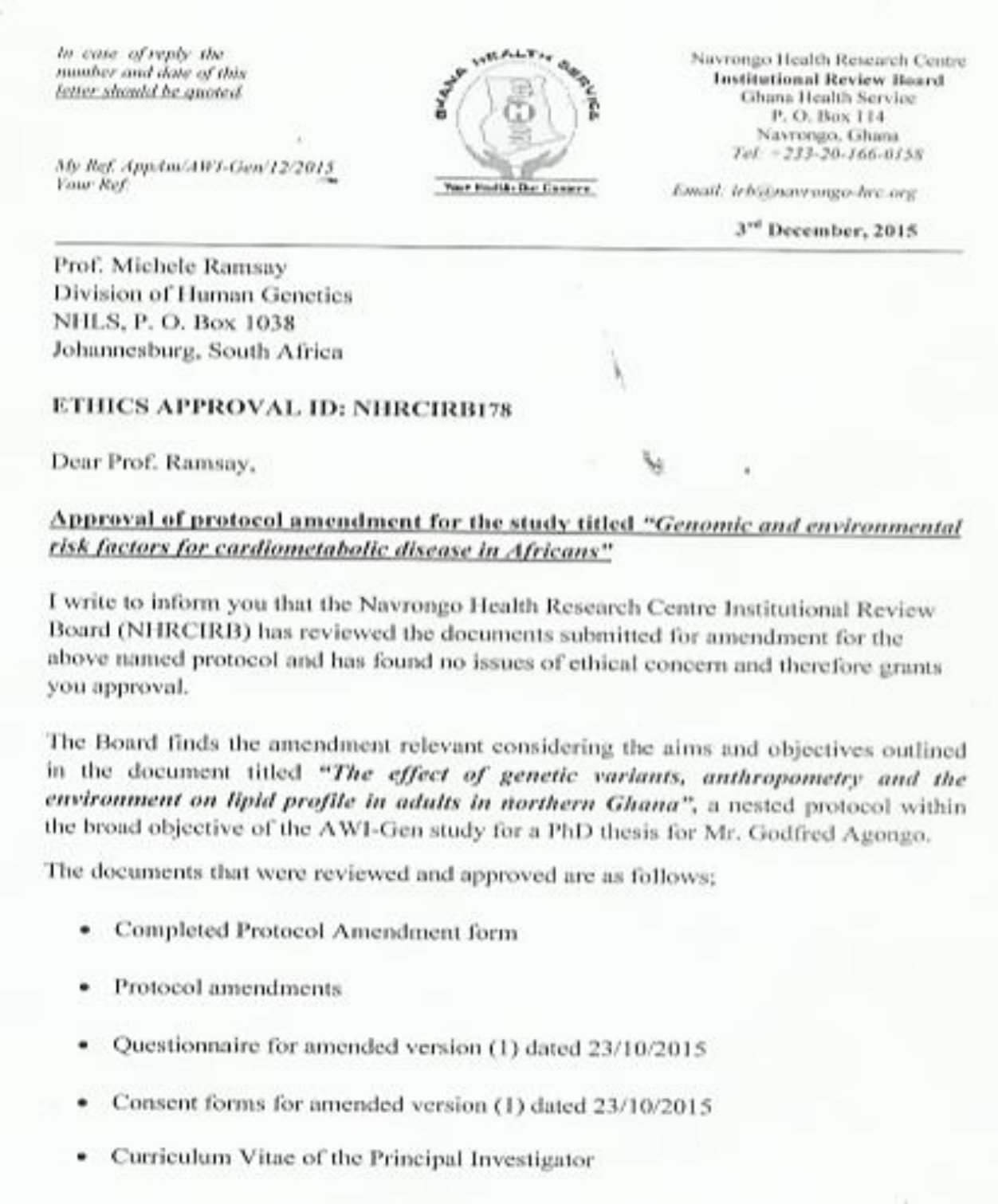
DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature Date 27/11/2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

7.3.2. Appendix C2: Navrongo Health Research Centre Institutional Review Board approval



In case of reply the number and date of this letter should be quoted

My Ref: Appiah/AWI-Gen/12/2015
Your Ref:

Navrongo Health Research Centre
Institutional Review Board
Ghana Health Service
P. O. Box 114
Navrongo, Ghana
Tel: +233-20-166-0158

Email: irb@navrongo-hrc.org

3rd December, 2015

Prof. Michele Ramsay
Division of Human Genetics
NHLS, P. O. Box 1038
Johannesburg, South Africa

ETHICS APPROVAL ID: NHRCIRB178

Dear Prof. Ramsay,

Approval of protocol amendment for the study titled *"Genomic and environmental risk factors for cardiometabolic disease in Africans"*

I write to inform you that the Navrongo Health Research Centre Institutional Review Board (NHRCIRB) has reviewed the documents submitted for amendment for the above named protocol and has found no issues of ethical concern and therefore grants you approval.

The Board finds the amendment relevant considering the aims and objectives outlined in the document titled ***"The effect of genetic variants, anthropometry and the environment on lipid profile in adults in northern Ghana"***, a nested protocol within the broad objective of the AWI-Gen study for a PhD thesis for Mr. Godfred Agongo.

The documents that were reviewed and approved are as follows;

- Completed Protocol Amendment form
- Protocol amendments
- Questionnaire for amended version (1) dated 23/10/2015
- Consent forms for amended version (1) dated 23/10/2015
- Curriculum Vitae of the Principal Investigator

Please note that any further amendment to these approved documents must receive ethical clearance from the NHRCIRB before its implementation.

You are also to note that this approval expires on **2nd December, 2016.**

The Board wishes you all the best in this study.

Sincerely,

Dr. (Mrs) Nana Akosua Ansah
(Vice Chair, NHRC IRB)

Cc: The Director, NHRC

7.3.3. Appendix C3: Wits Human Research Committee ethics approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Professor Michele Ramsay

CLEARANCE CERTIFICATE	**M121029**
PROJECT	WITS-INDEPTH Partnership: Genomic and Environment Risk Factors for Cardiometabolic Disease in Africans
INVESTIGATORS	Professor Michele Ramsay.
DEPARTMENT	School of Pathology/Division of Human Genetics
DATE CONSIDERED	26/10/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 21/11/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

M. Ramsay 11 April 2013

7.4. Appendix D: Coefficient of variation for lipid measurements

7.4.1. Appendix D1: Coefficient of variation (CV) for the measurement of HDL-C

Analyte HDL - C
Units mmol/l
Method RANDOX DIRECT HDL-CHOLESTEROL

LEVEL 1 - Target 0.72 mmol/l

Run	Date	Replicate 1	Replicate 2	Replicate 3	Run Mean	(x1 - Mean)^2	(x2 - Mean)^2	(x3 - Mean)^2	Sum	(Run Mean - Mean)^2
1	26.10.15	0.770	0.750	0.760	0.760	0.00010	0.00010	0.00000	0.00020	5.37778E-05
2	27.10.15	0.730	0.770	0.790	0.763	0.00111	0.00004	0.00071	0.00187	0.000016
3	28.10.15	0.750	0.790	0.790	0.777	0.00071	0.00018	0.00018	0.00107	8.71111E-05
4	29.10.15	0.800	0.770	0.780	0.783	0.00028	0.00018	0.00001	0.00047	0.000256
5	30.10.15	0.750	0.750	0.760	0.753	0.00001	0.00001	0.00004	0.00007	0.000196

Mean	0.767	mmol/l	**Variance for daily means (B)**	0.000152222
Number of days	5		**Degrees of freedom**	10
Replicates per day	3		**Percent Point (C)**	20.48317735
		CV	**Within-Run Verification value**	0.04030406
Within-Run Imprecision	0.019	2.5%	**Total precision degrees of freedom (T)**	13.37029558
Total Imprecision	0.020	2.6%		

			Verification Limit	
Claimed Within-Run Imprecision	0.02816113	3.67%	0.04030406	Within claim
Claimed Total Imprecision	0.02892847	3.77%	0.03580582	Within claim
Levels	2			

LEVEL 2 - Target 1.01mmol/l

Run	Date	Replicate 1	Replicate 2	Replicate 3	Run Mean	(x1 - Mean)^2	(x2 - Mean)^2	(x3 - Mean)^2	Sum	(Run Mean - Mean)^2
1	26.10.15	0.890	0.880	0.880	0.883	0.00004	0.00001	0.00001	0.00007	0.000300444
2	27.10.15	0.850	0.890	0.940	0.893	0.00188	0.00001	0.00218	0.00407	5.37778E-05
3	28.10.15	0.870	0.920	0.960	0.917	0.00218	0.00001	0.00188	0.00407	0.000256
4	29.10.15	0.990	0.930	0.920	0.947	0.00188	0.00028	0.00071	0.00287	0.002116
5	30.10.15	0.850	0.860	0.880	0.863	0.00018	0.00001	0.00028	0.00047	0.001393778

Mean	0.90	mmol/l	**Variance for daily means (B)**	0.00103
Number of days	5		**Degrees of freedom**	10
Replicates per day	3		**Percent Point (C)**	20.48317735
		CV	**Within-Run Verification value**	0.047049565
Within-Run Imprecision	0.03	3.8%	**Total precision degrees of freedom (T)**	9.977064818
Total Imprecision	0.04	4.7%		

			Verification Limit	
Claimed Within-Run Imprecision	0.03287433	3.65%	0.04704957	Within claim
Claimed Total Imprecision	0.03071273	3.41%	0.04400639	Within claim

7.4.2. Appendix D2: Coefficient of variation (CV) for the measurement of LDL-C

Analyte LDL - C
Units mmol/l
Method RANDOX DIRECT LDL-CHOLESTEROL

LEVEL 1 - Target 2.28 mmol/l

Run	Date	Replicate 1	Replicate 2	Replicate 3	Run Mean	(x1 - Mean)^2	(x2 - Mean)^2	(x3 - Mean)^2	Sum	(Run Mean - Mean)^2
1	26.10.15	1.880	2.050	1.960	1.963	0.00694	0.00751	0.00001	0.01447	0.000981778
2	27.10.15	2.060	1.960	2.050	2.023	0.00134	0.00401	0.00071	0.00607	0.000821778
3	28.10.15	1.960	1.940	1.860	1.920	0.00160	0.00040	0.00360	0.00560	0.005575111
4	29.10.15	1.800	1.960	2.160	1.973	0.03004	0.00018	0.03484	0.06507	0.000455111
5	30.10.15	2.100	2.120	2.060	2.093	0.00004	0.00071	0.00111	0.00187	0.009735111

Mean	1.995	mmol/l	**Variance for daily means (B)**	0.004392222
Number of days	5		**Degrees of freedom**	10
Replicates per day	3		**Percent Point (C)**	20.48317735
			Within-Run Verification value	0.100772886
	CV		**Total precision degrees of freedom (T)**	12.94787176
Within-Run Imprecision	0.096	4.8%		
Total Imprecision	0.103	5.2%		

			Verification Limit	
Claimed Within-Run Imprecision	0.07041173	3.53%	0.10077289	Within claim
Claimed Total Imprecision	0.0879648	4.41%	0.11063904	Within claim
Levels	2			

LEVEL 2 - Target 3.42mmol/l

Run	Date	Replicate 1	Replicate 2	Replicate 3	Run Mean	(x1 - Mean)^2	(x2 - Mean)^2	(x3 - Mean)^2	Sum	(Run Mean - Mean)^2
1	26.10.15	3.290	3.450	3.350	3.363	0.00538	0.00751	0.00018	0.01307	0.000324
2	27.10.15	3.480	3.400	3.470	3.450	0.00090	0.00250	0.00040	0.00380	0.004715111
3	28.10.15	3.440	3.360	3.230	3.343	0.00934	0.00028	0.01284	0.02247	0.001444
4	29.10.15	3.160	3.310	3.480	3.317	0.02454	0.00004	0.02668	0.05127	0.004181778
5	30.10.15	3.410	3.490	3.400	3.433	0.00054	0.00321	0.00111	0.00487	0.002704

Mean	3.38	mmol/l	**Variance for daily means (B)**	0.003342222
Number of days	5		**Degrees of freedom**	10
Replicates per day	3		**Percent Point (C)**	21.70739075
			Within-Run Verification value	0.194292665
	CV		**Total precision degrees of freedom (T)**	13.76826587
Within-Run Imprecision	0.10	2.9%		
Total Imprecision	0.10	2.9%		

			Verification Limit	
Claimed Within-Run Imprecision	0.131872	3.90%	0.19429266	Within claim
Claimed Total Imprecision	0.10177813	3.01%	0.12779642	Within claim

7.4.3. Appendix D3: Coefficient of variation (CV) for the measurement of TC

TC
mmol/l
RANDOX TOTAL CHOLESTEROL

Date	Replicate 1	Replicate 2	Replicate 3	Run Mean	(x1 - Mean)^2	(x2 - Mean)^2	(x3 - Mean)^2	Sum	(Run Mean - Mean)^2
28.09.15	4.57	4.45	4.51	4.510	0.00360	0.00360	0.00000	0.00720	0.004715111
29.09.15	4.28	4.51	4.58	4.457	0.03121	0.00284	0.01521	0.04927	0.000235111
30.09.15	4.42	4.47	4.59	4.493	0.00538	0.00054	0.00934	0.01527	0.002704
01.10.15	4.45	4.68	4.20	4.443	0.00004	0.05601	0.05921	0.11527	4E-06
02.10.15	4.28	4.31	4.32	4.303	0.00054	0.00004	0.00028	0.00087	0.019044
							Variance for daily means (B)		0.006675556
							Degrees of freedom		10
							Percent Point (C)		20.48317735
							Within-Run Verification value		0.189421075
							Total precision degrees of freedom (T)		13.74141511

Mean	4.441	mmol/l		
Number of days	5			
Replicates per day	3			
		CV		
Within-Run Imprecision	0.137	3.1%		
Total Imprecision	0.139	3.1%		
			Verification Limit	
l Within-Run Imprecision	0.13235173	2.98%	0.18942107	Within claim
laimed Total Imprecision	0.13235173	2.98%	0.16158929	Within claim
Levels	2	Ricos %l		

Date	Replicate 1	Replicate 2	Replicate 3	Run Mean	(x1 - Mean)^2	(x2 - Mean)^2	(x3 - Mean)^2	Sum	(Run Mean - Mean)^2
28.09.15	7.500	7.220	7.360	7.360	0.01960	0.01960	0.00000	0.03920	0.004715111
29.09.15	7.340	7.410	7.380	7.377	0.00134	0.00111	0.00001	0.00247	0.007281778
30.09.15	7.340	7.400	7.310	7.350	0.00010	0.00250	0.00160	0.00420	0.003441778
01.10.15	7.300	7.480	7.290	7.357	0.00321	0.01521	0.00444	0.02287	0.004268444
02.10.15	6.990	7.060	6.990	7.013	0.00054	0.00218	0.00054	0.00327	0.077284
							Variance for daily means (B)		0.024247778
							Degrees of freedom		10
							Percent Point (C)		20.48317735
							Within-Run Verification value		0.400716144
							Total precision degrees of freedom (T)		5.651806798

Mean	7.29	mmol/l		
Number of days	5			
Replicates per day	3			
		CV		
Within-Run Imprecision	0.08	1.2%		
Total Imprecision	0.17	2.3%		
			Verification Limit	
l Within-Run Imprecision	0.2799872	3.84%	0.40071614	Within claim
laimed Total Imprecision	0.10134953	1.39%	0.19294199	Within claim

7.4.4. Appendix D4: Coefficient of variation (CV) for the measurement of TG

Analyte TG
Units mmol/l
Method RANDOX DIRECT LDL-CHOLESTEROL

LEVEL 2 - Target 6.36 mmol/l

Run	Date	Replicate 1	Replicate 2	Replicate 3	Run Mean	(x1 - Mean)^2	(x2 - Mean)^2	(x3 - Mean)^2	Sum	(Run Mean - Mean)^2
1	17.08.15	0.820	0.890	0.930	0.880	0.00360	0.00010	0.00250	0.00620	3.6E-05
2	18.08.15	0.840	0.840	0.830	0.837	0.00001	0.00001	0.00004	0.00007	0.001393778
3	19.08.15	0.830	0.810	0.800	0.813	0.00028	0.00001	0.00018	0.00047	0.003680444
4	20.08.15	0.820	0.770	0.980	0.857	0.00134	0.00751	0.01521	0.02407	0.000300444
5	21.08.15	1.030	0.980	0.940	0.983	0.00218	0.00001	0.00188	0.00407	0.011953778

Mean	0.874	mmol/l	**Variance for daily means (B)**	0.004341111
Number of days	5		**Degrees of freedom**	10
Replicates per day	3		**Percent Point (C)**	20.48317735
			Within-Run Verification value	0.041153426
	CV		**Total precision degrees of freedom (T)**	8.460183323
Within-Run Imprecision	0.059	6.8%		
Total Imprecision	0.082	9.3%		

			Verification Limit	
Claimed Within-Run Imprecision	0.0287546	3.29%	0.04115343	Exceeds claim
Claimed Total Imprecision	0.0306774	3.51%	0.04773394	Exceeds claim
Levels	2			

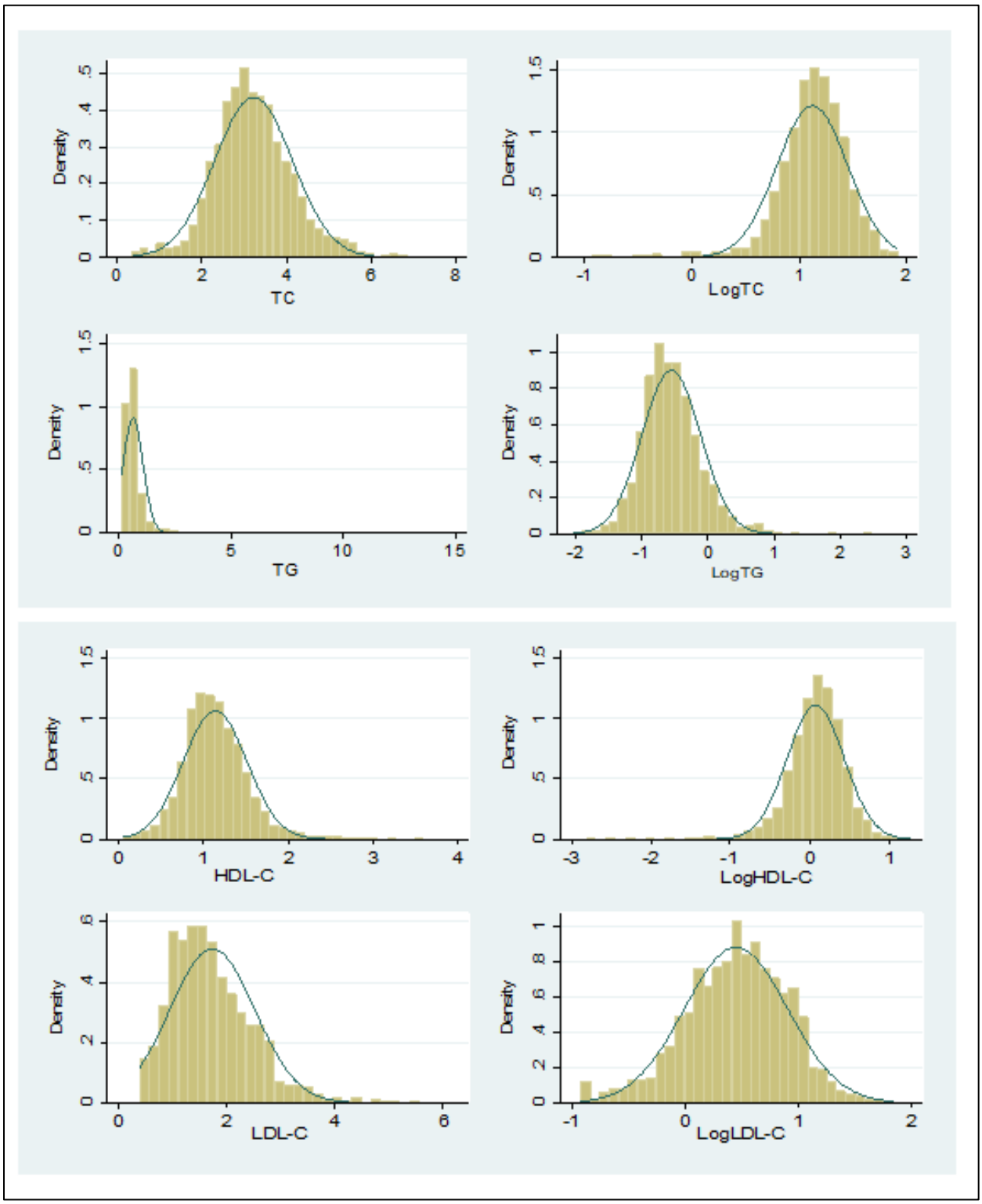
LEVEL 3 - Target 15.8mmol/l

Run	Date	Replicate 1	Replicate 2	Replicate 3	Run Mean	(x1 - Mean)^2	(x2 - Mean)^2	(x3 - Mean)^2	Sum	(Run Mean - Mean)^2
1	17.08.15	2.160	2.140	2.080	2.127	0.00111	0.00018	0.00218	0.00347	0.008961778
2	18.08.15	2.000	2.070	2.060	2.043	0.00188	0.00071	0.00028	0.00287	0.000128444
3	19.08.15	2.010	1.980	1.970	1.987	0.00054	0.00004	0.00028	0.00087	0.002055111
4	20.08.15	1.960	1.980	2.010	1.983	0.00054	0.00001	0.00071	0.00127	0.002368444
5	21.08.15	2.020	2.000	2.040	2.020	0.00000	0.00040	0.00040	0.00080	0.000144

Mean	2.03	mmol/l	**Variance for daily means (B)**	0.003414444
Number of days	5		**Degrees of freedom**	10
Replicates per day	3		**Percent Point (C)**	20.48317735
			Within-Run Verification value	0.045076902
	CV		**Total precision degrees of freedom (T)**	5.506287802
Within-Run Imprecision	0.03	1.5%		
Total Imprecision	0.06	3.1%		

			Verification Limit	
Claimed Within-Run Imprecision	0.031496	1.55%	0.0450769	Within claim
Claimed Total Imprecision	0.0524256	2.58%	0.10111431	Within claim

7.5. Appendix E: Histogram showing both non transformed and log-transformed lipid levels



7.6. Appendix F: DNA manual extraction protocol

Instructions for the preparation of solutions for Salting Out can be found below

SUCROSE-TRITON-X-LYSING BUFFER

1. Add 10ml 1M Tris-HCl pH8, 5ml 1M $MgCl_2$ and 10ml Triton-X 100 and make up to 1L with dH_2O.

2. Autoclave.

3. Keep solution chilled at 4°C.

4. Add 109.5g sucrose on the day it is meant to be used. Do not store solution for more than a day.

20mM TRIS 5mM EDTA (T20E5)

1. Add 20ml 1M Tris-HCl (pH 8.0), 10ml 0.5M EDTA (pH 8.0), and make up to 1L with dH2O.

2. Autoclave.

1X TRIS EDTA (TE) BUFFER

1. Add 10ml 1M Tris-HCl (pH 8), 2ml 05.M EDTA, and make up to 1L with dH2O.

2. Autoclave.

PROTEINASE-K MIX

1. For 8 extractions; add 400µl 10% SDS, 16µl 0.5M EDTA, 2.8ml autoclaved dH2O.

2. Add 800µl Proteinase-K (10mg/ml stock) just before use.

10% SDS

1. Add 10g SDS to 100ml autoclaved dH2O.

SATURATED SALT SOLUTION

1. Autoclave 100ml dH2O.

2. Slowly add 40g NaCl until saturated (some may precipitate out).

3. Before use, agitate and let NaCl settle. Use the clear supernatant.

0.5M EDTA

1. Add 93.06g EDTA and make up to 500ml with dH2O.

2. Use NaOH to get pH to 8.0. EDTA will only dissolve once the correct pH is reached.

3. Autoclave.

1M MgCl2

1. Add 101.66g MgCl2 and make up to 500ml with dH2O.

2. Autoclave.

1M TRIS-HCl pH 8.0

1. Add 121.1g Tris and make up to 1L with dH2O. Adjust pH with NaOH.

2. Autoclave.

Note: All samples in this process are centrifuged at 2400 rpm at 4°C.This procedure is performed over two days. Samples are frozen prior to extraction.

DAY 1:

1. Thaw sample at room temperature for about an hour.
2. 4.3.3. Fill each 50ml NUNC with cold sucrose-Triton X lysing buffer (which should be kept cold during this procedure). This detergent lyses the red blood cells.
3. 4.3.4. Invert the NUNC tubes several times to mix. Centrifuge for 10 minutes.
4. 4.3.5. Pour off and dispose of the supernatant, ensuring that the pellet is not dislodged.
5. Wash the pellet with 20-25ml of cold sucrose-Triton X lysing buffer.
6. Freeze tubes at -20°C for 5 minutes and then centrifuge for 5 minutes.
7. Pour off and dispose of the supernatant. Washes are carried out at least twice to ensure that most of the red cell debris has been removed.
8. Add 3ml T20E5, 200µl of 10% SDS and 500µl of Proteinase-K mix. This step lyses white blood cells and degrades proteins.
9. Mix components well by inversion and incubate overnight at 42°C.

DAY 2:

1. Add 1ml of saturated NaCl to the lysate and agitate vigorously for 15 seconds.
2. Freeze the tube at -20°C for 5-10 minutes and then centrifuge for half an hour.
3. A white pellet should be visible, if not spin again.
4. Transfer the supernatant containing the DNA to a new NUNC tube. Dispose of the old one appropriately.
5. Add 2 volumes of absolute ethanol kept at room temperature.
6. Agitate gently and spool the DNA out into a cryovial.
7. Wash DNA in ice cold 70% ethanol to remove excess salt.
8. Air dry DNA and resuspend in 400µl of TE buffer, or according to pellet size.
9. Aliquot DNA sample as per study requirements and store appropriately.

PRECIPITATION (This process is required if DNA is not visible at 6. at day 2.)

1. If DNA is not visible, precipitate by placing the sample at -70°C for 30 minutes.
2. Centrifuge for 20 minutes to pellet the DNA. Often the DNA will present as smudges on the sides of the tube, if it is visible spool out and proceed from 4.3.16 to 4.3.18.
3. Pour off the supernatant, being careful to avoid throwing the DNA out as well.
4. Wash the pellet in ~10ml 70% ethanol and centrifuge for 10 minutes.
5. Pour off the supernatant and invert tubes on paper towel to dry the pellet.
6. Resuspend in appropriate volume of TE buffer (usually 100-400µl) and store as appropriate.

7.7. Appendix G: R^2 values of significant variants in *CETP* and *PON1* associated with HDL-C levels in Ghanaians

7.7.1. Appendix G1: R^2 values of significant variants in *CETP* associated with HDL-C levels in Ghanaians

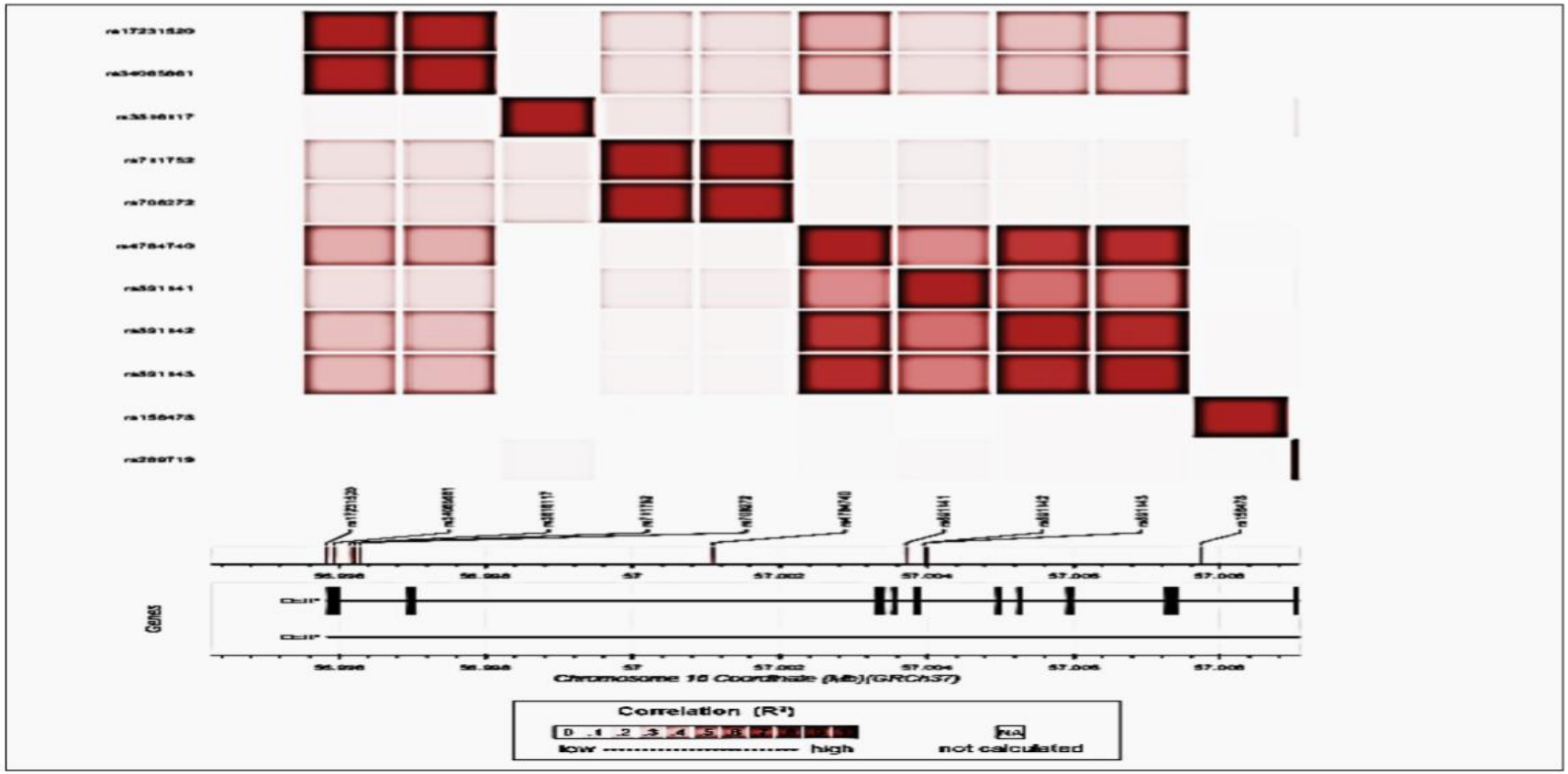
Rs number	rs17231520	rs34065661	rs3816117	rs711752	rs708272	rs4784740	rs891141	rs891142	rs891143	rs158478	rs289719
rs17231520	1.000	0.990	0.058	0.268	0.268	0.468	0.273	0.420	0.446	0.007	0.013
rs34065661	0.990	1.000	0.059	0.271	0.271	0.479	0.269	0.415	0.441	0.008	0.013
rs3816117	0.058	0.059	1.000	0.222	0.222	0.005	0.011	0.001	0.002	0.007	0.079
rs711752	0.268	0.271	0.222	1.000	1.000	0.102	0.161	0.085	0.088	0.011	0.000
rs708272	0.268	0.271	0.222	1.000	1.000	0.102	0.161	0.085	0.088	0.011	0.000
rs4784740	0.484	0.479	0.005	0.102	0.102	1.000	0.589	0.876	0.927	0.031	0.031
rs891141	0.273	0.269	0.011	0.161	0.161	0.589	1.000	0.672	0.636	0.001	0.048
rs891142	0.420	0.415	0.001	0.085	0.085	0.876	0.672	1.000	0.945	0.021	0.023
rs891143	0.446	0.441	0.002	0.088	0.088	0.972	0.636	0.945	1.000	0.019	0.023
rs158478	0.007	0.008	0.007	0.011	0.011	0.031	0.001	0.021	0.019	1.000	0.033
rs289719	0.013	0.013	0.079	0.000	0.000	0.031	0.048	0.023	0.023	0.033	1.000

7.7.2. Appendix G2: R^2 values of significant variants in *PON1* associated with HDL-C levels in Ghanaians

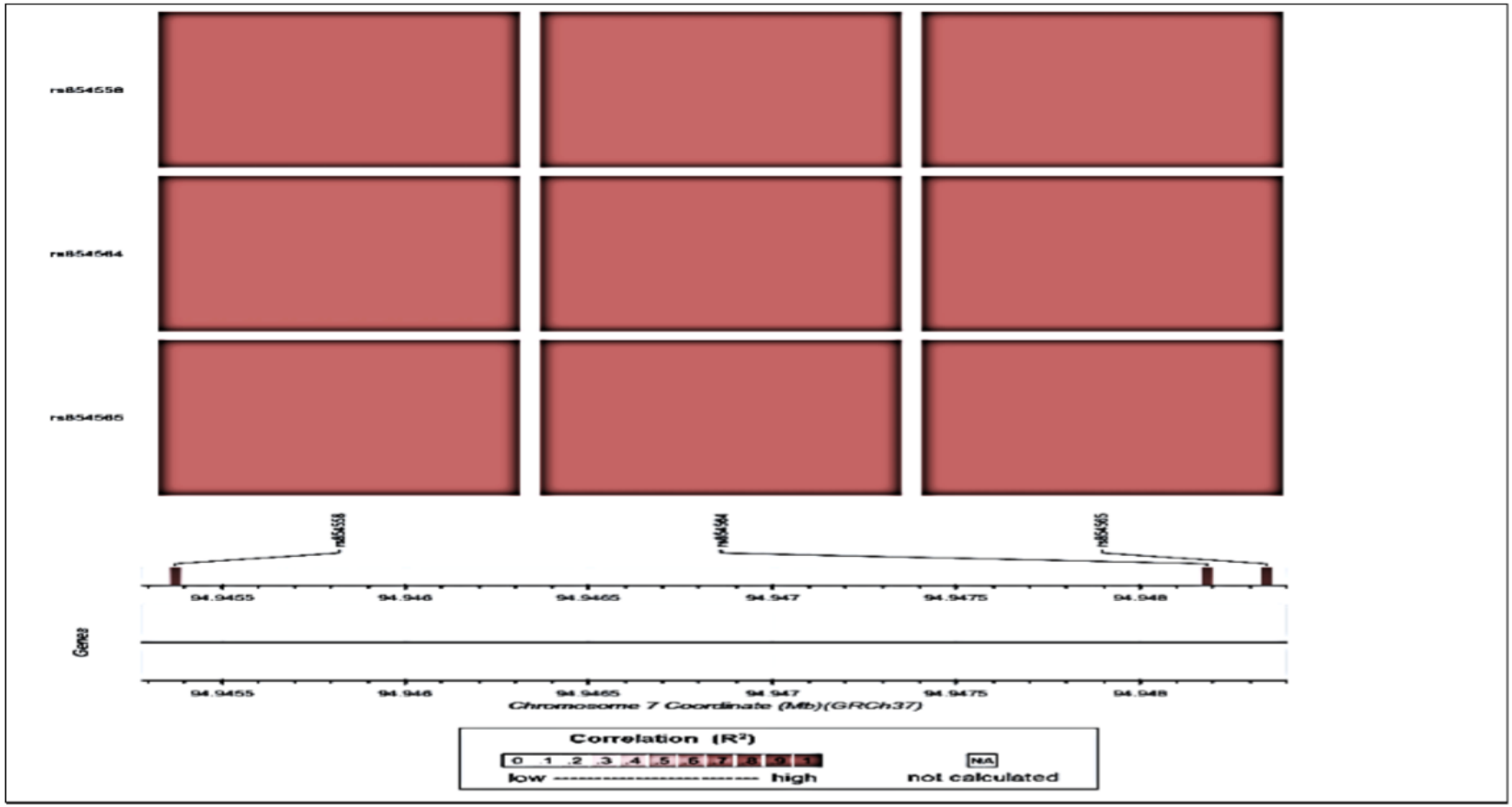
Rs number	rs854558	rs854564	rs854565
rs854558	1.000	0.993	0.993
rs854564	0.993	1.000	1.000
rs854565	0.993	1.000	1.000

7.8. Appendix H: LD plots associated with HDL-C in *CETP* and *LPL* genes

7.8.1. Appendix H1: LD plot of significant variants associated with HDL-C in *CETP* gene. LD was calculated using the Africa 1000 Genomes project data



7.8.2. Appendix H2: LD plot of significant variants associated with HDL-C in *LPL* gene. LD was calculated using the Africa 1000 Genomes project data



7.9. Appendix I: Published article and manuscript under review

7.9.1. Appendix I1: Published article related to this study

PLOS ONE

RESEARCH ARTICLE

The burden of dyslipidaemia and factors associated with lipid levels among adults in rural northern Ghana: An AWI-Gen sub-study

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OPEN ACCESS

Citation: Agongo G, Nonterah EA, Debpuur C, Amenga-Etego L, Ali S, Oduro A, et al. (2018) The burden of dyslipidaemia and factors associated with lipid levels among adults in rural northern Ghana: An AWI-Gen sub-study. PLoS ONE 13(11): e0206326. https://doi.org/10.1371/journal.pone.0206326

Editor: Bamidele O. Tayo, Loyola University Chicago, UNITED STATES

Received: February 3, 2018

Accepted: October 10, 2018

Published: November 28, 2018

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: We acknowledge the National Institutes of Health (NIH), URL www.https://grants.nih.gov/funding/index.htm, through the H3Africa AWI-Gen project (NIH grant number U54HG006938) and the Wits Non-Communicable Disease Research Leadership Program (NIH Fogarty International Centre grant number D43TW008330) for funding

Abstract

Dyslipidaemia is a primary risk factor for cardiometabolic disease, causing over 17 million deaths globally in 2015. However, the burden of dyslipidaemia and factors associated with lipid levels remain unknown in many rural African populations. Therefore, this study evaluated the association of socio-demographic, anthropometric and behavioural factors with lipid levels in rural Ghana. The prevalence of hypercholesterolaemia, hypertriglyceridaemia and elevated LDL-C in the total population of 1839 (846 men and 993 women) was 4.02%, 2.12%, and 5.55% respectively and did not differ between genders. The prevalence of low HDL-C levels was 60.30% and differed ($p = 0.005$) between men (56.86%) and women (63.24%). Subcutaneous abdominal fat was associated with TC ($\beta = 0.067$, $p = 0.015$) and TG ($\beta = 0.137$, $p<0.001$) among women and LDL-C ($\beta = 0.139$, $p = 0.006$) and TC ($\beta = 0.071$, $p = 0.048$) among men. Body mass index was associated with TC ($\beta = 0.010$, $p = 0.043$) among men while waist circumference was associated with LDL-C ($\beta = 0.116$, $p<0.001$) and TG ($\beta = 0.094$, $p<0.001$) among women. Hip circumference was negatively associated ($\beta = -0.053$, $p = 0.043$) while visceral fat was positively associated with TG ($\beta = 0.033$, $p = 0.022$) among women. Socioeconomic status, education, being unmarried and employment were associated with HDL-C ($\beta = 0.081$, $p = 0.004$), LDL-C ($\beta = 0.095$, $p = 0.004$) and TG ($\beta = 0.095$, $p = 0.001$) all among women, and TC ($\beta = 0.070$, $p = 0.010$) among men, respectively. Nankana women had lower TC ($\beta = -0.069$, $p = 0.001$), and men lower TG levels ($\beta = -0.084$, $p = 0.008$) than the other ethnic groups. Tobacco smoking ($\beta = 0.066$, $p = 0.024$) and alcohol intake ($\beta = 0.084$, $p = 0.001$) were associated with HDL-C levels among men and women respectively. Further studies are required to investigate whether high prevalence of low HDL-C levels in this population presents with any adverse cardiovascular disease outcomes. Associations of education, employment and adiposity with lipid levels suggest that future societal advances and increases in the prevalence of obesity may

this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The funding was received by MR.

Competing interests: The authors declare that no competing interests exist.

lead to associated adverse health consequences. Monitoring and interventions are required to limit these effects.

Introduction

Dyslipidaemia is a metabolic derangement that predisposes an individual to atherosclerosis and cardiovascular disease (CVD) which caused over 17 million deaths globally in 2015, an increase of 12.5% from 2005 [1]. The contribution of dyslipidaemia to CVDs is evident from several longitudinal studies which have demonstrated the association of high levels of low density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG) and low levels of high density lipoprotein cholesterol (HDL-C) with CVD [2–5]. While dyslipidemia is progressively declining in developed and high-income countries, the same is not observed in middle- and low-income countries. Thus, it has been shown that TC levels decreased by 0.2mmol/l from the 1980s to 2008 in high-income countries, but declined by only 0.08–0.09mmol/l in middle- and low-income countries [6].

Serum lipid levels are reported to be influenced by anthropometric, demographic, environmental and genetic factors [7–10]. Consequently, the burden of dyslipidaemia in sub-Saharan Africa has been attributed mainly to urbanization which is associated with environmental, behavioural and socio-cultural changes [11,12]. In Ghana, previous studies have examined the burden of, and the factors associated with, dyslipidemia in mainly urban and semi-urban communities but few have evaluated the factors associated with serum lipid levels in rural settlements [13–17]. To date only one study has been conducted on lipid modulating factors in a rural settlement in Ghana but only the effect of anthropometric parameters was evaluated [17]. In rural communities in Northern Ghana where agriculture is the mainstay of the population [18] pesticide use is common. Findings elsewhere have associated pesticide use with increased lipid levels but this has not been evaluated to date in rural Ghana [19,20].

Just as urban settings in sub-Saharan Africa are experiencing a rising burden of dyslipidaemia and its adverse health consequences, rural areas are also facing the same challenge [21–23]. Treatment and control of dyslipidaemia in resource-poor settings is expensive [24] and will require innovative approaches for prevention, diagnosis and treatment. Measuring the prevalence and understanding the factors associated with dyslipidaemia in rural settings will assist in channeling scarce resources toward its prevention. This study therefore determined the burden of dyslipidaemia and evaluated how anthropometric, demographic and environmental factors are associated with serum lipid levels among adults in rural Northern Ghanaian communities.

Materials and methods

Study area

This study was carried out in the two Kassena-Nankana districts of north-eastern Ghana. The study area lies between latitude 10.30' and 11.10' north and longitude 1.1' west, and covers a total land area of 1675km^2, bordering northwards along the Ghana-Burkina Faso border. The area is characterized by Guinea Savannah vegetation dominated by semi-arid conditions and vast grassland integrated with short trees. The districts form the coverage area of the Navrongo Health and Demographic Surveillance System (NHDSS) which is implemented by the Navrongo Health Research Centre (NHRC) [25]. The study area is typical of many rural areas in sub-Saharan Africa where agriculture is the mainstay of the local economy with about 90% of

the people being subsistence farmers. Poverty levels are high due to insufficient agricultural production resulting from a short rainy season and prolonged dry season [18]. The major ethnic groups in the area are the Kassena and Nankana, with several minority groups including the Bulsas [25].

Study design and population

This population based cross-sectional study was conducted as part of the Africa Wits-INDEPTH Partnership for Genomic studies (AWI-Gen) project under the broader Human Heredity and Health in Africa (H3Africa) Initiative [26]. Participants were recruited from February to October 2015 in the west and east zones of the NHDSS area. These zones which are mainly Kassena and Nankana speaking communities respectively were purposefully selected for this study. A list of participants in the age range of 40 to 60 years was generated using the NHDSS database from each of the zones. A combined sample size of 2200 participants, including 10% for non-response or refusal to participate, of roughly equal gender ratio and with fairly equal representation from the major ethnic groups, was randomly selected. Of this number, 1050 participants were sampled from the east and 1150 participants were sampled from the west zones. Individuals who were resident within the study area for at least 10 years were recruited into the study after witnessed informed consent was obtained. Pregnant women were excluded from the study [26]. Participants who had no data for one or more of the investigated variables were excluded from the analytical data set. Therefore, a sample size of 1839 comprising 993 women and 864 men were included in the analysis.

Data collection

Data on socio-demographic, anthropometric and behavioural factors were captured on a paper questionnaire [26] and uploaded into the Research Electronic Data Capture (REDCap) platform [27]. All participants were assigned a unique individual identification code on recruitment to ensure that both their identity, and data captured were anonymized. Data quality control included the checking of 10% of the entries for accurate data capture.

Anthropometric and blood pressure measurements

Standing height and weight of each participant were measured without shoes and in light clothes using a Harpenden stadiometer (Holtain, Crymych, Wales) fixed to the wall and a digital calibrated weighing scale, respectively. Waist and hip circumference were measured using a non-stretch measuring tape (Seca GmbH& Co. KG, Hamburg, Germany) according to the guidelines of the WHO 2008 report on waist and hip circumference [28]. Body mass index (BMI), calculated as weight in kg/ height in m^2, was categorized as underweight: $<18.5 kg/m^2$, normal weight:$18.5–24.9 kg/m^2$, overweight: $25.0–29.9 kg/m^2$ and obese: $\geq 30 kg/m^2$ according to WHO recommendation [29].

Visceral and abdominal subcutaneous fat tissue thicknesses were measured twice using a LOGIQ e ultrasound system with a 2–5.5 MHz 4C-RS curved transducer (GE, Healthcare, CT, USA) and the mean taken. The visceral fat thickness was defined as the distance in centimeters (cm) from the peritoneum to the vertebral bodies and subcutaneous fat thickness as the depth in cm from the skin to the linea alba. In order to visualize the relevant anatomical structures the scan depth was set at 15 cm for visceral fat and 9 cm for subcutaneous fat. The site for both measurements was where the xyphoid line and waist circumference meet.

Blood pressure was measured using a digital sphygmomanometer (Omron M6, Omron, Kyoto, Japan). The participant was seated on a chair and the feet firmly rested on the floor. The measurement was taken from the left hand which rested on a desk with the antecubital

fossa level with the heart and palm facing upwards. The measurements were repeated twice more, with two minutes between each interval. The mean of the last two measurements were used to calculate the systolic blood pressure (SBP) and diastolic pressure (DBP) of the participant. High blood pressure was defined as SBP>140mmHg or DBP>90mmHg or self-reported controlled treatment using hypertensive medication [30].

Biomarker analysis

Overnight fasting serum HDL-C, LDL-C, TG and TC were all measured directly using an automated chemistry analyzer (Randox RX Daytona+, Crumlin, Northern Ireland) at the University of the Witwatersrand Developmental Pathway for Health Research Unit (DHPRU), Chris Hani Baragwanath Hospital, Soweto, South Africa. A random selection of 150 samples were assayed in duplicate for glucose and all lipids to ascertain the coefficients of variation (CVs) of the assays. The CVs for glucose and the lipids (HDL-C, LDL-C, TC and TG) were 2.3% and less than 1.5%, respectively. Three control levels each were run for glucose and each of the lipid parameters. The mean bias relative to the reference standards [(measured mean-reference mean)/reference mean] for each of the levels for glucose was 0.12 for level 1, -0.03 for level 2 and 0.22 for level 3. The relative mean bias for each of the controls for HDL-C, LDL-C, TC and TG for levels 1, 2 and 3 respectively, were as follows: 0.07, 0.12 and 0.18; 0.14, 0.01 and -0.18; 0.05, 0.04 and -0.05; -0.27, -0.22 and -0.19, respectively.

All analyses were done in mmol/l, the equivalent in mg/dl being: mmol x 38.67 for HDL-C, LDL-C and TC; mmol/l x 88.57 for TG and mmol/l x 18 for glucose. Dyslipidaemia was defined as follows: high TC (>5.0mmol/l), high LDL-C (>3.0mmol/l), high TG (>1.7mmol/l), low HDL-C (<1.0mml/l for men and <1.2mmol/l for women) [31]. Participants needing treatment for hyperlipidaemia was defined according to the ATP III guidelines [32]. High blood glucose was defined as fasting serum glucose ≥7.0mmol/l [33].

Socio-demographic and lifestyle variables

Self-reported data on ethnicity, marital status, employment, education, alcohol and tobacco use, household amenities, physical activity and pesticide exposure were categorized using the definitions described below.

Ethnicity was defined by self-reported ethnic origin or ancestral lineage. Marital status: a participant who was staying with a partner for at least a year during the period of recruitment was considered currently married. Employment status: a person, who reported to be involved in any form of employment, whether formal or informal, was considered employed. Education: this was defined by self-reported attainment of formal education. Alcohol consumption: data on alcohol consumption was collected using the CAGE questionnaire [34] that was adapted and incorporated into the main AWI-Gen questionnaire. A person was defined as having a current problematic drinking pattern if they reported 'yes' to more than two of the following: they felt they should cut down on their drinking, felt bad or guilty about their drinking, people have annoyed them by criticizing their drinking, and ever had an alcoholic drink first thing in the morning to steady their nerves or get rid of a hangover. Current non-problematic drinking pattern was defined as the drinking of alcohol by a person who took alcohol but reported affirmative to less than three of the above listed attributes. Tobacco use was defined as present or past consumption of cigarettes, pipes or smokeless tobacco.

Socioeconomic status: this was assessed using the INDEPTH Health Equity tool which is an asset index generated by using principal component analysis to combine data on household possessions (http://indepth-network.org/resources/indepth-health-equity-tool-measuring-socio-economic-status). The household assets were first broken down into categorical

variables which were further converted into weights and principal components. The weights of the first principal component were used to develop an index from which scores of socioeconomic status of the households were derived. These scores were divided into quintiles with the first quintile being the poorest and the fifth the least poor. Physical activity was assessed using the Global Physical Activity Questionnaire (GPAQ) [35] which was incorporated into the main AWI-Gen questionnaire. Moderate to vigorous-intensity physical activity (MVPA) was calculated as minutes of physical activity per week (min/week). Pesticide exposure was defined by self-reported current working with pesticide or living close to a farm where pesticide was being used. Sleep duration was defined as the self-reported number of hours slept per night. Vegetable and fruit servings were estimated from self-reported quantities consumed per day using a serving size card. Malaria infection was defined as self-reported malaria fever within the past month. Menopausal status was categorized as follows: pre-menopausal status as having regular periods; menopausal transition as having irregular periods within the past 12 months; post-menopausal status as having no periods within the past 12 months [36].

Ethical approval

The H3Africa AWI-Gen project was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M12109), the Ghana Health Service Ethics Review Committee (ID No: GHS-ERC05/05/2015) and the Navrongo Institutional Review Board (ID No: NHRCIRB178). Community engagement was carried out prior to commencement of the study and signed or thump printed informed consent was obtained from each participant before being enrolled into the study.

Statistical analyses

All statistical analyses were performed using STATA 14.2 (StataCorp, College Station, Texas, 77845, US). All continuous variables were presented as means with standard deviations and compared among men and women using Student's non-paired t-test. Lipid levels were compared across age categories using ANOVA. Categorical variables were presented as percentages and compared between men and women using Pearson's χ^2 test. Awareness of dyslipidaemic or diabetes status was calculated using the number of participants who reported that they had been told by a health professional that they had high cholesterol or diabetes, respectively. Lipid levels were log-transformed to an approximate normal distribution and sex-stratified multivariable linear regression analyses were used to determine the association of each lipid species (dependent variable) with the selected study variables (independent variables). Variables that correlated with any of the lipids at $p<0.20$ in a univariate analysis were included in the multivariable linear regression model, and these models are displayed in the following tables. Multicollinearity among variables was assessed using the variance inflation factor (VIF). All variables in the univariate analyses had VIF<5.0 and were therefore all included in the multivariable analyses. Residuals were approximately normally distributed and omitted variable bias was ruled out ($p>0.05$).

Results

Characteristics of study participants

A comparison of socio-demographic, behavioural, cardiometabolic and anthroponetric variables across genders are presented in Table 1. The study population was 1839 participants and consisted of 54% women. The mean age (±SD) of the study participants was 51 ± 6 years. The majority ethno-linguistic groups were Kassena (51.93%) and Nankana (43.12%) with the

Table 1. Gender comparison of socio-demographic variables, food intake, exercise level, sleep duration, fasting blood glucose levels, blood pressure measurements and anthropometric variables.

Variables	Men	Women	Total	p-value
	(n = 846, 46%)	(n = 993, 54%)	(N = 1839)	Men vs women
Age (years)	50 ± 6.0	52 ± 6.0	51 ± 6.0	<0.001
Ethnicity				
Kassena	439 (51.89)	516 (51.96)	955 (51.93)	
Nankana	392 (46.34)	401 (40.38)	793 (43.12)	<0.001
Minority ethnic groups	15 (1.77)	76 (7.65)	91 (4.95)	
Educational status				
No formal education	517 (61.11)	768 (77.34)	1285 (69.87)	
Primary	192 (22.70)	161 (16.21)	353 (19.20)	<0.001
Secondary	111 (13.12)	55 (5.54)	166 (9.03)	
Tertiary	26 (3.07)	9 (0.91)	35 (1.90)	
Employment status				
Unemployed	292 (34.52)	390 (39.27)	682 (37.09)	
Employed	554 (65.48)	603 (60.73)	1157 (62.91)	<0.001
Marital status				
Currently married	717 (84.75)	632 (63.65)	1349 (73.36)	
Currently unmarried	129 (15.25)	361 (36.35)	490 (26.64)	
Household SES categories				
Poorest	129 (15.25)	204 (20.54)	333 (18.11)	
Very poor	139 (16.43)	192 (19.34)	331 (18.00)	
Poor	154 (18.20)	194 (19.54)	348 (18.92)	
Less poor	203 (24.00)	227 (22.86)	430 (23.38)	
Least poor	221 (26.12)	176 (17.72)	397 (21.59)	
Fruit Intake (servings/day)	1.01 ± 1.63	1.10 ± 1.69	1.06 ± 1.63	0.293
Vegetable Intake(servings/day)	3.43 ± 1.46	3.24 ± 1.51	3.33 ± 1.49	0.006
Vendor meals (times/week)	1.17 ± 1.68	0.79 ± 1.31	0.97 ± 1.50	<0.001
MVPA(hours/week)	40.07 ± 28.78	29.84 ± 27.72	34.55 ± 28.66	<0.001
Sleeping (hours/night)	7.71 ± 1.34	8.28 ± 1.32	8.02 ± 1.36	<0.001
Fasting blood glucose(mmol/l)	4.47 ± 0.76	4.61 ± 0.86	4.55 ± 0.82	<0.001
SBP(mmHg)	124.97 ± 20.44	123.28 ± 22.55	124.06 ± 21.61	0.094
DBP(mmol/l)	77.03 ± 12.86	77.12 ± 12.59	77.13 ± 12.72	0.760
BMI (kg/m^2)	20.87 ± 3.15	22.28 ± 3.85	21.63 ± 3.61	<0.001
Hip circumference (cm)	8.41 ± 0.79	8.93 ± 0.99	8.69 ± 0.94	<0.001
Waist circumference (cm)	7.33 ± 0.81	7.68 ± 0.95	7.52 ± 0.91	<0.001
Visceral fat (cm)	4.18 ± 1.21	3.54 ± 1.12	3.83 ± 1.20	<0.001
Subcutaneous fat (cm)	0.78 ± 0.38	1.15 ± 0.54	0.98 ± 0.51	<0.001

Data is given as mean ± SD or n (%)

https://doi.org/10.1371/journal.pone.0206326.t001

remainder constituting the minority ethnic groups (Bulsa, Dagaati, Sisaala, Mampruga, Frafra, Hausa, and Akan). The population was mainly illiterate (69.87%) and a small proportion had either secondary (9.03%) or tertiary education (1.90%) with men constituting a greater proportion ($p<0.001$). Over half (62.91%) of the participants reported having some form of employment and more men were employed ($p = 0.035$) compared to women. Similarly, more men were least poor according to household attributes and were currently married compared to women ($p<0.001$ for both).

There was no difference between men and women in reported average fruit servings per day ($p = 0.293$) but reported vegetable servings per day ($p = 0.006$) and vendor meals per week ($p<0.001$) were significantly higher in men. The MVPA of the study population was 34.55 hours/week with men being more physically active than women ($p<0.001$). The mean sleep duration per night for men was significantly lower than that for women ($p<0.001$). Though not presented in the table, malaria infection in the past month was not significantly different between men and women (16.54% and 16.84% respectively; $p = 0.868$), and the prevalence of self-reported HIV positive cases in the study population was 0.93%. The mean fasting blood glucose of the study population was 4.55 ±0.82 mmol/l with that of women being significantly higher that of men ($p<0.001$). The mean values of SBP and DBP of the study population were 124.06 ± 21.61 mmHg and 77.13 ± 12.72 mmHg, respectively with no significant difference between men and women. All the anthropometric indices were higher in women than men ($p<0.001$) except visceral fat which was higher in men ($p<0.001$).

Mean lipid levels stratified by sex and age category in the study population

The mean HDL-C, LDL-C, TC and TG were 1.14 ± 0.38 mmol/l, 1.73 ± 0.78 mmol/l, 3.23 ± 0.92 mmol/l and 0.64 ± 0.45 mmol/l respectively. There were no significant differences in measured lipid levels between men and women except for HDL-C level which was significantly higher in men than in women ($p = 0.001$) (Fig 1).

The distribution of each lipid species in males and females is shown in the supplementary data files (see S1 Fig). The mean lipid levels were stratified according to the following age categories: 40–44 years, 45–49 years, 50–54 years and 55–60 years in males and females, and the data shown in the supplementary data files (see S1 Table). Only in females were correlations observed between age and lipids, in this case positive correlations of age with both TG ($p = 0.006$) and TC ($p = 0.001$). Significant gender differences were noted for HDL at age

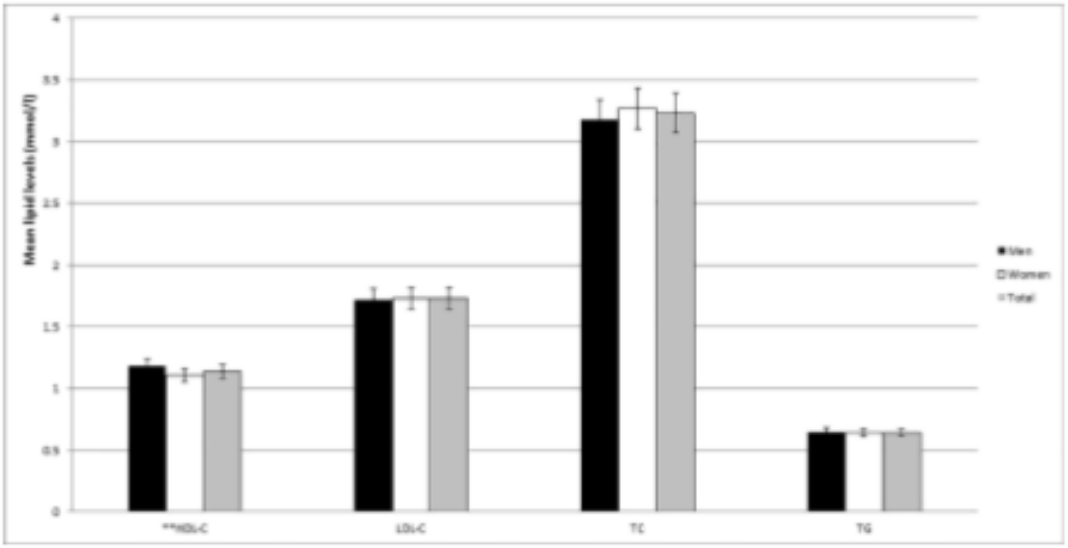

Fig 1. Mean lipid levels of the study population stratified by sex. **$p<0.005$ men vs women.

https://doi.org/10.1371/journal.pone.0206326.g001

categories 45–49 and 55–60 years, with values higher in males than females. Males had significantly lower TC and TG than females in the age group 55–60 years.

Prevalence of dyslipidaemia and other CVD risk factors in the study population stratified by sex

The prevalence of dyslipidaemia and other CVD risk factors among men and women is shown in Table 2. Smoking was far more common among men than women ($p<0.001$) but the use of smokeless tobacco did not differ between men and women ($p = 0.997$). Alcohol consumption was very prevalent with higher levels in males than females ($p<0.001$). Pesticide exposure was more common among men than women ($p<0.001$). Women were less physically active compared to men ($p<0.001$). The prevalence of overweight and obesity in the study population was 10.82% and 2.66% respectively with women being more overweight and obese than men ($p<0.001$). The prevalence of both high blood glucose (0.54%) and self-reported diabetes (0.76%) was very low with no difference between men and women ($p = 0.309$ and $p = 0.529$, respectively). The self-reported use of diabetes medication in the group with self-reported diabetes, was 50.0%, with no significant differences between genders ($p = 0.515$) There was no

Table 2. Comparison across genders of the prevalence of smoking, alcohol intake, pesticide exposure, low physical activity, obesity, high blood sugar, high blood pressure, dyslipidaemia and required therapy for dyslipidaemia.

Variable	Men (n = 846, 46%)	Women (n = 993, 54%)	Total (N = 1839)	p-value
Behavioural factors				
Current smoker	185 (21.87)	14 (1.41)	199 (10.82)	<0.001
Former/current smokeless tobacco use	86 (10.17)	101 (10.17)	187 (10.17)	0.997
Former/current alcohol drinker	782 (92.43)	777 (78.25)	1559 (84.77)	<0.001
Pesticide exposure	509 (60.17)	497 (50.05)	1006 (54.70)	<0.001
Low physical activity	57 (6.74)	133 (13.39)	190 (10.33)	<0.001
Body weight				
Overweight	50 (5.91)	149 (15.01)	199 (10.82)	
Obese	8 (0.95)	41 (4.13)	49 (2.66)	<0.001
Diabetes				
High blood glucose	3 (0.35)	7 (0.70)	10 (0.54)	0.309
Self reported diabetes	7 (0.84)	7 (0.70)	14 (0.76)	0.529
Self reported diabetes medication	5 (71.42)	6 (85.71)	7 (50.00)	0.515
High blood pressure	190 (22.46)	209 (21.05)	399 (21.70)	0.464
Dyslipidaemia				
Low HDL-C	481 (56.86)	628 (63.24)	1109 (60.30)	0.005
High LDL-C	49 (5.79)	53 (5.34)	102 (5.55)	0.671
High TC	28 (3.31)	46 (4.63)	74 (4.02)	0.150
High TG	16 (1.89)	23 (2.32)	39 (2.12)	0.528
Self reported dyslipidaemia	3 (0.35)	4 (0.40)	7 (0.38)	0.895
Self reported dylipidaemia medication	0 (0.00)	1 (25.00)	1 (16.67)	0.439
Needing therapy for dyslipidaemia	10 (1.18)	4 (0.40)	14 (0.76)	0.055
Menopausal status				
Pre-menopausal	—	372 (37.44)	372 (37.44)	—
Peri-menopausal	—	245 (24.62)	245 (24.62)	—
Post-menopausal	—	376 (37.94)	376 (37.94_	—

Data is given as n (%)

https://doi.org/10.1371/journal.pone.0206326.t002

difference in the prevalence of high blood pressure between men and women (p = 0.464). The prevalence of hypercholesterolemia, hypertriglyceridaemia and elevated LDL-C in the total population was 4.02%, 2.12% and 5.55%, respectively. There were no significant sex differences for any of these sub-types of dyslipidaemia. However, the prevalence of low HDL-C concentration in the population was 60.30% and differed (p = 0.005) between men (56.86%) and women (63.24%). Awareness of dyslipidemia in this population was low at 0.38% and did not differ between women and men (p = 0.895). Only one person reported the use of lipid–lowering medication. The proportion of the population needing treatment for hyperlipidaemia was also low at (0.76%) with more men than women requiring treatment, but this difference just failed to reach statistical significance (p = 0.055). Pre–menopausal status among the women in the population was 37.44%, peri–menopausal status was 24.62% and the rest were in their post–menopausal status.

Factors associated with lipid levels in males and females

Due to the very low self–reported use of anti–diabetic and lipid–lowering medication, and the low prevalence of self–reported HIV infection, these variables were not included in the regression models developed for each of the lipid species.

The association of each of the study variables with LDL-C in both genders is illustrated in Table 3. In the univariate linear regression models formal education, high SES, BMI, waist circumference, hip circumference, visceral and subcutaneous fat were each associated with LDL-C among women. Age, formal education and waist circumference were associated with LDL-C in the multivariable regression model which explained only 8.2% ($p<0.001$) of the variance in LDL-C. In men the factors associated with LDL-C at the univariate level were formal education, high SES, vendor meals, sleep duration, BMI, waist circumference, hip circumference, visceral and subcutaneous fat. Only subcutaneous fat remained significant in the multivariable model which explained 5.1% ($p<0.001$) of the variance in LDL-C levels.

The principal modulators of HDL-C levels among men and women are shown in Table 4. High SES, past or current drinking and sleep duration were associated with HDL-C among women in the univariate analyses but only high SES and past or current drinking remained significant in the multivariable model. Only 2.3% ($p<0.001$) of the variance in HDL-C among women was explained by this model. In men, Nankana ethnicity, past or current smoking, past or current drinking and waist circumference were associated with HDL-C levels in the univariate analysis. Only past or current smoking remained associated with HDL-C in the multivariable model and explained only 0.95% (p = 0.062) of the variance in HDL-C levels.

Table 5 shows the factors associated with TC levels among men and women. In the univariate regression models age, Nankana ethnicity, high SES, BMI, waist circumference, hip circumference and subcutaneous fat were each associated with TC level among women. However only age, Nankana ethnicity and subcutaneous fat remained significant in the multivariable regression model and these explained 4.9% ($p<0.001$) of the variance in TC. Among men SES, fruit consumption and BMI, were associated with TC concentration in the univariate models. Employment and BMI were significant in the multivariate analysis. This model explained 3.0% ($p<0.001$) of the variance in TC among men.

Table 6 shows the factors associated with TG after stratifying by sex. Age, Nankana ethnicity, education, being unmarried, high SES, waist and hip circumferences, visceral and subcutaneous fat were all associated with TG in the univariate analyses among women. In the multivariate linear regression analysis age, being unmarried, waist and hip circumferences, visceral and subcutaneous adipose tissue thickness were significant. This model explained 12.0% ($p<0.001$) of the variance in TG levels. The factors associated with TG among men were

Table 3. Factors associated with LDL-C levels among men and women.

Women				
Variable	**Univariate models**		**Multivariate model**	
	β-Coefficient(95%CI)	**p-value**	**β-Coefficient(95%CI)**	**p-value**
Age(years)	0.004(-0.001, 0.009)	0.092	0.007(0.002, 0.012)	0.004
Nankana ethnicity	-0.040(-0.095, 0.016)	0.161	0.010(-0.046, 0.065)	0.735
Some formal education[1]	0.131(0.067, 0.196)	<0.001	0.095(0.030, 0.160)	0.004
High SES[2]	0.135(0.065, 0.206)	<0.001	0.026(-0.049, 0.100)	0.500
Used smokeless tobacco	-0.077(-0.167, 0.013)	0.092	-0.036(-0.134, 0.053)	0.453
Vendor (meals/week)	0.018(-0.003, 0.039)	0.085	0.013(-0.007, 0.034)	0.191
Sleeping (hours/night)	-0.019(-0.040, 0.001)	0.065	-0.010(-0.030, 0.011)	0.359
BMI (kg/m^2)	-0.023(0.016, 0.030)	<0.001	-0.009(-0.025, 0.005)	0.201
Waist circumference (cm)	0.122(0.094, 0.149)	<0.001	0.116(0.060, 0.172)	<0.001
Hip circumference (cm)	0.089(0.062, 0.116)	<0.001	0.012(-0.032, 0.055)	0.596
Visceral fat (cm)	0.057(0.033, 0.081)	<0.001	0.001(-0.027, 0.028)	0.978
Subcutaneous fat (cm)	0.174(0.125, 0.223)	<0.001	0.046(-0.028, 0.119)	0.222
Men				
Variable	Univariate models		Multivariate model	
	β-Coefficient(95%CI)	p-value	β-Coefficient(95%CI)	p-value
Some formal education[1]	0.072(0.006, 0.138)	0.034	0.022(-0.045, 0.090)	0.514
Employed	0.056(-0.012, 0.124)	0.109	-0.001(-0.073, 0.073)	0.998
Currently unmarried	-0.061(-0.151, 0.029)	0.184	-0.014(-0.104, 0.075)	0.747
High SES[2]	0.148(0.075, 0.221)	<0.001	0.044(-0.037, 0.125)	0.285
Past or current smoker[3]	-0.045(-0.113, 0.023)	0.197	-0.004(-0.048, 0.040)	0.860
Past or current drinker[4]	-0.113(-0.235, 0.010)	0.071	-0.028(-0.157, 0.100)	0.663
Vegetable (servings/day)	0.020(-0.002, 0.042)	0.081	0.021(-0.003, 0.044)	0.087
Vendor (meals/week)	0.021(0.001, 0.040)	0.036	0.008(-0.012, 0.028)	0.419
Sleeping (hours/night)	-0.034(-0.058, -0.010)	0.006	-0.023(-0.047, 0.002)	0.067
BMI (kg/m^2)	0.023(0.013, 0.033)	<0.001	0.001(-0.012, 0.014)	0.896
Waist circumference (cm)	0.114(0.074, 0.153)	<0.001	0.045(-0.011, 0.101)	0.117
Hip circumference (cm)	0.111(0.071, 0.151)	<0.001	0.032(-0.025, 0.088)	0.270
Visceral fat (cm)	0.034(0.007, 0.061)	0.013	0.001(-0.028, 0.030)	0.968
Subcutaneous fat (cm)	0.243(0.159, 0.326)	<0.001	0.139(0.040, 0.238)	0.006

CI: Confidence Interval; all lipid values were logged

[1]education was coded as some formal education vs. no education

[2]SES was coded as those with highest vs. those with lowest SES

[3]smoking status was coded as those who are current or past smokers vs. those who never smoked

[4]alcohol intake was coded as those who had ever drunk alcohol vs. those who had never drunk.

https://doi.org/10.1371/journal.pone.0206326.t003

Nankana ethnicity, high SES, use of smokeless tobacco, fruit consumption, waist circumference and visceral and subcutaneous fat thickness in the univariate analyses. In the multivariate analysis only Nankana ethnicity had a significant association with TG levels. This model accounted for 3.3% ($p<0.001$) of the variance in TG levels among men.

Factors associated with lipid levels in total population

The association of factors with lipid levels in the combined population is shown in S2 Table for LDL and HDL and in S3 Table for TC and TG. All factors associated with lipid levels in either women or men or both show the same association in the combined population. The

Table 4. Factors associated with HDL-C levels stratified by sex.

Women				
Variable	**Univariate models**		**Multivariate model**	
	β-Coefficient(95%CI)	**p value**	**β-Coefficient(95%CI)**	**p value**
High SES[1]	0.077(0.022, 0.132)	0.006	0.081(0.025, 0.136)	0.004
Past or current smoker[2]	0.099(0.018, 0.216)	0.096	0.087(-0.030, 0.203)	0.144
Used smokeless tobacco	0.060(-0.009, 0.129)	0.090	0.061(-0.008, 0.130)	0.084
Past or current drinker[3]	0.086(0.035, 0.136)	0.001	0.084(0.033, 0.134)	0.001
Sleeping (hours/night)	-0.016(-0.032, -0.001)	0.044	-0.013(-0.029, 0.003)	0.110
Men				
Variable	**Univariate models**		**Multivariate model**	
	β-Coefficient(95%CI)	**p value**	**β-Coefficient(95%CI)**	**p value**
Age(years)	0.006(-0.001, 0.008)	0.126	0.002(-0.003, 0.007)	0.463
Nankana ethnicity	0.056(0.003, 0.106)	0.039	0.041(-0.018, 0.100)	0.170
Past or current smoker[2]	0.081(0.028, 0.135)	0.003	0.066(0.009, 0.124)	0.024
Past or current drinker[3]	0.118(0.021, 0.214)	0.017	0.052(-0.053, 0.156)	0.334
Pesticide exposure	-0.043(-0.095, 0.010)	0.112	-0.005(-0.064, 0.055)	0.872
MVPA (hours/week)	0.001(-0.001, 0.002)	0.153	0.002(-0.001, 0.001)	0.796
Malaria in past month	-0.054(-0.125, 0.017)	0.133	-0.042(-0.114, 0.029)	0.243
BMI (kg/m^2)	-0.007(-0.015, 0.001)	0.107	0.001(-0.011, 0.013)	0.876
Waist circumference (cm)	-0.033(-0.065, -0.001)	0.043	-0.029(-0.074, 0.017)	0.215
Hip circumference (cm)	-0.027(-0.060, 0.005)	0.102	0.006(-0.040, 0.052)	0.800

CI: Confidence Interval

[1]SES was coded as those with highest vs. those with lowest SES

[2]smoking status was coded as those who are current or past smokers vs. those who never smoked

[3]alcohol intake was coded as those who had ever drunk alcohol vs. those who had never drunk.

https://doi.org/10.1371/journal.pone.0206326.t004

only exception is sleep duration which was associated with none of the lipid levels in the gender stratified analyses but was associated with LDL-C in the combined analyses. Interestingly, male gender was associated with higher HDL levels in the univariate analysis, but this association was lost in the multivariable model (S2 Table). This suggests that one of the variables in the multivariable analysis was confounding the gender effect. To isolate this variable we performed a univariate regression analysis of HDL with only gender included as an independent variable. We then systematically added each variable from the multivariable analysis into the model, one at a time, until we observed a variable that attenuated the gender effect to non-significance. The variable that did this was smoking. Smoking was more common in males than females (see Table 2) and correlated strongly with HDL levels in both the univariate and multivariate analyses (S2 Table). The relationship between smoking and HDL was investigated further to determine if other variables were modifying this association. The main variable chosen was alcohol intake, as this correlated with HDL in the univariate and multivariate models and was very prevalent in the population (see Table 2). In a univariate regression model for HDL, smoking correlated positively with the lipid in alcohol drinkers ($\beta = 0.031$, $p<0.001$, $n = 1559$), but in non-drinkers the relationship was negative but not significant ($\beta = -0.014$, $p = 0.69$, $n = 280$).

Discussion

The current study shows that the prevalence of dyslipidaemia, as defined by high serum levels of TC, LDL-C or TGs, was relatively low (<6.0% for all 3 lipid species) in this rural Ghanaian

Table 5. Factors associated with TC among men and women.

Women				
Variable	**Univariate models**		**Multivariate model**	
	β -Coefficient(95%CI)	**p value**	**β -Coefficient(95%CI)**	**p value**
Age(years)	0.006(0.003, 0.009)	0.001	0.007(0.003, 0.010)	<0.001
Nankana ethnicity	-0.086(-0.126, -0.046)	<0.001	-0.069(-0.109, -0.029)	0.001
Currently unmarried	0.035(-0.006, 0.076)	0.095	0.025(-0.016, 0.066)	0.233
High SES[1]	0.055(0.004, 0.107)	0.035	0.027(-0.027, 0.081)	0.325
Sleeping (hours/night)	-0.012(-0.026, 0.003)	0.129	-0.008(-0.023, 0.007)	0.280
BMI (kg/m^2)	0.013(0.006, 0.020)	0.001	-0.009(-0.019, 0.001)	0.083
Waist circumference (cm)	0.042(0021, 0.062)	<0.001	0.014(-0.026, 0.053)	0.493
Hip circumference (cm)	0.039(0.020, 0.059)	<0.001	0.030(-0.001, 0.062)	0.061
Subcutaneous fat (cm)	0.089(0.053, 0.125)	<0.001	0.067(0.013, 0.120)	0.015
Men				
Variable	**Univariate models**		**Multivariate model**	
	β -Coefficient(95%CI)	**p value**	**β -Coefficient(95%CI)**	**p value**
Employed	0.074(0.026, 0.122)	0.109	0.070(0.021, 0.118)	0.005
High SES[1]	0.063(0.011, 0.115)	0.018	0.011(-0.046, 0.068)	0.710
Used smokeless tobacco	-0.050(-0.126, 0.026)	0.197	-0.048(-0.124, 0.027)	0.210
Pesticide exposure	0.042(-0.005, 0.089)	0.079	0.018(-0.030, 0.066)	0.463
Fruit (servings/day)	0.021(0.007, 0.035)	0.004	0.016(0.002, 0.030)	0.059
BMI (kg/m^2)	0.013(0.006, 0.020)	0.001	0.010(0.001, 0.019)	0.043

CI: Confidence Interval

[1]SES was coded as those with highest vs. those with lowest SES

https://doi.org/10.1371/journal.pone.0206326.t005

community, and the proportion of subjects that needed treatment for high cholesterol was very low at 0.76%. However the prevalence of low HDL levels was very high at just over 60.0%. The major determinants of serum lipid levels, identified through multivariable linear regression analysis, were varied and included socio-demographic, behavioural and anthropometric factors. Ethnic grouping also influenced lipid levels with women and men from the Nankana ethnic group having lower TC and TG respectively than participants from the other ethnic groups.

The burden of dyslipidaemia in this population, as described by high LDL-C, TC or TG levels, was lower than that observed in Ghanaian urban settings [13,15] and lower than that noted in urban environments in other sub-Saharan African countries [12,37]. A previous study from Ghana comparing lipid profiles between urban and rural populations also demonstrated higher TC and LDL-C levels in the urban population, but higher TG levels in the rural cohort [15]. The higher prevalence of dyslipidaemia in urban settings may be due to reduced physical activity, westernization of diets and sedentary employment [38–40]. Our study also showed that very few dyslipidaemic participants were aware of their status. This may be due to the low background prevalence of hypercholesterolaemia and lack of access to appropriate medical facilities. A recent study in a rural South African population also showed a low level of awareness of dyslipidaemia [41].

Despite the low frequency of high TC, LDL-C and TG levels, we observed a very high prevalence of low HDL-C levels, as has also been observed in other studies from sub-Saharan Africa [42,43]. This raises the question of whether the current cut points used for diagnosing dyslipidaemia in African populations are appropriate, and whether the high prevalence of low HDL-C levels observed in this population suggest heightened CVD risk. One study has

Table 6. Factors associated with TG among men and women.

Women				
Variable	**Univariate models**		**Multivariate model**	
	β-Coefficient(95%CI)	**p-value**	**β-Coefficient(95%CI)**	**p-value**
Age(years)	0.007(0.002, 0.012)	0.006	0.008(0.003, 0.012)	0.002
Nankana ethnicity	-0.075(-0.131, -0.018)	0.010	-0.013(-0.071, 0.045)	0.668
Some formal education[1]	0.097(0.031, 0.164)	0.004	0.046(-0.020, 0.113)	0.173
Currently unmarried	0.111(0.53, 0.168)	<0.001	0.095(0.037, 0.152)	0.001
High SES[2]	0.088(0.015, 0.160)	0.018	-0.030(-0.085, 0.066)	0.802
Vegetable (servings/day)	-0.017(-0.054, 0.003)	0.096	-0.012(-0.030, 0.006)	0.188
Malaria in past month	0.072(-0.004, 0.148)	0.062	0.055(-0.017, 0.127)	0.135
MVPA (hours/week)	-0.001(-0.002, 0.001)	0.090	-0.001(-0.001, 0.001)	1.000
Waist circumference (cm)	0.125(0.097, 0.153)	<0.001	0.094(0.043, 0.144)	<0.001
Hip circumference (cm)	0.068(0.041, 0.096)	<0.001	-0.053(-0.094, -0.011)	0.014
Visceral fat (cm)	0.083(0.059, 0.107)	<0.001	0.033(0.005, 0.062)	0.022
Subcutaneous fat (cm)	0.238(0.188, 0.287)	<0.001	0.137(0.065, 0.209)	<0.001
Men				
Variable	**Univariate models**		**Multivariate model**	
	β-Coefficient(95%CI)	**p-value**	**β-Coefficient(95%CI)**	**p-value**
Nankana ethnicity	-0.119(-0.174, -0.059)	<0.001	-0.084(-0.147, -0.022)	0.008
High SES[2]	0.110(0.041, 0.178)	0.002	0.041(-0.032, 0.115)	0.270
Used smokeless tobacco	-0.109(-0.209, -0.009)	0.032	-0.078(-0.177, 0.022)	0.125
Fruit (servings/day)	0.025(0.006, 0.043)	0.008	0.016(-0.002, 0.035)	0.085
Vendor (meals/week)	0.015(-0.003, 0.033)	0.095	0.007(-0.011, 0.025)	0.433
MVPA (hours/week)	-0.001(-0.001, 0.001)	0.090	-0.001(-0.001, 0.002)	0.347
Waist circumference (cm)	0.059(0.022, 0.096)	0.002	0.038(-0.016, 0.072)	0.207
Visceral fat (cm)	0.029(0.004, 0.054)	0.023	0.003(-0.024, 0.030)	0.844
Subcutaneous fat (cm)	0.153(0.074, 0.231)	<0.001	0.076(-0.016, 0.167)	0.105

CI: Confidence Interval

[1]education was coded as some formal education vs. no education

[2]SES was coded as those with highest vs. those with lowest SES

https://doi.org/10.1371/journal.pone.0206326.t006

reported high rates of fatal CVD events in black African subjects with high HDL-C levels [44], and it has been shown that HDL-C levels in black participants are higher than in white participants even though the occurrence of CVDs is increasing more in the black population [45,46]. Furthermore, CVD risk is greater in subjects with low HDL in combination with high LDL or high triglyceride when compared to those with isolated low HDL levels [47]. This suggests that isolated HDL-C may not be a reliable indicator of CVD risk and supports the call for the shift of focus to HDL-C quality (HDL functionality and subclasses) rather than quantity [46,48] and the use of HDL in combination with other lipid species for predicting CVD risk [47].

Reports of sex differences in lipid levels in African populations are inconsistent. While some findings show that men have more favourable lipid profiles than women [23] other studies report no sex differences in lipids [49,50]. In this population men and women did not have significantly different lipid levels, with the exception of HDL-C which was lower in women. However, after adjusting for tobacco smoking within a multivariable linear regression model, the association between gender and HDL levels was no longer found to be significant. Furthermore, HDL levels correlated positively with smoking in males and in the total population. This is contrary to the literature, which consistently describes a negative relationship between these

variables [51–53]. These data therefore suggested that the higher HDL levels in males were due to their higher frequency of tobacco smoking. Further analyses demonstrated that the positive relationship between smoking and HDL levels was only observed in alcohol drinkers, with the relationship being an inverse one (but not significant) in those who did not drink. This combined effect of smoking and alcohol intake on HDL levels is a novel finding, and requires further investigation in a larger sample size to assess the interaction between these variables and to disentangle the relative effects of smoking and alcohol intake on serum HDL levels. However, it must be noted that the positive association of smoking with HDL levels may be due to residual confounding. This problem may be minimized in future studies by using more objective measures of both smoking and possible confounding variables, including alcohol intake.

Our study did not show significant association between HDL-C levels and age. It has been suggested that low HDL-C is associated with age after 60 years [54]. Similarly studies have reported decreased HDL-C levels with age in cohorts with upper age ranges >60 years [55,56]. The lack of association of HDL-C with age in our study could possibly be due to the younger upper age range of our cohort. The positive association between age and LDL-C, TC and TG levels among women in this population reflects similar findings in previous studies [57,58]. The molecular mechanisms involved in the increase of serum cholesterol and TG levels with aging is not fully understood, although a number of possible causes have been suggested [59] including changes in hormone levels during menopause transition [60].

Lipid levels are known to show ethnic variation [61,62]. Our results show that the Nankana women have significantly lower TC and the Nankana men lower TG levels than their counterparts in the other ethno-linguistic groups analysed in this study. To our knowledge this is the first study in sub-Saharan Africa to assess differences in lipid levels between ethno-linguistic sub-groups within the same broad ethnic group (black Africans). This suggests possible genetic variability since the differences in lipid levels in these groups persist even after adjusting for anthropometric, behavioural and socio-demographic variables. However, these results must be confirmed in further studies that systematically compare lipid levels between these ethno-linguistic groups and adjust for a larger array of possible confounding factors.

The current study found a positive association of SES with HDL-C but a lack of association with LDL-C, TC and TG. This is contrary to several findings which indicate poorer lipid profiles with higher SES [63–65]. This may be due to the use of household assets in computing SES in our study rather than a combination of household assets, highest levels of education attained and employment status used in other studies [64,65].

There are mixed findings regarding the influence of employment status and education level on lipid levels. While some studies have associated low education levels and unemployment with higher lipid levels [65,66] others have reported the contrary [64,67,68]. The results of our study showed independent positive associations of formal education and employment with LDL-C among women and TC among men, respectively.

The positive association of alcohol consumption with HDL-C levels corroborate the findings of other studies [69,70]. It has been suggested that alcohol consumption may raise HDL-C levels by elevating the transport rate of the major apolipoproteins, Apo-I and Apo-II [71].

Pesticide exposure among the study population was high due to agricultural activities in the study area but there was no association between pesticide exposure and lipid levels [18]. This finding contradicts results of other studies which show a positive association of LDL-C, TC, TG and negative association of HDL-C levels with pesticide or chemical exposure [19,20]. The lack of association of pesticide exposure with lipid levels in our study may be due to the evaluation of the association of the exposure as opposed to the blood concentration of pesticides with lipid levels. Further studies are required to evaluate the association of pesticide concentration on lipid levels in this population.

There is a lack of consistent evidence regarding the influence of sleep duration on lipid levels. While some studies show an association of unfavourable lipid levels with increased night time sleep duration other studies show the contrary [72,73]. Though our study did not show association of sleep duration with any of the lipids in the gender stratified analysis, there was a negative association of sleep duration with LDL-C levels in the combined analysis. This calls for further studies on rural African adults to ascertain the influence of sleep duration on lipid levels.

Though diet (vendor meals, vegetable and fruit intake) is known to influence lipid levels our study did not find any association of lipid levels with diet. The lack of association between lipids and diet in our study may also be due to a lack of detailed information on daily food intake.

Despite the low level of obesity in this rural study, our results show that increased BMI was positively associated with TC among men and waist circumference was associated with both LDL-C and TG among women. Subcutaneous fat was associated with both TC and TG among women and both LDL-C and TC among men. The association of these markers of obesity with lipid levels are consistent with results of studies elsewhere [74–76]. Whilst visceral fat was associated with only TG, subcutaneous adipose tissue was associated with increasing LDL-C, TC and TG in the study population. These findings, unlike studies elsewhere that have indicated visceral adipose tissue as a better marker of dyslipidaemia [77,78], suggest that subcutaneous adipose tissue is an important factor influencing lipid levels in this population. Furthermore TG was negatively associated with hip circumference and this is consistent with other findings [77]. Hip circumference is a good measure of gluteofemoral fat which has been shown to protect against CVD risk factors such as insulin resistance, hypertension and dyslipidaemia [77,78].

Conclusion

A longitudinal study is required to investigate whether high prevalence of low HDL-C really is a CVD risk factor in this community. The association of Nankana ethnicity with a healthier lipid profile in older adults is interesting and suggests that further studies, including genetic analyses, are required to understand this association. The association of education and employment with higher lipid levels suggests that societal advances will lead to increasing lipid levels in future, and so this must be monitored over time to allow early interventions. Likewise, the association of anthropometric variables with high lipid levels in a population with a low level of obesity suggests that any increase in the prevalence of obesity in this community may lead to high TC and TG levels. Hence, interventions should be developed to restrict this. Also, the association of hip circumference with lower TG levels suggests an attenuation effect of this adipose tissue depot at least on TGs but again this requires a longitudinal study to determine the magnitude of this effect.

Strengths and limitations

This study has several strengths in terms of sample size, approach to biomarker analysis and the measurement of a large set of potential risk factors for dyslipidaemia. The sample of 1839 participants and the population-based study design is ideal for providing general baseline data. Many studies use derived LDL-C levels from the Friedewald equation, whereas we used a direct measurement method and implemented stringent quality control processes [75,79]. The study is anchored on the strength of the HDSS which provides an ideal platform for follow-up studies to establish the cause of dyslipidaemia among participants. Limitations are that participant age was often estimated based on an events calendar, and therefore may not be accurate,

and lifestyle data were collected based on self-reported responses from the participants and could not be independently verified. The dietary data was limited and did not provide enough detail to truly establish whether food intake was associated with lipid levels. Finally, this was a cross-sectional study that could only investigate factors associated with lipid levels but could not establish causality. To further investigate the causes of abnormal lipid levels in the community the study will be extended to build a cohort that will allow for the collection of similar data from all participants five years after the baseline data collection.

Supporting information

S1 Table. Mean lipid levels of the study population stratified by sex and age category. Correlation performed using Pearson analysis; *p<0.05, **p<0.005 vs women.
(DOCX)

S2 Table. Factors associated with LDL-C and HDL-C levels in the total population.
(DOCX)

S3 Table. Factors associated with TC and TG levels in the total population.
(DOCX)

S1 Fig. Distribution profiles for LDL-C, HDL-C, TC and TG in males and females.
(DOCX)

S1 File. Dataset.
(CSV)

Acknowledgments

We express our appreciation to the study participants who provided data and samples to this project. We are equally grateful to the chiefs and elders of the Kassena-Nankana traditional area whose cooperation and support made this study successful. We appreciate the staff and management of the Navrongo Health Research Centre, Ghana, and the Sydney Brenner Institute for Molecular Bioscience (SBIMB), University of the Witwatersrand, South Africa, who have provided the platform, the material and human support for this project. We express our gratitude to the INDEPTH Secretariat in Accra, Ghana for diverse support towards the AWI-Gen study. We are also grateful to Prof Shane Norris, Yussif Gumah and the staff of the Developmental Pathway for Health Research Unit (DHPRU) laboratory in the Chris Hani Baragwanath hospital in Soweto where all lipid analysis were conducted.

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7.9.2. Appendix I2: Erratum to published article related to this study

PLOS ONE

CORRECTION

Correction: The burden of dyslipidaemia and factors associated with lipid levels among adults in rural northern Ghana: An AWI-Gen sub-study

Godfred Agongo, Engelbert Adamwaba Nonterah, Cornelius Debpuur, Lucas Amenga-Etego, Stuart Ali, Abraham Oduro, Nigel J. Crowther, Michèle Ramsay, as members of AWI-Gen and the H3Africa Consortium

There are errors in the Hip circumference and Waist circumference values in Table 1. Please see the corrected Table 1 below.

There is an error in reference 14. The correct reference is: Asare-Anane H, Bawah AT, Osa-Andrews B, Adanu R, Ofori EK, Tagoe, Bani S A, Ateko EA R.O, AK Nyarko. Lipid Profile In Ghanaian Women With Gestational Diabetes Mellitus. Int J Sci Technol Res. 2013;2(4):168–75.

Citation: Agongo G, Nonterah EA, Debpuur C, Amenga-Etego L, Ali S, Oduro A, et al. (2019) Correction: The burden of dyslipidaemia and factors associated with lipid levels among adults in rural northern Ghana: An AWI-Gen sub-study. PLoS ONE 14(2): e0213233. https://doi.org/10.1371/journal.pone.0213233

Published: February 27, 2019

Table 1. Gender comparison of socio demographic variables, food intake, exercise level, sleep duration, fasting blood glucose levels, blood pressure measurements and anthropometric variables.

Variables	Men	Women	Total	p value
	(n = 846, 46%)	(n = 993, 54%)	(N = 1839)	Men vs women
Age (years)	50 ± 6.0	52 ± 6.0	51 ± 6.0	<0.001
Ethnicity				
Kassena	439 (51.89)	516 (51.96)	955 (51.93)	
Nankana	392 (46.34)	401 (40.38)	793 (43.12)	<0.001
Minority ethnic groups	15 (1.77)	76 (7.65)	91 (4.95)	
Educational status				
No formal education	517 (61.11)	768 (77.34)	1285 (69.87)	
Primary	192 (22.70)	161 (16.21)	353 (19.20)	<0.001
Secondary	111 (13.12)	55 (5.54)	166 (9.03)	
Tertiary	26 (3.07)	9 (0.91)	35 (1.90)	
Employment status				
Unemployed	292 (34.52)	390 (39.27)	682 (37.09)	
Employed	554 (65.48)	603 (60.73)	1157 (62.91)	<0.001
Marital status				
Currently married	717 (84.75)	632 (63.65)	1349 (73.36)	
Currently unmarried	129 (15.25)	361 (36.35)	490 (26.64)	
Household SES categories				
Poorest	129 (15.25)	204 (20.54)	333 (18.11)	
Very poor	139 (16.43)	192 (19.34)	331 (18.00)	
Poor	154 (18.20)	194 (19.54)	348 (18.92)	
Less poor	203 (24.00)	227 (22.86)	430 (23.38)	
Least poor	221 (26.12)	176 (17.72)	397 (21.59)	
Fruit Intake (servings/day)	1.01 ± 1.63	1.10 ± 1.69	1.06 ± 1.63	0.293
Vegetable Intake(servings/day)	3.43 ± 1.46	3.24 ± 1.51	3.33 ± 1.49	0.006
Vendor meals (times/week)	1.17 ± 1.68	0.79 ± 1.31	0.97 ± 1.50	<0.001
MVPA(hours/week)	40.07 ± 28.78	29.84 ± 27.72	34.55 ± 28.66	<0.001
Sleeping (hours/night)	7.71 ± 1.34	8.28 ± 1.32	8.02 ± 1.36	<0.001
Fasting blood glucose(mmol/l)	4.47 ± 0.76	4.61 ± 0.86	4.55 ± 0.82	<0.001
SBP(mmHg)	124.97 ± 20.44	123.28 ± 22.55	124.06 ± 21.61	0.094
DBP(mmol/l)	77.08 ± 12.86	77.12 ± 12.59	77.13 ± 12.72	0.760
BMI (kg/m^2)	20.87 ± 3.15	22.28 ± 3.85	21.63 ± 3.61	<0.001
Hip circumference (cm)	84.1 ± 7.9	89.3 ± 9.9	86.9 ± 9.4	<0.001
Waist circumference (cm)	73.3 ± 8.1	76.8 ± 9.5	75.2 ± 9.1	<0.001
Visceral fat (cm)	4.18 ± 1.21	3.54 ± 1.12	3.83 ± 1.20	<0.001
Subcutaneous fat (cm)	0.78 ± 0.38	1.15 ± 0.54	0.98 ± 0.51	<0.001

Data is given as mean ± SD or n (%)

https://doi.org/10.1371/journal.pone.0213233.t001

Reference

1. Agongo G, Nonterah EA, Debpuur C, Amenga-Etego L, Ali S, Oduro A, et al. (2018) The burden of dyslipidaemia and factors associated with lipid levels among adults in rural northern Ghana: An AWI-Gen sub-study. PLoS ONE 13(11): e0205325. https://doi.org/10.1371/journal.pone.0205325 PMID: 30485283

7.9.3. Appendix J: Manuscript related to this study under review

Candidate gene analysis reveals strong association of *CETP* variants with high density lipoprotein cholesterol in Ghanaian adults: an AWI-Gen sub-study

Running title: *CETP* variants associated with High density lipoprotein cholesterol in Ghanaians

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Number of figures: 3

Word count: 4078

Manuscript should be formatted in British English

Abstract

Variations in lipid levels are attributed partly to genetic factors. Genome-wide association studies (GWASs) mainly performed in European, African American and Asian cohorts have identified variants associated with LDL-C, HDL-C, total cholesterol (TC) and triglycerides (TG), but few studies have been performed in black Africans. This study evaluated the effect of single nucleotide variants (SNVs) in eight candidate loci (*ABCA1*, *LCAT*, *LPL*, *PON1*, *CEPT*, *PCSK9*, *MVK* and *MMAB*) on lipid levels among 1855 Ghanaian adults. All lipid levels were measured directly using an automated analyzer. DNA was extracted and genotyped using the H3Africa SNV array. Linear regression models were used to test the association between SNVs and log-

transformed lipid levels, adjusting for sex, age and waist circumference. In addition Bonferroni correction was performed to account for multiple testing. Several variants of *CETP*, *LCAT*, *PCSK9* and *PON1* (MAF>0.05) were associated with HDL-C, LDL-C and TC levels at $p<0.05$. The lead variants for association with HDL-C were rs17231520 in *CETP* ($\beta=0.139$, $p<0.0001$) and rs1109166 in *LCAT* ($\beta=-0.044$, $p=0.028$). Lower LDL-C levels were associated with an intronic variant in *PCSK9* (rs11806638 [$\beta=-0.055$, $p=0.027$]) and increased TC was associated with a variant in *PON1* (rs854558 [$\beta=0.040$, $p=0.020$]). Functional analyses indicated that these variants likely influence gene function through their effect on gene transcription. We validated a strong association between *CETP* variants and HLD-C in West Africans, with two potentially functional variants.

Keywords: lipid, single nucleotide variants, candidate genes, AWI-Gen, Ghanaians

1.0 Introduction

Cardiovascular disease (CVD) is a major health risk accounting for over 17 million deaths (about 30% of all deaths) globally each year. A major proportion (80%) of these deaths occurs in low and middle-income countries with the total number of annual deaths expected to reach 23.6 million by 2030 (Wang *et al.*, 2016). One major risk factor for CVD is dyslipidaemia which is a metabolic derangement that predisposes an individual to atherosclerosis (O'Donnell and Elosua, 2008; Reinikainen *et al.*, 2015). There is variation in the prevalence of dyslipidaemia across populations with the global adult prevalence of raised total cholesterol (TC) in 2008 estimated at 9.7% (WHO, 2011). Population variations are attributed partly to genetic ancestry (Willer, 2013).

It has been documented that approximately 25% to 80% of the inter-individual variation in lipid phenotypes is heritable (Perusse *et al.*, 1997; Beekman *et al.*, 2002). Single nucleotide variants (SNVs) in the following genes have been shown to be associated with differences in lipid levels: lipoprotein lipase (*LPL*) (Pirim *et al.*, 2014), cholesteryl ester transport protein (*CETP*) (Ridker *et al.*, 2009), paraoxonase 1 (*PON1*), (Durrington, Mackness and Mackness, 2001), ATP-binding cassette A1 (*ABCA1*) (Kelishadi *et al.*, 2014), lecithin-cholesterol acyltransferase (*LCAT*) (Hovingh *et al.*, 2005), protein convertase subtilisin/kexin type 9 (*PCSK9*) (Kotowski *et al.*, 2006), methylmalonic aciduria cb1B (*MMAB*) (Sun *et al.*, 2016) and mevalonate kinase (*MVK*) (Miao *et al.*, 2017). Evidence from candidate gene and genome-wide association studies (GWASs) on the influence of genetic polymorphisms on lipid level variations comes primarily from studies in cohorts of European origin, and an increasing number in Asians and populations of African descent, but few in black Africans (Willer *et al*, 2013; Dron and Hegele 2016). Identifying the common genetic variants associated with lipid levels in populations in Africa will assist in the detection of individuals at higher risk for dyslipidaemia.

The ability to identify gene loci that associate with serum lipid (high density lipoprotein cholesterol [HDL-C], low density lipoprotein cholesterol [LDL-C], triglycerides [TG] and total cholesterol [TC]) levels has been advanced by the use of genotyping arrays that were developed from several projects including the HapMap project (The International HapMap Consortium et al. 2007; Wu et al. 2013), the 1000 Genomes Project (The 1000 Genomes Project Consortium, 2010; Wood *et al.*, 2013) and the Genome of the Netherlands Project (Boomsma *et al.*, 2014; van Leeuwen *et al.*, 2015). These arrays are generally Eurocentric and under-represent common variants found in African populations. We therefore used the H3Africa Afrocentric array

(Mulder *et al.,* 2018) to investigate the influence of SNVs at selected loci on lipid levels in our study population.

The aim of our study was to examine the genetic association of SNVs in the transcribed regions of eight genes previously associated with LDL-C, HDL-C, TC and TG and to perform a replication study for the associated SNVs in another African cohort. This is the first African study to use an Afrocentric gene array to perform and replicate a candidate gene analysis of serum lipid levels.

2.0 Methods

2.1 Study design and population

This is a candidate gene study that was conducted in the Kassena-Nankana districts of northern Ghana as part of the Africa Wits-INDEPTH Partnership for Genomic research (AWI-Gen) project (Michele Ramsay *et al.*, 2016) under the broader Human Heredity and Health in Africa initiative (The H3Africa Consortium, 2014). A sample size of 2200 men and women aged 40-60 years and of roughly equal sex ratio, including 10% for non-response or refusal to participate, was randomly selected. Of this number, 2016 participants were recruited into the study (Agongo et al, 2018).

Given this sample size, a power analysis was performed using Quanto software version 1.2.4 (Gauderman, 2002). Using information from a previous study that reported a mean HDL-C level in West Africans of 1.016±0.321mmol/l with an effect size of 0.388mmol/l for the rs328 variant in the *LPL* gene at ~5% minor allele frequency (MAF) (Bentley *et al.*, 2014), we determined that our study had >90% power to detect an effect size of at least 0.129mmol/l for lipid traits at MAF>0.05 for a sample size of 1800 individuals. This study was carried out in accordance with the recommendations of the National Institutes of Health guidelines, Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M12109), the Ghana Health Service Ethics Review Committee (ID No: GHS-ERC:05/05/2015) and the Navrongo Health Research Centre Institutional Review Board (ID No: NHRCIRB178) with community engagement and written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ID No: M12109), the Ghana Health Service Ethics Review Committee (ID No: GHS-ERC:05/05/2015) and the Navrongo Health Research Centre Institutional Review Board (ID No: NHRCIRB178).

2.2 Demographic data, anthropometric measurements and lipid analyses

Since many of the participants did not know their exact birthdates, their ages were mainly estimated using information from the Navrongo Health and Demographic Surveillance System (Oduro *et al.,* 2012). Fasting whole blood samples were taken from participants for lipid measurements and DNA extraction for genetic analyses. Serum HDL-C, LDL-C, TC and TG were all measured directly using an automated chemistry analyzer and waist circumference was measured as described elsewhere (Ali *et al.*, 2018).

2.3 Selection of candidate genes

Genes were selected based on evidence from genome-wide association and candidate gene studies on lipid levels in peoples of African descent (Adeyemo *et al.*, 2012; Clara C. Elbers *et al.*, 2012; Bentley *et al.*, 2014) and black Africans (Abessolo *et al.*, 2014; Niemsiri *et al.*, 2015). We analysed polymorphic markers in 8 candidate genes: *ABCA1*, *LCAT*, *LPL*, *PON1*, *CETP*,

PCSK9, *MVK* and *MMAB* by selecting SNVs in the transcribed region of each gene (genome coordinates shown in Table 1).

2.4 DNA extraction and genotyping

The DNA was extracted using a modified protocol of the salting out method (Diego Chacon-Cortes, 2014). Genotyping was performed using the H3Africa single nucleotide variant (SNV) array designed on the Illumina platform. The array is enriched for common variation in African populations and contains >2.2 million SNVs (Mulder *et al.,* 2018).

2.5 Imputation and quality control processes

The following pre-imputation quality control (QC) filters were applied to the genotyping data of the entire AWI-Gen dataset of 10,903 individuals from six different sites of which our study site is one. Individuals with a missing call rate>0.05 and SNVs with a missing call rate>0.05, MAF<0.01 and Hardy-Weinberg equilibrium (HWE) p-value<0.0001 were removed from the data set. SNVs that did not match the GRCh37 reference alleles or strands were also removed. The cleaned dataset (with 1,729,661 SNVs and 10,903 individuals) was, pre-phased with EAGLE2 and imputed using the Sanger Imputation Server with the African reference panel. The default default Positional Burrows-Wheeler transform (PBWT) algorithm was used for imputation. At post imputation QC, poorly imputed SNVs (SNVs with IMPUTE2 information score<0.6), SNVs with MAF<0.01 and HWE p-value<0.00001 were excluded resulting in the final quality controlled imputed dataset containing 10,903 individuals and 13,980,000 SNVs. The data for this study was extracted from the larger dataset. The final data set included 1855 participants from Ghana. Only SNVs with MAF>0.05 in the regions described in Table 1 were used for data analysis.

2.6 Data analysis

Lipid levels were presented as medians with interquartile ranges and compared between men and women using Mann Whitney U test. The lipid levels were log-transformed and presented across genotypes of lead SNVs using analysis of covariance (ANCOVA) with adjustment for age, sex and waist circumference. PLINK version 1.90 was used for association analyses (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell *et al.*, 2007). Data on SNVs in the transcribed regions of selected genes were extracted from the H3Africa AWI-Gen genotyped data for further analyses. Pearson χ^2 test was used to assess deviation from HWE equilibrium by comparing observed to expected frequencies. Linear regression analyses were used to test for association between log-transformed lipid traits (Agongo et al, 2018) and SNVs. Standardized β values and confidence intervals were calculated using the major allele as reference. All p-values were corrected for multiple testing using the Bonferroni method (Bland and Altman, 1995) and adjusted for covariates (age, sex and waist circumference). LocusZoom plots were drawn using an online analysis tool at http://csg.sph.umich.edu/locuszoom by the University of Michigan, Department of Biostatistics, Centre for Statistical Genetics (Pruim *et al.*, 2010). All Bonferroni-adjusted p-values at 5% significance level after covariate adjustment were considered significant.

2.7 Functional analysis of significant SNVs

Functional variant analyses included localization of the variant within the gene region and combined annotation dependent depletion (CADD) scores to predict the damaging effect of the

variant on protein structure and function. Another annotation was loss of function tool (LoFtool) score which predicted genic intolerance and consequent susceptibility to disease. A CADD score above 10 implied a deleterious effect and a low LoFtool score indicated damaging effect of the mutations on the gene. The RegulomeDB (RDB) score (Boyle *et al.*, 2012) was used to assess the regulatory potential of the variants. All of these were analysed using Variant Effect Predictor (McLaren *et al.*, 2016). The 1000 Genomes database (accessed using https://www.ncbi.nlm.nih.gov/dbvar) was used to compare the MAFs of the SNVs analysed in the current study with those observed in other populations.

2.8 Replication

All variants with $p<0.05$ in either the covariate-adjusted or unadjusted models (n=21) were selected for replication using the Africa America Diabetes Mellitus study (AADM), which has been previously described (Rotimi *et al.*, 2001). Briefly, AADM is a genetic epidemiology study of type 2 diabetes (T2D), enrolling participants from university medical centers in Nigeria (Enugu, Lagos and Ibadan), Ghana (Accra and Kumasi), and Kenya (Eldoret). Analyses were conducted using linear mixed models of the log transformations of serum lipids in up to 4317 participants with available data. The first 2 PCs of the genotypes were included in the model. All models also included a genetic relationship matrix to account for the random effect of relatedness, as related individuals were included in AADM, and were adjusted for T2D, as this is a case-control study. Models were run using EPACTS (http://genome.sph.umich.edu/wiki/EPACTS) with and without adjustment for additional covariates including age, sex and waist circumference. Replication was defined as an association in a consistent direction with Bonferroni-corrected $p<0.0024$ (i.e. 0.05/21).

3.0 Results

3.1 Characteristics of the study population

The demographic characteristics and lipid levels of the study population, stratified by sex, are shown in Table 2. Participants who had no genetic data were excluded from the analyses resulting in a sample size of 1855. The average age of the study participants was 51 years with women being significantly older than men ($p=0.0001$). Waist circumference among women was significantly higher than that among men ($p<0.0001$). There was no significant difference in LDL-C ($p=0.427$), TC ($p=0.093$) and TG ($p=0.854$) levels between men and women but HDL-C levels were significantly lower ($p=0.0009$) among women. Though not presented in the results, there was no self-reported dyslipidaemia or self-reported lipid lowering treatment among the study participants.

3.2 Variants associated with lipid levels

We tested the association between variants within the transcribed regions of selected genes (*ABCA1*, *CETP*, *LCAT*, *LPL*, *MMAB*, *MVK*, *PCSK9* and *PON1*) and each lipid fraction as a continuous variable (HDL-C, LDL-C, TC and TG). There were associations between variants of *CETP*, *LCAT*, *PCSK9* and *PON1* and lipid levels at $p<0.05$ after adjustment for age, sex and waist circumference (Table 3). The lead associated SNVs were the following: rs17231520 and rs34065661 in *CETP* and rs1109166 in *LCAT* for HDL-C, rs11806638 in *PCSK9* for LDL-C and rs854558 in *PON1* for TC. For *CETP*, there were 11 SNVs that were significantly associated with HDL serum levels, with the strongest associations being observed at rs17231520 and rs34065661 (Table 3). All but one of these SNVs (rs3816117) showed a positive association

between the minor allele (relative to the major allele) and HDL-C levels. One SNV within the *LCAT* gene (rs1109166) showed a significant negative association with HDL-C levels. With regards to LDL-C, 4 SNVs within the *PCSK9* gene were identified with significant associations with serum LDL-C levels. However, only one of these (rs11806638) remained significant ($p<0.05$) after adjustment for age, sex and waist circumference. All these variants demonstrated negative associations with LDL-C levels. Five SNVs in the *PON1* gene were significantly associated with serum TC levels, but only 3 (rs854558, rs854564 and rs854565) of these remained significant after adjustment for age, sex and waist circumference. All these SNVs were positively associated with TC serum levels. No significant gene associations were found for serum TG levels.

The LocusZoom plots for the significant associations are shown in Figures 1A and 1B and lead SNVs from *CETP*, *LCAT*, *PCSK9* and *PON1* are shown. Figure 2 shows the median serum lipid levels given each genotype for each lead SNV. The rs17231520 SNV within *CETP* shows an additive effect, with serum HDL-C levels increasing in the presence of the minor allele (A). The SNV within *LCAT*, rs1109166, showed a dominant and negative effect of the minor allele (T) on serum HDL-C levels. The minor allele (A) at rs11806638 in the *PCSK9* gene demonstrated a negative effect on LDL-C levels, in a recessive manner. The serum levels of TC were positively associated with the minor allele (T) at rs854558 in the *PON1* gene, with the T allele acting in a dominant fashion.

3.3 Functional annotation of significantly associated variants

Results of functional analyses of the significantly associated variants are summarised in Table 4. The functional annotation shows that the leading associated variant (rs17231520: p=4.44e-09) in the *CETP* region was an upstream variant with an RDB score of 5 suggesting possible regulation of the rate of transcription. The second lead SNV in *CETP* (rs34065661: p=5.03e-09) was a missense variant which also had an RDB score of 5 but with a CADD score>10 which suggested a possible deleterious effect on gene structure and protein function. All other significantly associated variants were located in the introns of three four (*CETP, LCAT, PCSK9* and *PON1)* and had RDB scores of 4 or 5 which indicated possible effects on binding sites and regulation of transcription. The LoFtool score of all mutations in *PON1* was 0.787 suggesting benign susceptibility of these mutations to high TC levels. The LoFtool scores of rs1109166 in *LCAT* and rs11806638 in *PCSK9* were 0.127 sand 0.467 respectively suggesting possibly damaging effects of the mutations on gene function.

The frequencies of the minor alleles for our lead SNVs were similar to what have been observed in 1KG African populations (Table 4). Notably, some of our associated variants, including rs17231520 and rs34065661 in *CETP*, are observed only in those with African ancestry.

3.4 Linkage disequilibrium among significant variants

Linkage disequilibrium (LD) among significantly associated SNVs was assessed using LDlink, a web-based tool (Machiela and Chanock, 2015). The value of r^2 in *CETP* ranged from 0.000 to 1.000 (Table S1) while that of *PON1* ranged from 0.993 to 1.000 (Table S2). In *CETP*, there was complete LD between two pairs of SNVs (rs711752 with rs708272 and rs17231520 with rs34065661) and strong LD between three pairs of SNVs (rs891142 with rs891143, rs4784740

with rs891142 and rs4784740 with rs891143) (Figure 3A). All the significant variants in *PON1* were in complete LD with each other (Figure 3B).

3.5 Replication results

Replication results of significant variants in our population using the AADM study are shown in Table 5. All the *CETP* variants in both the unadjusted and adjusted models were replicated with the two lead signals being rs34065661 (β=0.122, p=1.62e-07) and rs17231520 (β=0.120, p=2.68e-07) in the replication analyses. Similarly, all signals in *PCSK9* in the unadjusted model were replicated. However in the adjusted model the significant SNV (rs11806638) in our study was not replicated (study population: β=-0.055, p=0.02683; replication: β=-0.009, p=0.4535). None of the significant SNVs in *LCAT* and *PON1* was replicated.

4.0 Discussion

This study on adults in northern Ghana evaluated the associations between lipid levels and common nucleotide variants in the transcribed regions of eight candidate genes involved in lipid pathways. After adjustment for sex, age and waist circumference, SNVs in four genes (*CETP*, *LCAT*, *PCSK9* and *PON1*) were significantly associated with lipid levels. Variants in *CETP* and *LCAT* were significantly associated with HDL-C levels. One variant in *PCSK9* was significantly associated with LDL-C and another in *PON1* associated with TC levels.

Previous studies have suggested that African populations and populations of African descent tend to have higher HDL-C levels than Europeans suggesting a more favourable profile for CVD risk (Seedat et al. 1992; Chaturvedi et al. 1994; Johnson et al. 2004; Miljkovic-Gacic et al. 2006; D'Adamo et al. 2010). In this study higher HDL-C levels were strongly associated with an upstream variant, a missense variant and intronic variants in the *CETP* gene. The lead SNV (rs17231520) was in the upstream regulatory region and functional prediction suggests an impact on the rate of transcription. The high CADD score of the associated missense variant (rs34065661) is interesting as it suggests a deleterious effect on protein function. The two lead SNVs in *CETP* may be the causal variants for the region for high HDL-C in West Africans since they are functionally relevant, their effect sizes are larger than those of the others and they are common in African populations (>0.08), and were not observed in 1000 Genomes European and Asian populations. Additionally we identified several intronic SNVs in the *CETP* gene that were positively associated, except rs3816117, which was negatively associated, with HDL-C levels. Functional analysis suggests these intronic variants regulate gene transcription rate but their low CADD scores indicate that the effect of their regulatory activity on gene function is benign. The significantly associated variants in *CETP* probably impaired the ability of the gene to accelerate the transfer of cholesteryl esters (CE) from cholesteryl ester-rich HDL-C formed by *LCAT* to other lipoprotein particles resulting in increased HDL-C levels (Brown *et al.*, 1989).

Most of the SNVs in the *CETP* gene replicate previous GWAS findings in peoples of African descent (Buyske *et al.*, 2012; Clara C Elbers *et al.*, 2012; Pirim *et al.*, 2016). Our replication analysis, using the African American diabetes mellitus (AADM) study involving over 4000 East and West Africans (Rotimi *et al.*, 2001), reveals that the directions of effects of the SNVs in *CETP* were consistent with all those in our dataset. The minor allele frequencies of these variants in our population were similar to the allele frequencies in other Africans both in the 1000 Genomes data and replication population but the effect sizes of the variants in our population

were larger (Tables 3 and 5). The rs17231520 (A) and rs34065661 (G) alleles which are absent in European and Asian populations, were previously found to be positively associated with HDL-C levels among African Americans (Buyske *et al.*, 2012; Clara C. Elbers *et al.*, 2012) and Black Africans (Pirim *et al.*, 2016). As in our study, the two variants were in strong LD in all these studies. In these studies the frequencies of the rs17231520 (A) and rs34065661 (G) alleles were similar to those in our populations but the effect sizes of these alleles were smaller in our study. Similarly our study replicated rs711752, rs708272, rs891141, rs891143 and rs289719 variants in *CETP* that were previously found to be associated with HDL-C in black Africans by Pirim et al. 2016. Though the MAFs of these variants in this African population were similar to those in our study, the effect sizes of the variants were generally larger in our population.

In our study the minor allele (T) of rs1109166 in *LCAT* was negatively associated with HDL-C levels. Since the T allele is the common non-African allele (0.76 to 0.90 in Europeans and Asians), its association with low HDL-C in Africans may not be functionally relevant, despite the fact that the low LoFtool score predicts a deleterious effect for this intronic variant (Le Hir et al., 2003; Chorev and Carmel, 2012). It may be tagging other functionally relevant variants through LD, which could interfere with the ability of *LCAT* to form CE and to promote cholesterol efflux from peripheral cells. Furthermore, this variant was not associated with HDL levels in the replication study. Our study is therefore the first to report this association in an African population and requires further investigation.

PCSK9 binds to the LDL receptor (LDLR) to form a complex which moves from the endosomal recycling pathway to the lysosome for degradation (Akram *et al.*, 2010). Since LDLR removes cholesterol-rich LDL particles from plasma (Poirier *et al.*, 2008), a loss of function or reduction in PCSK9 activity inhibits LDLR degradation and leads to the reduction of LDL-C in the blood (Park et al. 2004). In this study the rs11806638 (A) allele in *PCSK9* was negatively associated with LDL-C level. Though we did not replicate this variant association in other black Africans from the AADM study (Rotimi *et al.*, 2001) the rs11806638 (A) allele was previously associated with lower LDL-C levels in African Americans (Musunuru *et al.*, 2012). Interestingly the allele frequency and effect size of the association in African Americans were similar to those in our population.

PON1 encodes an enzyme that exists within the HDL particle and protects HDL and LDL from peroxidation by degrading or hydrolyzing specific oxidized cholesteryl esters and phospholipids contained in oxidized lipoproteins (Barter *et al.*, 2004). This process contributes mainly to the antioxidation properties of the HDL particle which results in improved macrophage-mediated cholesterol efflux (Parthasarathy et al. 1990). Genetic variation in the gene is therefore associated with increased TC levels. Our results show positive associations of rs854558-T, rs854564-G, and rs854565-A alleles in *PON1* with TC in the study population. To the best of our knowledge this study is the first to report the association of these variants with TC levels, although other studies have shown associations of other SNVs in the *PON1* gene with serum lipid levels (Chang *et al.*, 2010; Bentley *et al.*, 2014; Luo *et al.*, 2018). The similar association observed in the lead variant and other signals (rs854564 and rs854565) in the gene with TC levels could be due to the complete LD between these signals and the lead variant. Though the frequency of the variant allele of rs854558 is substantial (0.385) in the study population, its effect size is small and it is unlikely to play a major role in hypercholesterolaemia and CVD risk.

4.1 Strengths and limitations of the study

The study had several strengths. Firstly, individuals were genotyped using an array enriched for gene variants that are common in African populations. Secondly, it is one of very few studies to evaluate the association of SNVs with lipid levels in Africa thereby contributing knowledge on the influence of genetic factors on lipid levels in under-studied African populations. The West and East African replication cohort (Rotimi *et al.*, 2001) supported the association of *CETP* variants with high HDL-C levels.

In terms of limitations, the sample size was relatively small and therefore underpowered to detect variants with small effect sizes. However, power calculations suggested that there was sufficient power to replicate previously reported gene associations with lipids. Candidate genes were selected from studies involving people of African descent, but not resident in Africa, and thus genes chosen may not be appropriate for indigenous African populations. Furthermore, only eight genes were included in the study and therefore additional genes that contribute more strongly to modulating lipid levels may have been overlooked. However, a GWAS for lipid levels is underway for the entire AWI-Gen dataset. The Bonferroni method to reduce false discovery rate assumes that pairwise tests are independent and this is considered by many to be overly conservative, as many SNVs from the same gene locus would be in linkage disequilibrium. Therefore results from sets of tests may not be independent (Lewis, 2002). Lipid lowering medications may affect the direction of associations and mask the genetic effect on lipid levels, but since no person reported taking such medication this was not a concern for the study. We equally are mindful of the potential masking effect of comorbidity in our study sample on the signals of association with lipid levels. Further research is needed on gene-gene interaction and gene-environment interaction to fully elucidate the factors influencing lipid levels in this African population.

4.2 Conclusion

Our findings showed that several variants in *CETP*, *LCAT*, *PCSK9* and *PON1* in this Ghanaian population were significantly associated with HDL-C, LDL-C and TC with the strongest signal being that of rs117231520 (*CETP*) with serum HDL levels. Some of these variants replicate previously reported lipid trait GWAS loci in African Americans and strengthen the evidence of the influence of these signals on lipid levels in peoples of African descent. In addition to our study being the first of its kind to investigate association of genetic polymorphisms with lipid levels in Ghanaians it is also the first to show an association of rs854558, rs854564 and rs854565 in *PON1* with TC levels.

Acknowledgement

We are grateful to the chiefs and people of the Kassena-Nankana traditional area (Ghana) for their cooperation and provision of samples that made the study possible. We appreciate the support and contribution of the management and staff of the Navrongo Health Research Centre (NHRC) and the Sydney Brenner Institute for Molecular Science (SBIMB). We are particularly grateful to Prof Scot Hazelhurst of the SBIMB for playing a lead role in the AWI-Gen genetic data curation. We acknowledge the roles of Ms Immaculate Anati of the NHRC for processing the buffy coats and Ms Cassandra Soo and Dr Stuart Ali both of the SBIMB for the DNA extraction and the management of the AWI-Gen phenotype data respectively. We also appreciate the effort of Prof Osman Sankoh, formerly Director of INDEPTH and co-principal investigator, on the AWI-Gen project.

Author contribution statement

GA, LA, NE, CD and AO collected the data. AC performed genetic data curation. GA conducted the data analysis and drafted the manuscript. GA, LA, NC and MR interpreted the results. AB and CR performed the replication assessment. LA, NE, CD, AO, NC, AC, AB, CR and MR edited the draft. MR was the principal investigator and team leader in the AWI-Gen project and provided scientific leadership in the development of the research protocol. All authors read and approved the final draft.

Conflict of interest statement

The authors declare no personal, professional or financial conflict of interest.

Funding

This study was funded by the National Institutes of Health (NIH) through the H3Africa AWI-Gen project (NIH grant number U54HG006938) and the Wits Non-Communicable Disease Research Leadership Programme (NIH Fogarty International Centre grant number D43TW008330). The study was supported in part by the Intramural Research Program of the National Institutes of Health in the Center for Research on Genomics and Global Health (CRGGH). The CRGGH is supported by the National Human Genome Research Institute, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the Center for Information Technology (CIT), and the Office of the Director at the National Institutes of Health (1ZIAHG200362).

Data availability statement

All datasets analysed for this study are included in the manuscript and the supplementary files

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Table 1: Number of variants tested within selected genes following quality control of data

Gene symbol	Chromosome position	Start transcript	End transcript	Number of SNVs
LPL	8	19,759,228	19,824,769	286
LCAT	16	67,973,653	67,978,034	6
CEPT	16	111,682,249	111,727,724	117
PON1	7	94,926,988	94,954,019	94
ABCA1	9	107,543,283	107,690,518	632
MVK	12	110,011,060	110,035,922	55
MMAB	12	109,991,542	110,011,679	84
PCSK9	1	55,505,221	55,530,525	118

Table 2: Basic characteristics of the study population in northern Ghana

Variable	Men (n=866)	Women (n=989)	Total (n=1855)	p value
Age (years)	50 (46-56)	52 (47-56)	51 (46-56)	0.0001
Waist circumference (cm)	72 (69-77)	75 (71-82)	74 (69-79)	<0.0001
HDL-C (mmol/l)	1.14 (0.91-1.40)	1.08 (0.89-1.30)	1.11 (0.90-1.35)	0.0009
LDL-C (mmol/l)	1.60 (1.15-2.19)	1.61 (1.20-2.15)	1.61 (1.18-2.16)	0.6599
TC (mmol/l)	3.11 (2.59-3.70)	3.17 (2.68-3.78)	3.15 (2.62-3.75)	0.0929
TG (mmol/l)	0.56 (0.43-0.73)	0.55 (0.43-0.74)	0.56 (0.43-0.73)	0.8541

Data given as median (interquartile range)

Table 3: Association of single nucleotide variants of selected candidate genes with lipid levels among the total study group (N=1855)

					Without covariates			With covariates[2]		
SNV(Gene)	A1/A2	Position[1]	MAF	pHWE	β (SE)	p_R value	p_B value	β (SE)	p_R value	p_B value
HDL-C										
rs17231520(*CETP*)	A/G	56995827	0.089	1.000	0.1386 (0.0209)	4.19e-11	4.90e-09	0.1387 (0.0209)	3.80e-11	4.44e-09
rs34065661(*CETP*)	G/C	56995935	0.090	1.000	0.1372 (0.0207)	4.47e-11	5.23e-09	0.1371 (0.0207)	4.30e-11	5.03e-09
rs711752(*CETP*)	A/G	56996211	0.246	0.573	0.0833 (0.0137)	1.35e-09	1.58e-07	0.0845 (0.0137)	7.45e-10	8.72e-08
rs708272(*CETP*)	A/G	56996288	0.245	0.531	0.0832 (0.0137)	1.42e-09	1.66e-07	0.0844 (0.0137)	7.82e-10	9.14e-08
rs891142(*CETP*)	T/C	57003977	0.098	1.000	0.0984 (0.0200)	9.29e-07	1.09e-04	0.0983 (0.0200)	9.55e-07	1.11e-04
rs891143(*CETP*)	T/C	57003980	0.097	0.692	0.0976 (0.0200)	1.16e-06	1.36e-04	0.0973 (0.0200)	1.24e-06	1.45e-04
rs4784740(*CETP*)	G/C	57001085	0.091	0.780	0.1008 (0.0208)	1.35e-06	1.58e-04	0.1009 (0.0208)	1.31e-06	1.53e-04
rs3816117(*CETP*)	T/C	56996158	0.378	0.459	-0.0539 (0.0124)	1.48e-05	1.73e-03	-0.0551 (0.0124)	9.17e-06	1.07e-03
rs158478(*CETP*)	C/A	57007734	0.300	0.294	0.0487 (0.0129)	1.59e-04	0.01858	0.0488 (0.0129)	1.49e-04	0.01740
rs891141(*CETP*)	G/T	57003723	0.168	0.115	0.0576 (0.0157)	2.46e-04	0.02875	0.0589 (0.0157)	1.76e-04	0.02053
rs289719(*CETP*)	T/C	57009941	0.440	0.963	0.0430 (0.0120)	3.62e-04	0.04236	0.0443 (0.0120)	2.30e-04	0.02685
rs1109166(*LCAT*)	T/C	67977382	0.219	0.946	-0.0406 (0.0145)	0.00510	0.03060	-0.0408 (0.0145)	0.00476	0.02853
LDL-C										
rs11806638(*PCSK9*)	A/C	55518160	0.396	0.961	-0.0601 (0.0152)	7.87e-05	9.29e-03	-0.0549 (0.0149)	2.27e-04	0.02683
rs11804420(*PCSK9*)	G/A	55520445	0.298	0.346	-0.0612 (0.0165)	2.02e-04	0.02389	-0.0513 (0.0161)	0.00147	0.1736
rs11800231(*PCSK9*)	A/G	55517940	0.215	0.149	-0.0682 (0.0184)	2.21e-04	0.02601	-0.0635 (0.0180)	4.25e-04	0.05017
rs45545732(*PCSK9*)	T/A	55519231	0.273	0.860	-0.0605 (0.0168)	3.08e-04	0.03634	-0.0521 (0.0164)	0.00147	0.17400
TC										
rs854558(*PON1*)	T/C	94945374	0.385	0.659	0.0435 (0.0110)	7.40e-05	6.95e-03	0.0404 (0.0109)	2.13e-04	0.02005
rs854564(*PON1*)	G/T	94948182	0.386	0.807	0.0412 (0.0110)	1.76e-04	0.01654	0.0383 (0.0109)	4.68e-04	0.04398
rs854565(*PON1*)	A/G	94948344	0.386	0.807	0.0412 (0.0110)	1.76e-04	0.01654	0.0383 (0.0109)	4.68e-04	0.04398
rs854566(*PON1*)	A/G	94948749	0.377	0.401	0.0405 (0.0110)	2.22e-04	0.02090	0.0374 (0.0109)	6.23e-04	0.05858
rs854567(*PON1*)	A/G	94948784	0.377	0.401	0.0405 (0.0110)	2.22e-04	0.02090	0.0374 (0.0109)	6.23e-04	0.05858

A1/A2: Minor/major allele; MAF: Minor allele frequency in the study population; pHWE: Hardy-Weinberg p value; N: total number of participants; SE: standard error; [1]Genomic position and chromosome in build 37; p_R: raw p value; p_B: Bonferroni-adjusted p value; [2] Covariates were sex, age and waist circumference

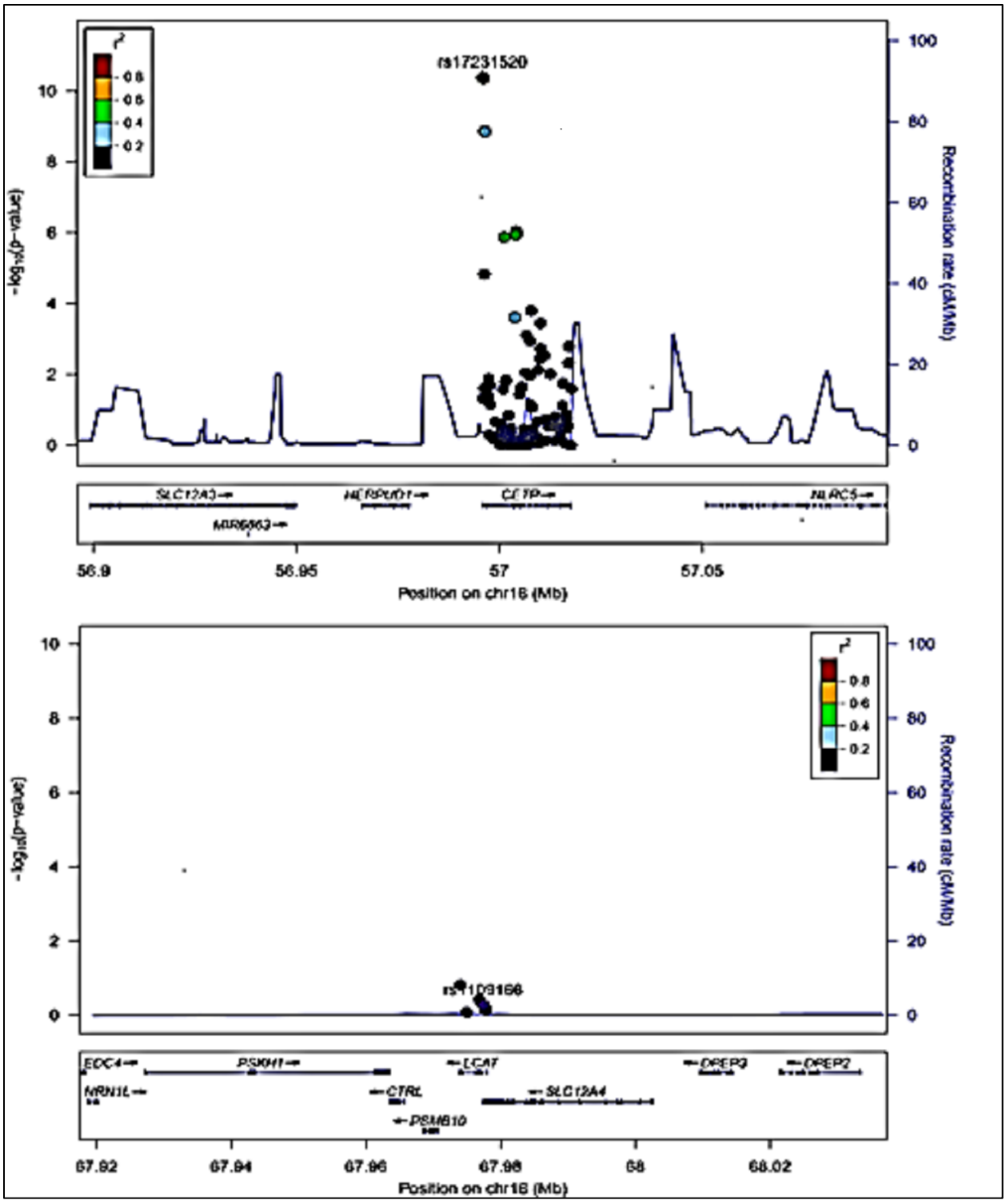

Figure 1A: LocusZoom plots of rs17231520(*CETP*) and rs1109166(*LCAT*) associated with higher and lower levels of HDL-C respectively after adjustment for sex, age and waist circumference

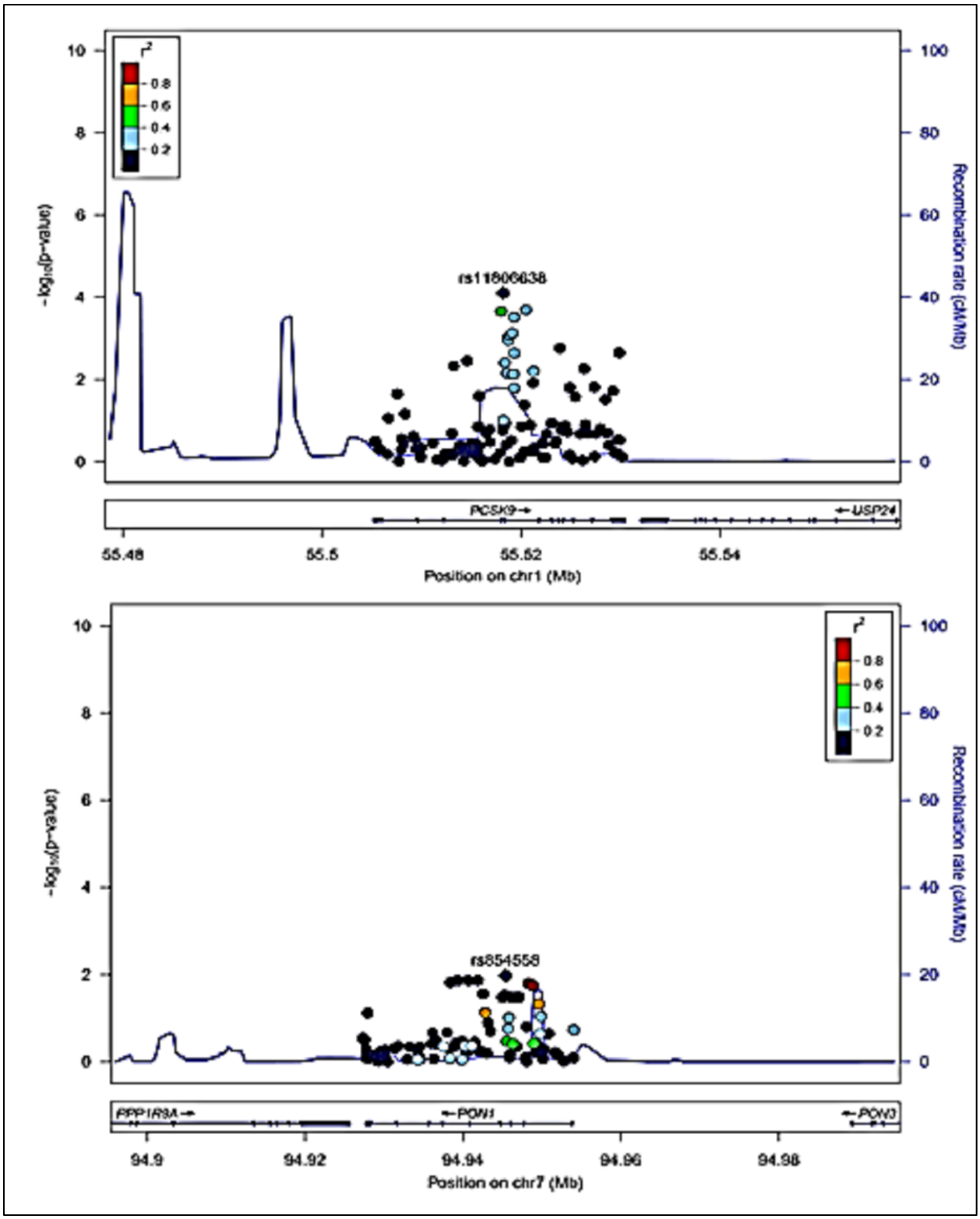

Figure 1B: LocusZoom plots of rs11806638(*PCSK9*) and rs854558(*PON1*) associated with lower levels of LDL-C and higher levels of TC respectively after adjustment for sex, age and waist circumference

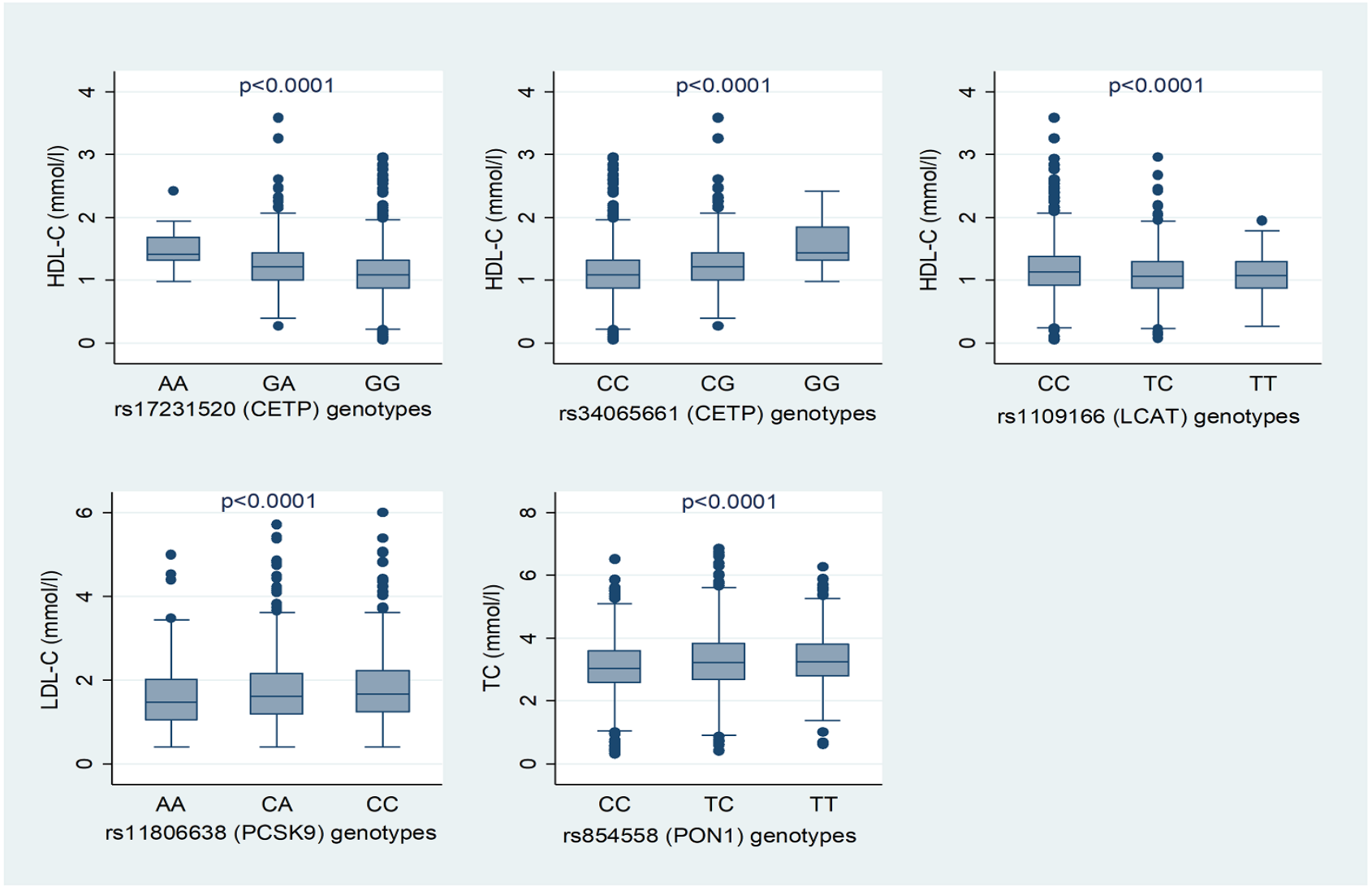

Figure 2: Median lipid levels for the genotypes of the lead SNVs among Ghanaian adults. The p value is adjusted for age, sex and waist circumference

Table 4: Functional annotations of lipid trait associated SNVs in Ghanaian adults and their minor allele frequencies in comparison to other population groups

Variant(Gene)	Localization	CADD score	RDB score	LoFtool score	Minor allele	Minor allele frequencies per population				
						Study population	Africa	Europe	East Asia	South Asia
rs17231520(*CETP*)	Upstream variant	4.38	5	0.970	A	0.089	0.082	0.000	0.000	0.000
rs34065661(*CETP*)	Missense variant	12.64	5	0.970	G	0.090	0.083	0.000	0.000	0.000
rs711752(*CETP*)	Intron variant	8.46	5	0.970	A	0.246	0.246	0.426	0.375	0.451
rs708272(*CETP*)	Intron variant	0.46	5	0.970	A	0.245	0.247	0.425	0.375	0.448
rs891142(*CETP*)	Intron variant	2.69	--	0.970	T	0.098	0.108	0.000	0.021	0.010
rs891143(*CETP*)	Intron variant	1.39	--	0.970	T	0.097	0.103	0.000	0.000	0.000
rs4784740(*CETP*)	Intron variant	0.86	6	0.970	G	0.091	0.096	0.000	0.000	0.000
rs3816117(*CETP*)	Intron variant	0.77	5	0.970	T	0.378	0.404	0.516	0.518	0.397
rs158478(*CETP*)	Intron variant	1.57	--	0.970	C	0.300	0.284	0.524	0.555	0.428
rs891141(*CETP*)	Intron variant	0.17	5	0.970	G	0.168	0.153	0.001	0.021	0.013
rs289719(*CETP*)	Intron variant	2.45	5	0.970	T	0.440	0.441	0.330	0.310	0.452
rs1109166(*LCAT*)	Intron variant	3.06	4	0.217	T	0.219	0.260	0.815	0.904	0.761
rs11806638(*PCSK9*)	Intron variant	3.86	5	0.467	A	0.396	0.356	0.064	0.027	0.064
rs854558(*PON1*)	Intron variant	1.92	--	0.787	T	0.385	0.340	0.714	0.702	0.614
rs854564(*PON1*)	Intron variant	3.01	5	0.787	G	0.386	0.341	0.286	0.299	0.387
rs854565(*PON1*)	Intron variant	2.23	--	0.787	A	0.386	0.341	0.286	0.298	0.387

CADD: combined annotation dependent depletion score predicts damaging effect of the variant on protein structure and function; LoFtool: loss of function tool score predicts gene intolerance and consequent susceptibility to disease; RDB: RegulomeDB score assesses the regulatory potential of the variant; the frequencies are for the 1000 Genomes data

Table 5: Replication results of lipid trait associated variants in East and West Africans (AADM study)

					Without covariates		With covariates[2]	
SNV(Gene)	A1/A2	Position[1]	N	MAF	β (SE)	p value	β (SE)	p value
HDL-C								
rs17231520(*CETP*)	**A/G**	**56995827**	**4317**	**0.039**	**0.2688 (0.0431)**	**5.04e-10**	**0.1202 (0.0233)**	**2.68e-07**
rs34065661(*CETP*)	**G/C**	**56995935**	**4317**	**0.039**	**0.2720 (0.0426)**	**1.94e-10**	**0.1218 (0.0232)**	**1.62e-07**
rs711752(*CETP*)	**A/G**	**56996211**	**4317**	**0.112**	**0.1574 (0.0267)**	**3.84e-09**	**0.0733 (0.0149)**	**8.50e-07**
rs708272(*CETP*)	**A/G**	**56996288**	**4317**	**0.112**	**0.1572 (0.0267)**	**3.94e-09**	**0.0734 (0.0149)**	**8.83e-07**
rs891142(*CETP*)	**T/C**	**57003977**	**4317**	**0.445**	**0.1609 (0.0381)**	**2.44e-05**	**0.0776 (0.0208)**	**0.00019**
rs891143(*CETP*)	**T/C**	**57003980**	**4317**	**0.455**	**0.1890 (0.0397)**	**2.05e-06**	**0.0975 (0.0218)**	**8.07e-06**
rs4784740(*CETP*)	**G/C**	**57001085**	**4317**	**0.041**	**0.2055 (0.0413)**	**6.89e-07**	**0.1055 (0.0227)**	**3.42e-06**
rs3816117(*CETP*)	**T/C**	**56996158**	**4317**	**0.285**	**-0.1134 (0.0228)**	**6.95e-07**	**-0.0480 (0.0125)**	**0.00012**
rs158478(*CETP*)	**C/A**	**57007734**	**4317**	**0.151**	**0.0327 (0.0246)**	**0.18320**	**0.0113 (0.0138)**	**0.41120**
rs891141(*CETP*)	**G/T**	**57003723**	**4317**	**0.427**	**0.1638 (0.0322)**	**3.82e-07**	**0.0902 (0.0176)**	**3.24e-07**
rs289719(*CETP*)	**T/C**	**57009941**	**4317**	**0.284**	**0.07487 (0.0223)**	**0.00081**	**0.0415 (0.0125)**	**0.00094**
rs1109166(*LCAT*)	T/C	67977382	4317	0.382	-0.0302 (0.0272)	0.26750	-0.0003 (0.0147)	0.98330
LDL-C								
rs11806638(*PCSK9*)	A/C	55518160	4287	0.189	-0.0826 (0.0238)	0.00051	-0.0089 (0.0147)	0.45350
rs11804420(*PCSK9*)	G/A	55520445	4287	0.128	-0.1151 (0.0265)	1.42e-05	-0.0279 (0.0118)	0.03157
rs11800231(*PCSK9*)	A/G	55517940	4287	0.099	-0.1035 (0.0292)	0.00039	-0.0258 (0.0130)	0.07349
rs45545732(*PCSK9*)	T/A	55519231	4287	0.115	-0.09416 (0.0274)	0.00060	-0.0257 (0.0134)	0.05602
TC								
rs854558(*PON1*)	T/C	94945374	4317	0.323	0.03918 (0.0237)	0.09874	0.0149 (0.0087)	0.08815
rs854564(*PON1*)	G/T	94948182	4317	0.322	0.03824 (0.0237)	0.10680	0.0150 (0.0087)	0.08526
rs854565(*PON1*)	A/G	94948344	4317	0.322	0.03677 (0.0237)	0.12050	0.0144 (0.0087)	0.09786
rs854566(*PON1*)	A/G	94948749	4317	0.329	0.03672 (0.0237)	0.12400	0.0153 (0.0088)	0.08096
rs854567(*PON1*)	A/G	94948784	4317	0.329	0.03827 (0.0237)	0.10890	0.0163 (0.0088)	0.06418

A1/A2: Minor/major allele; MAF: Minor allele frequency in AADM study; pHWE: Hardy-Weinberg p value; N: total number of participants; SE: Standard error; [1]Genomic position and chromosome in build 37; [2] Covariates were age, sex, waist circumference and T2D; results of replicated variants indicated in bold