***Original Research Article***

# Impact of SLC22A1 and CYP2C9 Gene Variants on Glycemic Response to Metformin/Sulfonylurea Therapy in T2DM Patients in Port Harcourt

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## ABSTRACT


**Aim:** To evaluate the influence of some variants of SLC22A1 and CYP2C9 genes on glycaemic response in T2DM subjects on metformin/Sulphonylureas combination therapy in Port Harcourt.
**Study design:** Cross-sectional Study.
**Place and Duration of Study:** Port-Harcourt, Nigeria, Inqaba Biotec (molecular analysis) and Evashalom Medical Diagnostic services, between November 2022 and April 2023.
**Methodology:** Ninety (90) subjects between aged between 18 - 70years among which 54 were females and 26 males were recruited. Individuals with diagnosed T2DM of at least 1 year, and on continuous metformin/sulphonylureas combination therapy for at least six (6) months prior to the study were selected for this study. Ten (10.0) ml of overnight fasting blood sample was obtained from each subject. This was after completing the questionnaire. Their body weight in kilogram, height in meter was also be measured and recorded. Eight SNPs, four each from SLC22A1 and CYP2C9 genes respectively were selected and genotyped using Mass Array. Blood glucose, total cholesterol (TCHOL), triglycerides (TG) and high-density lipoprotein (HDL-c) were measured by enzymatic colorimetric methods and low-density lipoprotein cholesterol (LDL-c), HOMA_IR, HOMA-β were calculated for each sample. Statistical Analysis was carried out using GraphPad Prism 9.03. Statistical comparisons of the means between groups were made using t-test and oneway analysis of variance (ANOVA). Genotypic and allelic distribution employed the Hardy-Weinberg equilibrium test (HWE). The minor allele frequency (MAF) was calculated using Excel. Furthermore, the associations between the alleles of SNPs and glycaemic response response were assessed using the chi-square test and 95% confidence intervals. Statistical significance was set at $p < 0.05$.
**Results:** This study showed that the AA genotype of rs622342 (SLC22A1) was significantly associated ($p=0.003$) with controlled T2DM in response to metformin/sulphonylureas combination therapy. The results in uncontrolled T2DM showed an association between variants of rs594709(AG/GG) with reduced level of HOMA-IR ($p<0.0331$), rs622342 (CA/AA) with reduced level of HOMA-IR ($p<0.0313$), and rs 9332214 variant (TT) with reduced level of AIP ($p<0.0245$). Moreover, in Obese, the variants of rs594709(AG/GG) was found to be associated with a reduced level of TCHOL($p<0.0054$), rs622342 (CA/AA) with a reduced level of Lipo A ($p=0.0400$), and rs 9332214 (TC/CC) with a reduced level of HbAIc ($p<0.0265$).On the other hand, in hypertensives, an association was found between the variant rs622342 (CA/AA) with a reduced level of BMI ($p=0.0416$).
**Conclusion:** Individuals with the AA genotype of rs622342 are likely to have a better glycaemic response as compared to those carrying the AC/CC genotype receiving metformin/sulphonylurea combination therapy.

*Keywords: SLC22A1 and CYP2C9 genes variants, glycaemic response, T2DM, metformin/Sulphonylureas therapy, Port Harcourt*

## 1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a non- communicable and chronic disorder characterized by hyperglycaemia because of defects in insulin secretion, action or a combination of both which could be because of the involvement of some environmental and genetic risk factors. Globally, it is regarded as an epidemic comprising of about 90% of people with diabetes [1]. It is estimated to affect over 400 million people because of its rising prevalence worldwide [2], especially in low- and middle-income countries [3]. It has been reported that the prevalence in Nigeria ranges from 0.8-11% in both urban and rural populations [4]. Pancreatic βeta-cells (PBC) dysfunction, insulin resistance, disturbed renal glucose transport, arising through the interplay of environmental and genetic risk factors are the main pathological causes of T2DM [5]. It is often associated with complications and long-term damage, dysfunction and failure of different organs resulting to neuropathy, renal failure, blindness and other microvascular complications [5]. Furthermore, T2DM as a common metabolic disorder predisposes to diabetic cardiomyopathy and atherosclerotic cardiovascular disease which could possibly result in heart failure through several mechanisms including chronic pressure overload and myocardial infarction.

Human studies have suggested that most of the variants identified (causal or tagging a causal variant) are linked with impaired beta cell function, whereas insulin secretion shows to be more heritable than insulin resistance. The genetic architecture of insulin action and beta cell function seem to differ, in humans, measures of determining beta cell function are more amenable to genetic approaches than measures of insensitivity. Applying adiposity as a modulator of insulin resistance or concentrating on genetic investigation on more elegant and precise measures of insulin sensitivity has yielded more loci, the majority of which seem to influence beta cell function [6]. Fewer loci, less frequent variants or those with more modest effects or a stronger environmental component might influence insulin resistance. These variations in genetics may influence drug response, this genetic information may be used to guide medication in choices of T2DM which is the standard care for monogenic diabetes. A polymorphism identified in metformin transporter is likely to affect glycemic response to metformin [7]. and a GWAS for metformin response has observed a polymorphism near the ATM gene that influences response to metformin in several independent cohorts [8]. Some sulfonylureas are metabolized by the cytochrome p450 enzyme CYP2C9 and individuals with loss of function variants in this gene are at a high risk of sulfonylurea-related hypoglycaemia [9]. Pharmacogenically, it is suggested that genes essential to drug response may be same as those that increase T2DM or they may be different.

A few candidate gene studies have made efforts to establish genetic predictors of metformin response. In a small cohort, missense variants encoded by SLC22A1 in the organic cation transporter (OCT1) were associated with a reduced response to metformin but the Genetics of Diabetes and Audit Research Tayside Study (GODARTS) failed to establish support for such association in an afterwards follow-up using a large clinical retrospective cohort of T2DM patients. On the other hand, when metformin is combined with drugs that causes OCT1 function impairment, loss -of-function variants present at this locus will increase the rate of gastrointestinal side effects [10]. Variability in response to this drug in type 2 diabetic patients could be associated with the single nucleotide polymorphisms in the poly specific organic cation transporters gene (SLC22A1) solute carrier family member 1 which plays a vital role in metformin influx into the hepatocytes and its excretion through the renal system. A number of SNPs found in SL22A1 have been linked with different responses of metformin in individuals with T2DM. For instance, carriers of rs72552763 in healthy individuals residing in Denmark exhibited reduced hepatic exposure and distribution of metformin, European carriers of rs36056065 may possess a reduced risk of gastrointestinal disturbances related with

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metformin [11]. Studies have also linked rs622342 in SLC22A1 with variability in efficacy of metformin therapy, Lebanese carriers of this SNP showed a greater reduction of HbAIc following a combination therapy of metformin and sulfonylureas [12].
Pharmacogenomic evidence shows a link between specific gene polymorphisms and inter-individual variability in the therapeutic effects of sulfonylureas. These polymorphisms are present in genes of molecules involved in their transport, metabolism and mechanism of action. The most common CYP2C9 variant alleles, *2 and *3 results in impaired metabolism of sulfonylureas and are linked with reduced clearance of sulfonylureas orally and high risk of drug induced hypoglycemia. The reduced response of sulfonylureas treatment is related to SNPs in KCNJ11 and ABCC8 genes that encode kir6.2 and SUR 1 subunits of potassium channels and cytochrome p4502C9 (CYP2C9), the metabolizing enzymes of sulfonylureas. Sulfonylureas are mainly metabolized by CYP2C9, T2DM patients having loss of function variants show a better glycaemic response than those with the wild-type allele [5].
Several SNPs in CYP2C9 are linked with reduced enzymatic activity, for instance, CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) are associated with poor metabolism phenotypes and impaired function [13]. Other genes such as the transcription factor-7-like 2 (TCF7L2) variants are linked to decrease in efficacy of drug and risk of T2DM through β-cell function reduction. Therefore, the aim of this study was to evaluate the influence of some variants of SLC22A1 and CYP2C9 genes on glycaemic response in T2DM subjects on metformin/Sulphonylureas combination therapy in Port Harcourt.

## 2. MATERIAL AND METHODS

### 2.1 Study Area

The study area was in Port Harcourt, capital of Rivers State, Southern Nigeria. It lies along the Bonny River and coordinates 4·49'27"N 7·2'1"E. It has an area of 369km2 and an estimated population of 1,865,000 as at 2016 [14]. It is a major industrial center having large numbers of multinational firms as well as other industrial concerns, particularly business related to the petroleum industry.

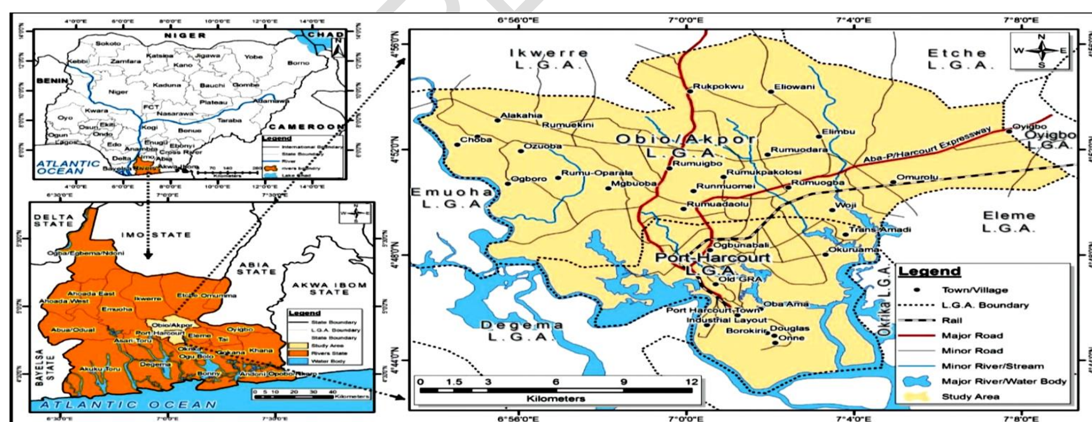


**Figure 1: Map of Port Harcourt [14]**

### 2.2 Research Design

This is a cross sectional study involving the evaluation of some genetic variants associated with glycemic response and to evaluate body mass index (BMI), blood pressure (SBP and DBP) and some biochemical markers such as glycated haemoglobin (HbAIc), insulin, homeostatic model assessment of β function (HOMA-β), homeostatic model assessment for insulin resistance (HOMA-IR¬), fasting blood sugar (FBS), Lipoproteins such as total

cholesterol (TCHOL), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), Non density lipoprotein cholesterol (Non-HDL-C), Lipoprotein A and atherogenic ratios such as Castelli Risk Index 1(CRI-1), Castelli Risk Index 11 (CRI-11), atherogenic index of plasma (AIP) and atherogenic coefficient (AC) in male and female type 2 diabetes mellitus subjects.. The biodata and medical history of the subjects were obtained using a questionnaire.

### 2.3 Validity of Instruments

Using manufacturer's specifications, all sensitive equipment was calibrated. Sample bottles were pre-treated accordingly before use. Appropriate labelling and all pre-analytical precautions were adhered to, so as to prevent errors.

## 2.4 Sample Size Calculation

A total of 90 adults were enrolled for this study. The incident rate of adult with T2DM in Nigeria is 3.84% cases [15]. Making prevalence rate a total of 0.038%. Using the formular,

N=Z2 × p (1-p) / d2
Where N= Minimum sample size
d = Desired level of significance (0.05)
z = Confidence interval (1.96)
p = Prevalence rate or proportion of occurrence (0.038%)
Therefore, N = 3.8416 × 0.038 (1-0.038)/ 0.0025
Minimum participants required =56

### 2.5 Eligibility Criteria

#### 2.5.1 Inclusion Criteria

Individuals with diagnosed T2DM of at least 1 year, aged between 18–70 years and on continuous metformin/sulphonylureas combination therapy for at least six (6) months prior to the study were included.

#### 2.5.2 Exclusion Criteria

Individuals treated with insulin in the last six months or on any other drug for T2DM prior to the study were excluded. Individuals who were also critically ill, particularly with cardiovascular disease, or pregnant were also excluded from study.

## 2.6 Sample Collection

Ten (10.0) ml of overnight fasting blood sample was obtained from each subject. This was after completing the questionnaire. Their body weight in kilograms, height in meters was also measured and recorded.

#### 2.6.1 Order of Dispensing and Volume of the Blood Sample

About 3.0 ml into vacutainer type plain tubes, 4.0 ml into vacutainer type EDTA K3 (1st Tube) & 3.0ml into vacutainer type EDTA K3 (2nd Tube). All the tubes were appropriately labelled. The sample in the plain tube was allowed to retract, then centrifuged at 3000 rpm. The serum was separated into two cryo tubes (one for enzyme-linked immunosorbent assay (ELISA). Insulin and one for Lipid profiles and Lipoprotein A), labelled and stored at -150C to -200C until analysis. The first EDTA tube was transported in cold box to Molecular Laboratory for DNA extraction and genotyping (Sequencing). The second EDTA tube for Glycated haemoglobin was stored at 2-80C until analysis. The fasting blood sugar was performed immediately using a controlled Kiptrack glucometer machine.

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## 2.7 Methodologies for Laboratory Determinants

### 2.7.1 Fasting Blood Glucose (FBG)

FBG was performed using Kiptrack blood glucose monitoring system by Taidoc Technology Corporation, Taiwan [16].

*2.7.1.1 Principle*

The glucose testing is based on the measurement of electric current generated by the reaction of glucose in the sample (blood) with the enzyme glucose oxidase on the test strip. When glucose undergoes a chemical reaction in the presence of the enzymes, electrons are produced. These electrons (i.e, the charges passing through the electrodes) are measured and this is proportional to the concentration of glucose in the sample.

### 2.7.2 Glycosylated Haemoglobin (HbA1c)

Quantitative determination of glycosylated Haemoglobin in blood was done using the modified Ion Exchange Resin method with kit from INTECO Diagnostics, UK [17].

*2.7.2.1 Principle*

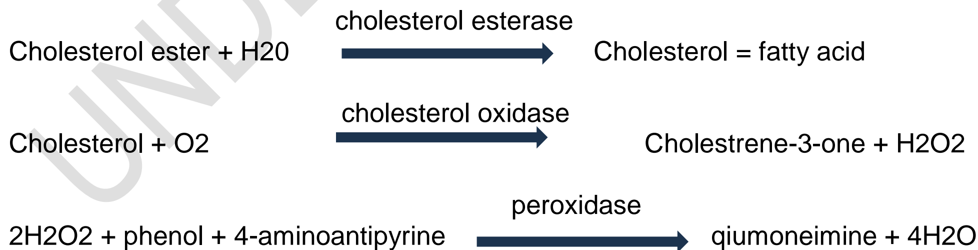
Glycosylated Haemoglobin has been defined operationally as the fast fraction of Haemoglobin A1 which elute first during column chromatography. A haemolysed preparation of whole blood is mixed continuously for 5 minutes with a weakly binding cation-exchange resin. The labile fraction is eliminated during the haemolysate preparation and during binding. During this mixing, the non-glycosylated binds to the ion exchange resin leaving the glycosylated Haemoglobin fraction free in the supernatant. After the mixing period, a filter separator is used to remove the resin from the supernatant. The percent glycosylated Haemoglobin is determined by measuring the absorbance of the ratio of the glycosylated Haemoglobin and the total Haemoglobin fraction [17].

### 2.7.3 Serum Total Cholesterol

Serum fasting cholesterol was performed by the cholesterol oxidase-peroxidase, enzymatic endpoint method using Erba Chem V5 Semi-Autoanalyzer and kit from Randox [18].

*2.7.3.1 Principle*

Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.




### 2.7.4 Serum Triglyceride

Serum fasting triglyceride was performed by a modified enzymatic colourimetric method using Erba Chem v5 Semi-auto analyzer and kit from Randox [19].

*2.7.4.1 Principle*

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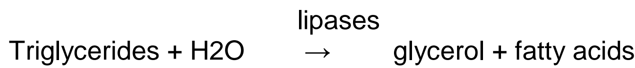
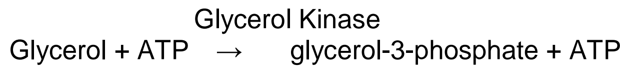
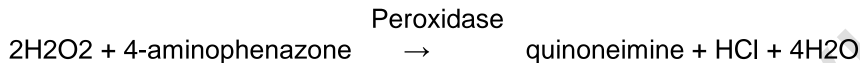
Triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under catalytic influence of peroxidase.






### 2.7.5 Serum HDL-Cholesterol

Quantitative in vitro determination of HDL-Cholesterol in serum was performed using phosphotungstic acid precipitation and the cholesterol oxidase-peroxidase method [20].

*2.7.5.1 Principle*

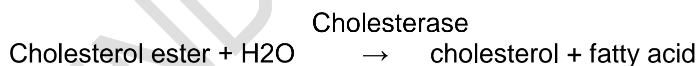
Low Density Lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (High Density Lipoprotein) fraction, which remains in the supernatant, is determined.

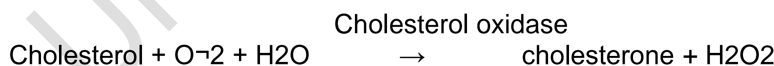
### 2.7.6 Serum LDL-Cholesterol

Quantitative in vitro determination of LDL-Cholesterol in serum was performed using Polyvinyl Sulphate/Polyethyleneglcol precipitation and the cholesterol oxidase-peroxidase method [21].

*2.7.6.1 Principle*

Low Density Lipoproteins (LDL) in the samples is precipitated with polyvinyl sulphate. The concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometrically measured by means of the coupled reactions described below.



### 2.7.7 Non-HDL-C and VLDL

Non-HDL-C and VLDL were calculated using values of lipid profile parameters in the following way;
Non-HDL-C =Total cholesterol- HDL-C
VLDL-C = TG/2.2.

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#### 2.7.8 Atherogenic Indices

Atherogenic indices [Atherogenic Index of Plasma (AIP), Cardiac Risk Ratio (CRR) & Atherogenic Coefficient (AC)] would be calculated using the values of lipid profile parameters in the following way:

AIP = Log (TG/HDL-C)

Where, the concentration of TG and HDL are in mmol/L.

Calculation of AIP was done using CZECH online calculator of atherogenic risk [22].

CR1 = TC/HDL-C
CR11 =LDL-C/HDL-C
AC = (TC-HDL-C)/HDL-C

#### 2.7.9 Lipoprotein a (Bio-Inteco ELISA Kit) Catalog No: E-EL-H0160

*2.7.9.1 Principle*

This Bio-Inteco catalogue kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human LP-a. Samples (or Standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human LP-a and Avidin Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human LP-a, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human LP-a. The concentration of Human LP-a in the samples can be calculated by comparing the OD of the samples to the standard curve [19].

#### 2.7.10 Serum Insulin (Bio-Inteco ELISA Kit) Catalog No: IN3745

*2.7.10.1 Principle*

The Insulin quantitative test is based on a solid phase enzyme-linked immunosorbent assay. The system utilizes one insulin antibody for solid phase (microtiter wells) immobilization and another anti-insulin antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the insulin antibody coated microtiter wells. Then anti-insulin antibody labeled with horseradish peroxidase (conjugate) is added. If human insulin is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the insulin molecules being sandwiched between the solid phase and the enzyme-linked antibodies. After a 1hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of 3,3′,5,5′-Tetramethylbenzidine (TMB) is added incubation and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is stopped with the addition of stop solution. The colour is changed to yellow and measured spectrophotometrically at 450 nm. The concentration on insulin is directly proportional to the colour intensity of the test sample [23].

#### 2.7.11 Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)

HOMA-IR index was calculated using the formula: Fasting Insulin (mU/L) × Fasting Glucose (mmol/L)/22.5 [19].

Healthy Range: 0.5 – 1.4

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**2.7.12 Homeostatic Model Assessment for Beta Cells (HOMA-β)**
HOMA-β index was calculated using the formula: Fasting Insulin (mU/L) × 20/Fasting Glucose (mmol/L)-3.5 [19].

**2.7.13 Genetic Analysis**

*2.7.13.1 Genomic DNA Extraction*

Genomic DNA extractions of the samples were performed using Geneaid DNA Mini Kit.

Principle
RBC Lysis Buffer and chaotropic salt are used to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column. Contaminants are removed using a wash buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt elution buffer, TE or water. The entire procedure can be completed within 25 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA, with approximately 20-30 kb, is suitable for use in PCR or other enzymatic reactions.

Stage 1 Guanidinium Thiocyanate or Guanidinium Isothiocyanate (GITC) Procedure

Ten mililitre (10 ml) of cold 1x Red blood cell lysis buffer (RCLB) was added to each sample and the tube properly closed and was mixed by inversion. The tube was then placed on ice for 10 minutes. The tubes were wiped carefully. The tubes were centrifuged at 4000 rpm for 7 minutes. The supernatant was carefully decanted into the waste bucket. Care was taken not to lose the cell pellet. Ten mililitre (10 ml) of cold 1x RCLB was again added to the cell pellet, mixed by vortexing and steps 3-5 above was repeated. Where there were still traces of red cells, 10 ml of cold RCLB was added to the cell pellet again, mixed by vortexing and steps 3-5 repeated. Ten mililitre (10 ml) of sterile Phosphate buffered saline (PBS) was added to the cell pellet, mixed by vortexing and centrifuged at 4000 rpm for 7 min. The supernatant was decanted, 5 ml of sterile PBS was added into each tube and mixed by vortexing and centrifuged at 4000 rpm for 5 min. The supernatant was decanted into the waste bucket carefully not to discard the pellet and the 15 ml tubes were drained on a clean towel. While draining, the GITC buffer was prepared by adding 10 ul beta-mercaptoethanol (BME) to the 1 ml of GITC.One mililitre (1ml) of activated GITC buffer containing BME was added to the white cell pellets in one tube. A blunt end 18G needle and 2 ml syringe was used to homogenise the GITC lysate 18 times. A sterile Pasteur pipettes was used to transfer the GITC lysate into 2 ml cryovial and labelled accordingly for storage at minus -200C. (The lysate can also be used irnmediately for nucleic acid extraction). For quality control (QC) purpose, also 1ml of the GITC buffer containing BME was transferred into a cryovial, label as QC control and treated as a sample during nucleic acid extractions.

Stage 2 DNA Extraction (Geneaid Genomic DNA Mini Kit)

*2.7.13.2 Selection of SNPs and Genotyping*
Each patient's genomic DNA was analyzed to determine the genotypes of the following genetic variants in the genes indicated: rs594709 (SLC22A1), rs12208357 (SLC22A1), rs622342 (SLC22A1), rs72552763 (SLC22A1), rs1057910 (CYP2C9), rs1799853(CYP2C9), rs41291560 (CYP2C9), and rs9332214 (CYP2C9). Two multiplex Mass ARRAY systems (Agena BioscienceTM) were designed and optimized by Inqaba Biotechnical Industries (Pretoria, South Africa) in January 2017. Each multiplex was used to genotype selected SNPs, using an assay that is based on a locus-specific PCR reaction. This reaction is followed by a single base extension using the mass-modified dideoxynucleotide terminators of an oligonucleotide primer, which anneals upstream of the site of mutation. Matrix-assisted laser

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desorption/ionization—time-of-flight (MALDI-TOF) mass spectrometry was used to identify the SNP of interest.

### 2.8 Statistical Analysis

The general characteristics of the participants were expressed as frequency (percentages), mean (X) and standard deviation (SD). Statistical Analysis was carried out using GraphPad Prism 9.03. Statistical comparisons of the means between groups were made using t-test and oneway analysis of variance (ANOVA). Genotypic and allelic distribution employed the Hardy-Weinberg equilibrium test (HWE). The minor allele frequency (MAF) was calculated using Excel. Furthermore, the associations between the alleles of SNPs and glycaemic response response were assessed using the chi-square test and 95% confidence intervals. Statistical significance was set at p < 0.05. Odd ratios were calculated using the Baptista-Pike test. Statistical significance was set at p<0.05

## 3. RESULTS AND DISCUSSION

**Table 1. Association of SLC22A1 (rs594709, rs622342, rs72552763) and CYP2C9 (rs9332214) Polymorphisms with Glycaemic Response in Type 2 Diabetic Subjects using Dominant Model**

| SNPs | Genotype | Total Sample N=90(n%) | Controlled T2DM N=39 | Uncontrolled T2DM N=51 | OR (95% CI) | p-Value |
|---|---|---|---|---|---|---|
| **rs 594709 A>G** | AA | 66 (73.33%) | 27 (69.23%) | 39 (76.47%) | 0.69 (0.27 - 1.77) | 0.442 |
| | AG | 15 (16.67%) | 12(30.77%) | 12(23.53%) | | |
| | GG | 9 (0.10%) | | | | |
| **rs 622342 A>C** | AA | 57(63.33%) | 18(46.15%) | 39(76.47%) | 0.26 (0.11 - 0.66) | **0.003*** |
| | AC | 33(36.67%) | 21(53.85%) | 12(23.53%) | | |
| | CC | 0(0.00%) | | | | |
| **rs 72552763** | ATGATATGAT | 84(93.33%) | 35(89.74%) | 49(96.08%) | 0.36 (0.06 - 2.06) | 0.233 |
| | ATGATAT | 6(6.67%) | 4(7.69%) | 2(3.92%) | | |
| | ATAT | 0(0.00%) | | | | |
| **rs 9332214 T>C** | TT | 84(93.33%) | 38(97.44%) | 46(90.20%) | 4.13 (0.46 - 36.89) | 0.173 |
| | CT | 6(6.67%) | 1(2.56%) | 5(9.80%) | | |
| | CC | 0(0.00%) | | | | |

Chi-square test was used to test the distribution of each genetic variant between controlled and uncontrolled T2DM groups, p value < 0.05(significant), OR= Odd Ratio

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**Table 2: Association of rs594709 Variant of SLC22A1 Gene Based on Glycaemic Response (HbA1c ≥ 6.5) on Metabolic traits, Lipid Particles, and Atherogenic Indices of T2D Subjects using Dominant Model**

| Parameters | AA (n=39) | AG/GG (n=12) | GLR, P value | PC, p value |
|---|---|---|---|---|
| BMI | 31.46±5.64 | 31.03±6.390 | 0.2953 | 0.2953 |
| FBS (mmol/L) | 8.19±2.52 | 8.18±2.42 | 0.2290 | 0.2290 |
| HbA1c (%) | 8.26±1.79 | 8.38±2.00 | 0.1759 | 0.1759 |
| TCHOL (mmol/L) | 4.63±1.12 | 4.62±1.05 | 0.2921 | 0.2921 |
| HDL (mmol/L) | 1.18±0.37 | 1.08±0.14 | 0.6566 | 0.6566 |
| LDL (mmol/L) | 2.77±1.00 | 2.79±1.07 | 0.1118 | 0.1118 |
| TG (mmol/L) | 1.54±0.80 | 1.58±0.89 | 0.7303 | 0.7303 |
| AIP | 0.09±0.05 | 0.11±0.07 | 0.3428 | 0.3428 |
| CRI-1 | 4.13±1.16 | 4.32±0.98 | 0.6339 | 0.6339 |
| CRI-1I | 2.46±0.97 | 2.61±0.97 | 0.5629 | 0.5629 |
| AC | 3.13±1.16 | 3.32±0.98 | 0.6339 | 0.6339 |
| Insulin (ng/ml) | 4.61±0.61 | 5.23±2.07 | 0.3376 | 0.3376 |
| HOMA-IR | 1.60±1.19 | 1.17±0.72 | ***p=0.0331** $R^2$=0.3788 | ***p=0.0331** r=0.6155 |
| Lipo A | 7.75±3.86 | 5.88±1.90 | 0.0683 | 0.0683 |

GLR=General Linear Regression, PC=Pearson's Correlation, A= Wild (Dominant), G=Polymorphic. *Significant Association, $R^2$ =Coefficient of Determination, r= Pearson's coefficient, n= number of subjects with Dominant or Polymorphic Alleles

**Table 3: Association of rs622342 Variant of SLC22A1 Gene Based on Glycaemic Response (HbA1c≥6.5) on Metabolic traits, Lipid Particles, and Atherogenic Indices of T2D Subjects Using Dominant Model**

| Parameters | AA (n=39) | CA/CC (n=12) | GLR, P value | PC, p value |
|---|---|---|---|---|
| BMI | 31.46±5.64 | 31.03±6.39 | 0.2953 | 0.2953 |
| FBS (mmol/L) | 8.19±2.52 | 8.18±2.42 | 0.2290 | 0.2290 |
| HbA1c (%) | 8.26±1.79 | 8.38±2.00 | 0.1759 | 0.1759 |
| TCHOL (mmol/L) | 4.63±1.12 | 4.62±1.05 | 0.2921 | 0.2921 |
| HDL (mmol/L) | 1.18±0.37 | 1.08±0.14 | 0.6566 | 0.6566 |
| LDL (mmol/L) | 2.77±1.00 | 2.79±1.07 | 0.1118 | 0.1118 |
| TG (mmol/L) | 1.54±0.80 | 1.58±0.89 | 0.7303 | 0.7303 |
| AIP | 0.09±0.04 | 0.11±0.06 | 0.3428 | 0.3428 |

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| | | | | |
|---|---|---|---|---|
| CRI-1 | 4.13±1.16 | 4.32±0.99 | 0.6339 | 0.6339 |
| CRI-1I | 2.46±0.97 | 2.61±0.97 | 0.5629 | 0.5629 |
| AC | 3.13±1.16 | 3.32±0.98 | 0.6339 | 0.6339 |
| Insulin ng/ml | 4.61±3.82 | 3.15±1.69 | 0.4712 | 0.4712 |
| HOMA-IR | 1.60±1.19 | 1.16±0.72 | **p=0.0313** $R^2$ =0.3850 | **p=0.0313** R=0.6205 |
| Lipo A | 7.746±3.861 | 5.883±1.904 | 0.0683 | 0.0683 |

GLR=General Linear Regression, PC=Pearson's Correlation. A= Wild (Dominant), C=Polymorphic. *Significant Association, $R^2$ =Coefficient of Determination, r= Pearson's coefficient, n= number of subjects with Dominant or Polymorphic Alleles

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**Table 4: Association of rs9332214 Variant of CYP2C9 Gene Based on Glycemic response (HbA1c >6.5) to metformin/sulphonylureas therapies/on metabolic traits, Lipid Particles, and Atherogenic Indices of T2D Subjects using Dominant Model**

| **Parameters** | **TT (n=46)** | **TC/CC (n=5)** | **GLR, P value** | **PC, p value** |
|---|---|---|---|---|
| BMI | 31.64±5.619 | 28.80±7.096 | 0.6671 | 0.6671 |
| FBS (mmol/L) | 8.165±2.390 | 8.360±3.466 | 0.3470 | 0.3470 |
| HbA1c (%) | 8.237±1.717 | 8.740±2.887 | 0.6774 | 0.6774 |
| TCHOL (mmol/L) | 4.654±1.145 | 4.400±0.4528 | 0.3886 | 0.3886 |
| HDL (mmol/L) | 1.174±0.3448 | 1.000±0.1581 | 0.5943 | 0.5943 |
| LDL (mmol/L) | 2.798±1.043 | 2.580±0.6140 | 0.6906 | 0.6906 |
| TG (mmol/L) | 1.507±0.7637 | 1.980±1.228 | 0.1984 | 0.1984 |
| AIP | 0.07826±0.03894 | 0.2200±0.1158 | **p=0.0245 $R^2$ =0.8550** | **p=0.0245 R=-0.9247** |
| CRI-1 | 4.139±1.128 | 4.520±1.071 | 0.6803 | 0.6803 |
| CRI-1I | 2.474±0.9630 | 2.700±1.061 | 0.7771 | 0.7771 |
| AC | 3.139±1.128 | 3.520±1.071 | 0.6803 | 0.6803 |
| Insulin ng/ml | 4.861±0.7311 | 3.780±0.8237 | 0.4330 | 0.4330 |

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| | | | | |
|---|---|---|---|---|
| HOMA-IR | 1.700±1.661 | 1.400±0.8155 | 0.2372 | 0.2372 |
| Lipo A | 7.387±3.739 | 6.580±1.252 | 0.1709 | 0.1709 |

GLR=General Linear Regression, PC=Pearson's Correlation. T= Wild (Dominant), C=Polymorphic. *Significant Association, $R^2$ =Coefficient of Determination, r= Pearson's coefficient, n= number of subjects with Dominant or Polymorphic Alleles.

In this study, the effect of three SLC22AI (rs594709, rs622342, rs72552763) and one CYP2C9(rs9332214) polymorphisms on glycaemic response to metformin/sulfonylureas combination therapy in T2DM patients was investigated. The AA genotype of rs622342 was found to be a protective factor and significantly associated with a good glycaemic response, this can be useful in predicting the response to metformin in combination therapy in Nigerian T2DM patients. The SLC22A1 gene is the main hepatic transporter of metformin hence association of polymorphisms associated with this gene have been observed with metformin response in patients with T2DM. It has been reported that patients with AA genotype had a 5.6 times better chance to respond to metformin when compared to those with CC genotype [24]. In a South African population, Masilela et al. [25] demonstrated a significant association of CC genotype with uncontrolled T2DM and suggested the use of this polymorphism as a predictor of metformin/sulfonylureas efficacy among patients of African origin with T2DM. In 2019, Ebid et al. [26] in an Egyptian population showed that AA alleles carriers of rs622342 variants were more responsive in a metformin/sulfonylurea's combination therapy likewise the study of Naja et al. [27], demonstrated a better glycaemic control in Lebanese carriers with the AA/AC genotype undergoing metformin/sulfonylureas combination therapy. This study is however in contrast to the study of Tkac et al., [7] that observed no significant association of the variant rs622342 with reduction in HbAIc level. Furthermore, no statistically significant effect on glycaemic response was observed with the other SNPs (rs 594709, rs72552763, rs9332214). The variant rs 72552763 has been reported to show no effect on glycaemic response in a study of Genetics of Diabetes Audit and Research in Tayside Scotland (GoDarts) database in 1531 patients with T2DM on metformin [5], however, Peng et al. [28], observed in his study that the variant rs 72552763 was significantly associated with glycaemic response in African, South Asian and Latin American populations but showed no association in East Asian and European populations.

This study found an association between variant rs594709 (AG/GG) with reduced levels of HOMA-IR ($p<0.0331$) and TCHOL ($p<0.0054$) which is consistent with the study of Xiao et al.,2016[] which reported that rs594709 polymorphism interaction with rs2289669 increases HOMA-IR in the AA genotype as opposed to aa allele, G allele carriers showed a higher decrease in TCHOL. The variants of rs594709 and rs622342 may influence insulin sensitivity and glucose metabolism contributing to individual difference in insulin resistance. The rs594709 variant reflects the role of ABCA1 to influence the efficiency of cholesterol efflux and HDL formation, affecting overall cholesterol levels in the bloodstream. An association of rs622342 (CA/AA) with reduced levels of HOMA-IR ($p<0.0313$), Lipo A (0.0400) and BMI (0.0416) was also observed, our findings were inconsistent with those obtained by Kunrong et al. [29], which indicated that patients with the minor allele of rs622342 had significantly lower HOMA-IR value than those with the common genotype (AA) implying that the C allele of SLC22A1 rs622342 was associated with a lower risk of insulin resistance in patients with type 2 diabetes their study as well reported no significant association with HOMA-β however. The rs622342 variant can be regarded as a predictor of insulin resistance in patients. The rs9332214 variant (TC/CC) with reduced levels of AIP ($p<0.0245$) and HbA1c ($p<0.0265$). The mechanism by which rs622342 variant influences Lipo A is not clearly understood but it is thought to involve the regulation of lipid metabolism and transport pathways. However, it is important to note that Lipoprotein A and BMI undergoes a complex genetic regulation involving

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multiple genes and environmental factors which may result in variations in cardiovascular risk, body weight and composition.

## 4. CONCLUSION

In conclusion, this study revealed that the individuals with the AA genotype of rs622342 are likely to have a better glycaemic response as compared to those carrying the AC/CC genotype receiving metformin/sulphonylurea combination therapy. The variants of rs594709, rs72552763 and rs9332214 showed no significant association with glycaemic response.

## CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

## ETHICAL APPROVAL

Ethical approval and permission were sought and obtained from the Rivers State Health Research Ethics Committee of Rivers State Health Management Board. Informed consent of the participants involved was obtained and anthropometric data was also obtained via a questionnaire.

**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Details of the AI usage are given below:

1.

2.

3.

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