GENETIC ASSOCIATION OF SOLUTE CARRIER TRANSPORTER GENE VARIANTS WITH METFORMIN RESPONSE

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by elevated blood glucose levels and is influenced by both genetic and environmental factors. It is treated with various classes of oral antidiabetic drugs, however, response to treatment is highly variable with patients failing to achieve adequate glycemic control. Treatment response variability has been associated with single nucleotide polymorphisms (SNPs) which influence the pharmacokinetics and pharmacodynamics of drug(s). The aim of this study was to evaluate the genetic association of 17 SNPs and the response to metformin therapy in patients diagnosed with diabetes from the indigenous Nguni population of South Africa. One hundred and forty indigenous African patients diagnosed with T2DM were recruited and genotyped using the MassARRAY® system. Therapeutic response of patients was ascertained by a change in Hb A1c. Two SNPs (rs1801282 and rs6265) were monomorphic. All other variants were within the Hardy-Weinberg equilibrium (HWE). The T allele of the SLC variant rs316009 [odds ratio (OR) = 0.25, 95% confidence interval (95% CI) = 0.01-0.09, \( p \) value = 0.044] and the CT genotype of the PCK1 variant rs4810083 (OR = 2.80, 95% CI = 1.01-7.79, \( p \) value = 0.049) were associated with an improved response to treatment after adjustment. No association was observed with post Bonferroni correction. Moreover, this study provides important additional data regarding possible associations between genetic variants and metformin therapy outcomes. In addition, this is one of the first studies providing genetic data from the understudied indigenous sub-Saharan African populations.

Keywords: Single nucleotide polymorphisms (SNPs); SLC variants; South African cohort; Treatment response; Type 2 diabetes mellitus (T2DM).

INTRODUCTION

The prevalence of diabetes mellitus (DM) across the world is constantly rising. It is estimated that 642 million cases of DM will be reported by the year 2040 [1]. In the African region alone, it was found that 15.5 million adults were living with DM and, of these, 7.0% originate from South Africa [2]. Diabetes mellitus is defined as a chronic metabolic disease characterized by prolonged hyperglycemia [3].

The prolonged hyperglycemia experienced by diabetic patients can result in macro- and microvascular complications that increases the risk for heart disease, stroke, and damage to the nervous system, retina, kidneys and other organs [4,5]. Therefore, DM treatment aims to maintain a blood glucose level within the physiological range [5]. Therapies implemented include dietary and lifestyle modification and the administration of oral antidiabetic drugs.

The preferred first line treatment in most clinical guidelines for the management of type 2 diabetes mellitus (T2DM), accounting for ~90.0% of all DM cases, is metformin [6,7]. However, 38.0% of T2DM patients respond poorly to metformin [8]. In addition to biguanides, several
other classes of drugs are being prescribed to treat T2DM; these include: sulfonylureas, meglitinides, thiazolidinedi-
one, α-glucosidase inhibitors, dipeptidyl peptidase-4 in-
hibitors, glucagon-like peptide-1 agonist, sodium glucose
cotransporter-2 inhibitors, insulin and its analogues [9-11].

Type 2 diabetes mellitus has been linked to variability in
candidate genes that interfere with the management of
glycemic control [9]. These candidate genes are involved in
drug absorption, transportation, distribution, metabolism and
the signaling cascade of oral anti diabetic drugs [12].

As metformin is the most common drug prescribed for
the treatment and management of T2DM, numerous studies
have been conducted to determine the therapeutic effects of
metformin in the presence of genetic variants. Amongst
the variants investigated, the SLC variants feature quite
often. Tzvetkov et al. [17] observed a variation in the renal
clearance of metformin in Caucasian males with genetic
polymorphisms in SLC22A1, SLC22A2 and SLC22A3. The
renal transport of metformin was associated with a
glucose lowering effect in combination with SLC47A1 and
SLC22A1 genetic variants in a Dutch cohort [18]. Chen et
al. [19] observed a very rare SLC22A1 (R206C) variant in
Asian patients diagnosed with T2DM. Patients with this
rare variant demonstrated an altered response to metformin
treatment. These studies and others like it, demonstrate the
impact that SLC variants and other genetic variants, have
on the efficacy and toxicity of prescribed drugs.

Pharmacogenomic and pharmacokinetic studies have
been conducted on the treatment response to T2DM in
various populations across the world [20-23]. However,
even though numerous studies have been conducted, lim-
ited data is available for sub-Saharan African populations
and other African populations, regardless of the human
genomic diversity found on this continent. Genetic di-
versity presented by indigenous populations across the
world, in this instance South Africa, should be explored for
improved diagnostic techniques and treatment plans for
conditions such as diabetes, cardiovascular disease and
cancer. The indigenous Nguni population of South Africa
was selected for investigation in this study. The Nguni
population is comprised of the Xhosa, Zulu, Ndebele and
Swati clades [24-26].

Loci identified in previously studied populations ob-
served anti diabetic drug efficacy may or may not affect
efficacy in South African populations because of ethnic
genetic differences. Seventeen single nucleotide polymor-
phism (SNP) biomarkers selected for investigation in this
study, have previously been associated with T2DM in vari-
ous populations across the world [17-23,27-28]. The aim
of this study was to investigate the genetic association of
these 17 SNP biomarkers and the response to anti diabetic
treatment to determine their suitability for individualized
metformin therapy in patients diagnosed with T2DM in
the Nguni indigenous population of South Africa.

MATERIALS AND METHODS

Patients and Study Design. All participants were
briefed about the project and a consent form was com-
pleted and submitted by each participant before the ex-
periment was conducted. Ethics clearance for this study
was obtained from the Senate Research Committee of the
University of the Western Cape [Ethics clearance number
BM/16/5/19].

Study Participants. A total of 140 T2DM outpatients
belonging to the indigenous Nguni population of South Africa
[Swati (n = 10), Xhosa (n = 81) and Zulu (n = 49)]
were recruited from the Cecilia Makhewane Hospital (East
London, Eastern Cape) and Piet Retief Hospital (Mkhondo,
Mpumalanga). Type 2 diabetes mellitus, according to the
WHO criteria of 1999: plasma glucose level between 7-13
mmol/L with glycated hemoglobin (Hb) level between
7.0 and 11.0%. As some patients had other comorbidities
(i.e. hypertension and dyslipidaemia) in this study, T2DM
was diagnosed as a plasma glucose level between 6.0-27.0
mmol/L. Each patient participating in the study had Hb A1c
levels measured within 6 month (baseline) and 12 month
(follow-up) periods. Based on Hb A1c levels, patients were
prescribed an average metformin dose of 1.95 mg per day
(with a maximum of 2.55 mg). Patients were categorized as
controlled if they demonstrated a decreased Hb A1c value
less than 8.0% at 12 months in comparison to the baseline
prior to the study. Uncontrolled patients demonstrated an
increased Hb A1c value more than 8.0% at 12 months in comparison to the baseline prior to the study. The classifi-
cation used herein for controlled and uncontrolled T2DM
has been described previously [29,30].

In this pool of study subjects, 53 patients demonstrat-
ed a controlled T2DM (responders to metformin therapy),
with the remaining 87 patients demonstrated an uncon-
trolled T2DM (non-responders to metformin therapy).
Patients were included in the study if they were 18 years
or older and had been on treatment for at least 1 year
prior to the study. All patients were on metformin mono-
therapy. Patients with other diseases such as type 1 diabetes
mellitus (T1DM), malignancies, hyperlipidaemia, chronic
kidney and liver diseases, as well as pregnant patients,
were excluded from the study. Information about age, family
history, medical history, demographic parameters and
medication used was obtained via medical reports and
Interviews. In addition to this, some patients were also on antihypertensive drugs, however, while the present study does not exclude drug-drug interactions, studies have not shown that other drugs co-administered with metformin have any influence on the outcome of a genetic association with metformin response.

**Data Collection and Laboratory Measurements.**
A trained research nurse took clinical measurements of: weight, height and blood pressure (BP). Measurements were taken with all participants wearing minimal clothing and no shoes. Body mass index (BMI) for each patient was calculated as weight (kg) divided by height (m²) (Table 1).

Random venous blood was collected to measure serum glycosylated Hb (Hb A¹c) levels. Furthermore, lipid profile [which includes: total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL)] was obtained (Table 1). All blood samples were sent to relevant clinical laboratory centers for analysis.

**Single Nucleotide Polymorphism(s) Selection and Genotyping.** The 17 relevant pharmacogenomic variants selected for this study were chosen based upon previous publications, where association was made between SNPs and response to treatment with metformin. In addition to this, variants were also cross-referenced and selected based upon an evidence level ranging between 2B-4 dictated by the pharmacogenomics knowledge base, accessed on February 5 February 2019; PharmGKB (http://www.pharmgkb.org) [31].

Genomic DNA was isolated from buccal swabs using a standard salt lysis method [32]. Samples were stored at −20 °C. DNA was quantified using a NanoDrop™2000/2000c UV/VIS Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The SNPs were genotyped using the MassARRAY®System IPLEX extension reaction (Agena Bioscience, San Diego, CA, USA). Genotypes of the selected SNP variants were determined for all the study participants (Table 2).

**Statistical Analyses.** Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 25 software (www.ibm.com/spss.statistics). Clinical laboratory data and anthropometric measurements were expressed as mean ± SD. Hardy-Weinberg equilibrium (HWE) p values were calculated for all SNPs using MedCalc version 2.2.0.0. (MedCalc Software, Ostend, Belgium), where p value(s) of <0.05 were considered to be significant and implied that the population was not in HWE. Association between variant(s) and response to diabetic treatment was measured using odds ratios (ORs), 95% confidence interval (95% CI) and p value(s) derived from logistic regression. The threshold for significance in association studies was set at $p = 0.05$.

**RESULTS**

Table 1 displays the clinical and biochemical demographics of the study population. All SNPs are within HWE with two SNPs (rs1801282 and rs6265) being mono-
Table 2. Single nucleotide polymorphism information and Hardy-Weinberg p values in the study population.

| SNP       | Gene/Closet Gene     | Chromosomal Position | Location       | Allele Change | Amino Acid Change | HWE p Value |
|-----------|----------------------|----------------------|----------------|---------------|------------------|-------------|
| rs10783050| EEF1A1P11-RPL7P9     | 1:96571527            | intergenic     | T>C           | –                | 0.932       |
| rs1143623 | IL1B                 | 2:112838252           | intergenic     | C>G           | –                | 0.309       |
| rs1326634 | SLC30A8              | 8:117172544           | missense       | C>A/T         | Arg325Trp        | 0.532       |
| rs1337631 | FMIO1                | 1:17166603            | intron         | A>G           | –                | 0.903       |
| rs1801282 | PPARG                | 3:12351626            | missense       | C>G           | Pro12Ala         | monomorphic |
| rs249429  | PRKAA1               | 5:40782137            | intron         | C>T/G         | –                | 0.299       |
| rs2815752 | NEGR1                | 1:72346757            | intergenic     | G>A           | –                | 0.636       |
| rs316009  | SLC22A2              | 6:160254732           | missense       | C>T/G         | –                | 0.595       |
| rs316019  | SLC22A2              | 6:160249250           | missense       | C>A           | Ala270Ser        | 0.808       |
| rs391300  | SRR                  | 17:2312964            | intron         | C>T/G         | –                | 0.739       |
| rs461473  | SLC22A1              | 6:16012530            | intergenic     | G>A           | –                | 0.898       |
| rs4810083 | PCK1                 | 20:57545215           | intergenic     | C>T/G         | –                | 0.145       |
| rs578427  | –                    | 6:91702432            | intergenic     | T>C           | –                | 0.909       |
| rs622342  | SLC22A1              | 6:160151834           | intron         | A>C           | –                | 0.218       |
| rs6265    | BDNF; BDNF-AS        | 11:27658369           | missense       | C>T           | Val66Met         | monomorphic |
| rs819     | SLC2A2               | 3:171007094           | intron         | C>T           | –                | 0.674       |

Table 3. Genotype and allele frequencies of 13 single nucleotide polymorphism(s) demonstrating no significant association to type 2 diabetes mellitus treatment response.

| SNP       | Genotype/Allele | Control n (%) | Uncontrolled n (%) | OR (95% CI) | p Values |
|-----------|-----------------|---------------|--------------------|-------------|----------|
| rs10783050| TT/TC/TC        | 52 (98.1)     | 1 (1.9)            | reference   | 0.331    |
|           | T/T/105 (99.1)  |              |                    | 0 (0.0)     | reference |
|           | C/1 (0.9)       |              |                    | 0 (0.0)     | 0.332    |
| rs1143623 | CC/CG/C        | 48 (90.6)     | 5 (9.4)            | reference   | 0.553    |
|           | C/C/96 (90.6)   |              |                    | 11 (12.6)   | reference |
|           | G/10 (9.4)      |              |                    | 11 (9.1)    | 0.369    |
| rs1326634 | CC/CT/C        | 50 (94.3)     | 3 (5.7)            | reference   | 0.444    |
|           | C/100 (94.3)    |              |                    | 8 (9.2)     | reference |
|           | T/6 (5.7)       |              |                    | 164 (94.3)  | 0.709    |
| rs1337631 | AA/GG/AG       | 16 (30.2)     | 11 (20.8)          | reference   | 0.818    |
|           | G/25 (13.3)     |              |                    | 37 (42.5)   | 0.860    |
|           | AG/47 (44.3)    |              |                    | 81 (46.6)   | 0.811    |
| rs1801282 | CC/C/100 (100)  | 53 (100.0)    | 106 (100.0)        | reference   | 0.567    |
|           | C/106 (100.0)   |              |                    | 86 (98.9)   | monomorphic|
| rs249429  | TT/CC/CT       | 28 (52.8)     | 3 (5.7)            | reference   | 0.650    |
|           | CT/22 (41.5)    |              |                    | 40 (46.0)   | 0.510    |
|           | T/78 (73.6)     |              |                    | 120 (69.0)  | 0.500    |
| rs2815752 | GG/AA/G        | 18 (34.0)     | 13 (24.5)          | reference   | 0.630    |
|           | GA/22 (41.5)    |              |                    | 43 (49.4)   | 0.254    |
|           | G/87 (50.0)     |              |                    | 87 (50.0)   |           |
|           | A/83 (47.7)     |              |                    | 83 (47.7)   | 0.567    |

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Table 4. Genotype and allele frequencies of four single nucleotide polymorphism(s) demonstrating no significant association to type 2 diabetes mellitus treatment response.

| SNP         | Genotype/Allele | Controlled n (%) | Uncontrolled n (%) | OR (95% CI) | p Values | Unadjusted | OR (95% CI) | p Values | Adjusted | OR (95% CI) | p Values | Bonferroni Corrected p Values |
|-------------|-----------------|------------------|--------------------|-------------|----------|------------|-------------|----------|----------|-------------|----------|-------------------------------|
| rs34834489  | GG              | 46 (86.7)        | 80 (92.0)          | reference   | 0.361    |             |             |          |          |             |          |                                |
|             | GA              | 6 (11.3)         | 6 (6.9)            | 0.58 (0.18-1.89) |          |          |             |          |          |             |          |                                |
|             | G               | 98 (92.5)        | 166 (95.4)         | 0.59 (0.19-1.88) | 0.373    |          |             |          |          |             |          |                                |
|             | A               | 6 (5.7)          | 6 (3.4)            | reference   |          |          |             |          |          |             |          |                                |
| rs391300    | CC              | 9 (17.0)         | 17 (19.5)          | reference   |          |          |             |          |          |             |          |                                |
|             | TT              | 18 (34.0)        | 24 (27.6)          | 0.71 (0.26-1.94) | 0.500    |          |             |          |          |             |          |                                |
|             | CT              | 26 (49.1)        | 44 (50.6)          | 0.90 (0.35-0.260 | 0.819    |          |             |          |          |             |          |                                |
|             | C               | 44 (41.5)        | 78 (44.8)          | reference   |          |          |             |          |          |             |          |                                |
|             | T               | 62 (58.5)        | 92 (52.9)          | 0.84 (0.51-1.37) | 0.477    |          |             |          |          |             |          |                                |
| rs461473    | GG              | 52 (98.1)        | 84 (96.6)          | reference   | 0.863    |          |             |          |          |             |          |                                |
|             | GA              | 1 (1.9)          | 2 (2.3)            | reference   | 0.864    |          |             |          |          |             |          |                                |
|             | G               | 105 (99.1)       | 170 (97.7)         | 1.24 (0.11-1.99) |          |          |             |          |          |             |          |                                |
|             | A               | 1 (0.9)          | 2 (1.1)            | reference   |          |          |             |          |          |             |          |                                |
| rs622342    | AA              | 33 (62.3)        | 48 (55.2)          | reference   | 0.075    |          |             |          |          |             |          |                                |
|             | CC              | 3 (5.7)          | 12 (13.8)          | reference   | 0.0017   |          |             |          |          |             |          |                                |
|             | CA              | 18 (34.0)        | 26 (29.9)          | 0.99 (0.47-2.10) | 0.985    |          |             |          |          |             |          |                                |
|             | A               | 84 (79.2)        | 122 (70.1)         | reference   |          |          |             |          |          |             |          |                                |
|             | C               | 22 (20.8)        | 50 (28.7)          | 1.56 (0.88-2.78) | 0.113    |          |             |          |          |             |          |                                |
| rs6265      | CC              | 53 (100.0)       | 86 (98.9)          | monomorphic |          |          |             |          |          |             |          |                                |
|             | C               | 106 (100.0)      | 172 (98.9)         | monomorphic |          |          |             |          |          |             |          |                                |
| rs8192675   | CC              | 42 (79.2)        | 58 (66.7)          | reference   | 0.508    |          |             |          |          |             |          |                                |
|             | TT              | 1 (1.9)          | 3 (3.4)            | 2.17 (0.22-21.62) |          |          |             |          |          |             |          |                                |
|             | CT              | 9 (17.0)         | 25 (28.7)          | 2.01 (0.85-4.75) | 0.111    |          |             |          |          |             |          |                                |
|             | C               | 93 (87.7)        | 141 (81.0)         | reference   |          |          |             |          |          |             |          |                                |
|             | T               | 11 (10.4)        | 31 (17.8)          | 1.86 (0.89-3.88) | 0.099    |          |             |          |          |             |          |                                |

OR: odds ratio; 95% CI: 95% confidence interval.
Percent does not account for missing allele(s) at specific loci.

OR: odds ratio; 95% CI: 95% confidence interval.
Percent does not account for missing allele(s) at specific loci. Significant p values (<0.05) are bold.
morphic (Table 2). Hardy-Weinberg equilibrium \( p \) values ranged between 0.145-0.932 for all of the studied SNPs in the population. Genotype and allele distribution of the 17 SNPs were determined in all the study participants (Table 3). Among the SNPs selected for this study, four displayed significant association between T2DM and/or genotype or allele frequencies prior to adjustment (Table 4). The four significantly associated SNPs identified are: rs316009, rs316019, rs4810083 and rs578427. Prior to adjustment, the heterozygous genotype, i.e. CT, and the minor allele \( T \) of rs316009 demonstrated significant association between T2DM and treatment response \([p = 0.023; \text{OR}: 2.80; 95\% \text{Cl}: 1.01-7.79]\) could still be associated with an increased treatment response to metformin (Table 4). The minor A allele of rs316019 with a \( p \) value of 0.026 (OR: 0.35; 95\% CI: 0.14-0.88) also showed a significant association (Table 4). The heterozygous genotype, i.e. CT of rs4810083 with a \( p \) value of 0.021 (OR: 0.38; 95\% CI: 0.17-0.86) and the homozygous minor genotype CC of rs578427 with a \( p \) value of 0.022 (OR: 4.67; 95\% CI: 1.25-1.83) also demonstrated a significant association between T2DM and response (Table 4). However, after adjustment, only the \( T \) allele of rs316009 with a \( p \) value of 0.044 (OR: 0.85; 95\% CI: 0.01-0.93) and the CT genotype of rs4810083 with a \( p \) value of 0.049 (OR: 2.80; 95\% CI: 1.01-7.79) could still be associated with a response to treatment. Lastly, after Bonferroni correction, both rs316009 with a \( p \) = 0.088 and rs4810083 with a \( p \) = 0.098, showed a lack of association.

**DISCUSSION**

In this study the genetic association of 17 pharmacogenomic biomarkers and response to metformin treatment in the indigenous Nguni population of South Africa was determined. Previously, the MATE2K variant, rs12943590 and the variant rs12752688, had been suggested for inclusion in pharmacogenomic profiling of the Nguni population [24]. This study will provide additional pharmacogenomic biomarker information about possible associations between genetic variants and response to metformin therapy in the Nguni population.

All SNPs, besides rs1801282 and rs6265 (which were shown to be monomorphic), were within HWE and showed \( p \) values ranged between 0.145-0.932 in the study population (Table 2). The two monomorphic SNPs (i.e. rs1801282 and rs6265) are rare variants, however, they were included in the study because of the important roles they play in the development and progression of the diabetes disease. The PPARG variant, rs1801282, is important in the development of obesity as well as adipose and muscle tissue metabolism [33]. This variant has recently been investigated in the development of early visual impairment in T2DM Chinese Han population [33] and been associated with obstructive sleep apnea in Chinese Han and Indian subjects diagnosed with T2DM [34,35]. Obesity is a known comorbid disease of diabetes and sleep apnea has also been associated with diabetes, therefore, this variant was included for investigation.

The BDNF gene theoretically plays a significant role on the well-being and health of individuals, as it has diverse roles throughout the body and brain [36]. The BDNF variant, rs6265, has been linked to obesity and T2DM in Chinese populations [36,37] and BMI in Korean [38] and British populations [39]. Because this variant could affect T2DM, comorbid diseases related to diabetes and other physical indicators of the progression of diabetes, it was selected for the study, regardless of its rarity in African populations.

Genotype and allele distribution of the 17 SNPs were determined in all the study participants (Tables 3 and 4). Among the SNPs analyzed, 13 of the SNPs selected for this study showed no statistically significant association between treatment response and the SNP variant (Table 3). The remaining four variants however, i.e. rs316009 (genotype \( p \) value 0.023; allele \( p \) value 0.027), rs316019 (genotype \( p \) value 0.026), rs4810083 (genotype \( p \) value 0.021) and rs578427 (genotype \( p \) value 0.022), showed a significant association between variant and treatment response prior to adjustment (Table 4). This study showed an increased treatment response to metformin for T2DM patients with SNP variants rs316009, rs316019 and rs4810083. In contrast, rs578427 demonstrated a decrease in response to treatment. However, post adjustment, only the \( T \) allele of rs316009 (\( p \) value 0.044) and the CT genotype of rs4810083 (\( p \) value 0.049) were associated with treatment response. Post Bonferroni correction rs316009 (\( p \) value 0.088) and rs4810083 (\( p \) value 0.098), demonstrated a lack of association. However, this can be attributed to the small sample size of the study cohort.

The rs316009 variant is located in a transcription factor binding motif and is in linkage disequilibrium with the non synonymous variant rs316019 [21,40-47]. In previous studies, the TT genotype of rs316009 showed an increase response to metformin in comparison to the CC and CT genotypes [41]. Unfortunately, the homozygous TT genotype was not observed in this study population. From the data available, the CT genotype demonstrated a better response to treatment in comparison to the CC genotype (Table 4). The rs316019 is the most common variant of SLC22A2 in many populations and has displayed contradictory results, linked to both decreased and increased renal clearance of metformin in healthy subjects [5,40,42-45].

The interaction of metformin and other drugs in the presence of rs316019 was determined in silico by Sajib et al. [43]. Based upon the in silico data generated by Sajib et al.
an improved response to treatment. These results are in lactic acidosis, the TT genotype can thus be associated with hyperlactacemia within clinical doses of metformin [43]. Thus, dose adjustments based on the rs316019 variant may be beneficial to maximize treatment response.

Prior to correction, the A allele was significantly associated with an improved response to treatment. This is in contradiction to studies conducted by Song et al. [44] and Wang et al. [42], as well as the in silico data generated by Sajib et al. [43]. This data is however in agreement with studies conducted by Chen et al. [40]. Other studies also indicated no association between this variant and response to metformin treatment [17, 21].

The SNP variant rs4810083 T allele is not associated with a response to metformin treatment in T2DM patients [46]. The results obtained in this study, however, may suggest that the T allele is most likely to be associated with a decrease in response to diabetic treatment as more patients in the uncontrolled category carry the T allele in comparison to the controlled category. This study group also shows the CT genotype to be associated with an improved response to treatment (Table 4). To enable further clarity with regard to the significance of this SNP variant, more data is required from other population groups as well as a bigger sample cohort for the current study group.

In the case of the SNP rs578427, the TT genotype has been associated with an increased renal clearance and secretion clearance of metformin in comparison to the CC genotype in a healthy population [47]. As the accumulation of metformin in the body can result in the development of hyperlactacidemia, the TT genotype can thus be associated with an improved response to treatment. These results are in concordance with the data generated for this study population as the CC genotype was shown to be significantly associated with a decreased response to treatment (Table 4).

Contradictory, as well as inconclusive, results may have arisen for a number of reasons. The most relevant being sample size as well as SNP selection and the approach used to analyze individual SNPs. Because SNPs do not occur in isolation of each other, but rather as combinations forming defined haplotypes, the phenotypic effect of individual SNPs is not always consistent with functional effects. Thus, genotyping single or a few individual SNPs may fail to reflect the true functionality of genetic variants [48]. Therefore, it should be recommended that future studies evaluate haplotypes to establish the functional effects that a collection of SNPs may have on response to treatment.

**Conclusions.** In this study, two SNP variants (rs316009 and rs4810083) were significantly associated with improved response to diabetic treatment prior to Bonferroni correction. The greatest limitation of this study was the sample size and this has inadvertently affected the relevance of significantly associated SNP variants. Regardless of this, this study provides additional important data regarding possible associations between genetic variants and metformin therapy outcomes. In, addition, this study is one of the first studies providing genetic data from the understudied indigenous sub-Saharan African populations.

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