



# Distribution of the ACE Gene Polymorphisms in Type 2 Diabetes Mellitus Patients, Their Associations with Nephropathy Biomarkers and Metabolic Indicators at a Tertiary Hospital in Uganda

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**Purpose:** We aimed at determining the distribution of the ACE insertion/deletion gene polymorphisms among type 2 diabetic patients and their association with the nephropathy biomarkers and the metabolic indicators.

**Patients and Methods:** Data were collected from 237 adult type 2 diabetes mellitus patients receiving healthcare at the diabetic clinic of Mbarara Regional Referral Hospital. Peripheral blood genomic DNA was amplified using a conventional PCR technique and analyzed for the ACE homozygous forms of the insertion (II), deletion (DD) and heterozygous insertion deletion (ID) genotypes as well as their respective allele counts. Biomarkers of nephropathy were analyzed on a Beckman coulter AU480 chemistry analyzer using system compatible reagents.

**Results:** Majority of the participants were older persons (Median = 57, IQR = 49–64) and female 171 (72.2%). Most of them had the Deletion allele 198 (83.5%) and DD genotype 116 (48.9%). At multivariate logistic regression, the nephropathy biomarkers that is microalbuminuria, serum creatinine, urea, eGFR and electrolytes had no association with the ACE I/D alleles or genotypes ( $p > 0.05$ ). On the other hand, selected metabolic indicators had a positive relationship. The insertion allele was associated with increasing glycated hemoglobin (OR = 1.082,  $p = 0.019$ ) and decreasing serum glucose levels (OR = 0.891,  $p = 0.001$ ). Deletion allele was associated with decreasing glycated hemoglobin (OR = 0.924,  $p = 0.047$ ) and increasing serum glucose levels (OR = 1.208,  $p = 0.001$ ). ACE II genotype was associated with decreasing serum glucose levels (OR = 0.873,  $p = 0.029$ ). ACE DD genotype was associated with decreasing glycated hemoglobin (OR = 0.917,  $p = 0.010$ ) and increasing serum glucose levels (OR = 1.132,  $p = 0.001$ ). ACE ID genotype was associated with increasing glycated hemoglobin (OR = 1.077,  $p = 0.022$ ), triglyceride levels (OR = 1.316,  $p = 0.031$ ) and decreasing serum glucose levels (OR = 0.933,  $p = 0.038$ ).

**Conclusion:** The presence or absence of the ACE I/D alleles and genotypes affects the ultimate increase or decrease in the serum glucose, glycated hemoglobin and triglyceride levels. Although there was no significant association between the biomarkers of nephropathy and the ACE I/D alleles or genotypes, the above implicated metabolic indicators should be included in healthcare guidelines used when attending to type 2 diabetic patients.

**Keywords:** angiotensin converting enzyme gene, insertion, deletion, polymorphism, biomarkers, type 2 diabetes mellitus, diabetic nephropathy, Uganda

## Introduction

Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes mellitus.<sup>1,2</sup> It is a multifactorial and polygenic disorder that occurs in a quarter to a third of all patients with prolonged diabetes mellitus and has been widely implicated as the leading cause of end-stage renal disease (ESRD).<sup>3</sup> Typically, the disease is diagnosed after

significant damage has occurred to the kidney tissue.<sup>4</sup> While DN is known to cause glomerular and tubular basement membrane thickening, and ultimately nephron loss, diagnosis using kidney biopsies are not a common routine practice in resource-limited settings. This creates a pressing need to look for possible early-disease biological markers.

A cross-sectional study conducted in adult patients at the diabetic clinic of Mbarara Regional Referral Hospital (MRRH) in southwestern Uganda established a prevalence of diabetic nephropathy at 22.9% among 140 patients who were sampled.<sup>5</sup> Also, evidence from studies has shown that there is clustering of diabetic nephropathy in families, and it is hypothesized that this could be the reason for the observed large variation in its prevalence among diverse populations.<sup>3</sup> In the progression of diabetes mellitus and its complications, one's genetic background is considered to contribute to an inherent susceptibility to diabetic nephropathy.<sup>6</sup>

Angiotensin converting enzyme (ACE), a key enzyme in the renin-angiotensin-aldosterone system (RAAS),<sup>7,8</sup> catalyzes the conversion of angiotensin I to angiotensin II. Angiotensin II has been implicated in inducing progressive renal injury by increasing cellular growth, proliferation and matrix synthesis, leading to glomerular sclerosis as well as precipitating changes in the renal hemodynamic such as an increase in intra-glomerular pressure.<sup>9,10</sup> The ACE gene insertion/deletion polymorphisms are single nucleotide polymorphisms (SNPs), which have been closely associated with the development of nephropathy among type 2 diabetes mellitus patients. The ACE gene is made up of 21 kilobases on the long arm of human chromosome 17q23 forming three possible genotypes that is to say II, ID or DD.<sup>11</sup> These ACE genetic mutations have been considered to be putative mediators of renal disease,<sup>9</sup> although some studies are quite controversial.<sup>6,11</sup>

Some studies have demonstrated an association between ACE (angiotensin-converting enzyme) insertion/deletion (I/D) polymorphisms and various metabolic indicators in type 2 diabetes mellitus (T2D) patients, while others found otherwise. A study found an association between these polymorphisms with coronary heart disease and dyslipidemia in T2D patients.<sup>12</sup> Another found blood pressure with new onset T2D and essential hypertension to have no association with the ACE I/D polymorphisms.<sup>13</sup> A recent meta-analysis found that ACE I/D polymorphism was associated with insulin-resistant polycystic ovarian syndrome, especially among Asians.<sup>14</sup> It is important to note that the exact relationship between ACE I/D polymorphisms and metabolic indicators could vary depending on several other variables such as population demographics, study design, and other genetic or environmental factors. In the current study, we elucidate the relationship between the ACE I/D polymorphisms and metabolic indicators among T2DM patients.

Studies have elucidated that the II genotype protects against the development of diabetic nephropathy, while the DD genotype predicts poor renal disease outcomes.<sup>12</sup> In two separate meta-analyses, Ng et al reported that there was a noticeable protective role of the II genotype against diabetic nephropathy, especially among T2DM patients of Asian (Chinese, Japanese, and Korean) descent compared to Caucasians.<sup>13,14</sup> The authors reported that the presence of the ACE II genotype among Asians with T2DM was also associated with a lower risk of macroalbuminuria but was slightly associated with the risk of microalbuminuria. There is limited research that has validated these findings.

In this study, we determined the association between metabolic indicators, biomarkers of nephropathy and the ACE insertion/deletion gene polymorphisms among type 2 diabetic patients at Mbarara Regional Referral Hospital in southwestern Uganda.

## Materials and Methods

### Study Setting

The study was conducted at Mbarara Regional Referral Hospital (MRRH) located in Mbarara City, southwestern Uganda. The facility receives patients from neighboring districts and across the country's borders like Tanzania, Rwanda and Democratic Republic of Congo. The hospital is a government entity where all services are free, although a private wing also exists. It is also the teaching hospital of Mbarara University of Science and Technology Medical School. Mbarara Regional Referral Hospital has a bed capacity of 350. It provides in-patient, outpatient, and specialized services, which include a diabetic clinic, a nephrology unit, medical wards, an eye care center, and other specialized clinics. The hospital receives about 500–600 diabetic patients annually of which 10% are new cases.

## Study Design and Participants' Information

We enrolled 237 already-diagnosed type 2 diabetes mellitus adult patients receiving healthcare at the diabetic clinic of MRRH as participants in this cross-sectional study. We excluded patients who had a kidney disease diagnosis prior to the actual type 2 diabetes mellitus diagnosis and pregnant women with gestational diabetes. We consecutively enrolled participants who met the inclusion criteria until the target sample size was achieved as shown in [Figure 1](#).

## Data Collection

Following clinical examination at the diabetic clinic, 4 mills of blood were collected from each participant into a vacutainer without anticoagulant for the biochemical analyses, and the same amount was collected into an ethylenediamine tetraacetic acid (EDTA) tube for the genetic analyses. One spot urine samples were also obtained for subsequent measurement of microalbuminuria.

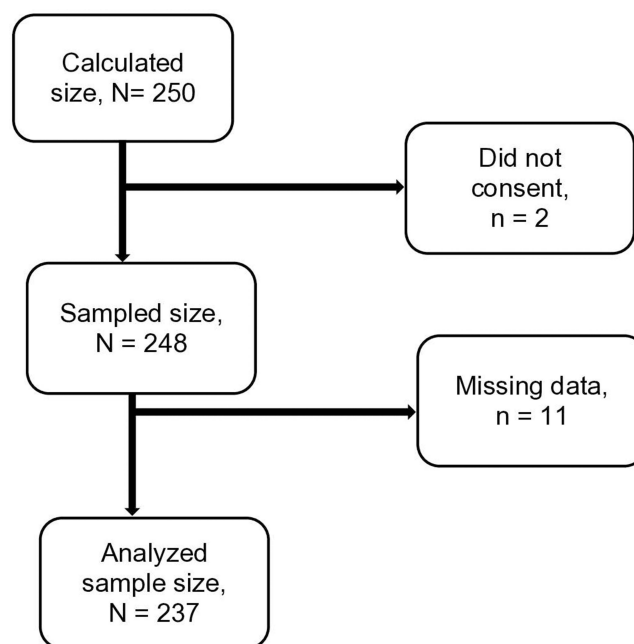
## Urine and Blood Sample Collection for Laboratory Analysis

Urine samples were collected during unrestricted daily life activities to measuring microalbumin. Samples of venous blood were collected and serum obtained following centrifugation at 12,000 revolutions per minute for 5 minutes. Sera was used to measure urea, creatinine, glycated hemoglobin, glucose, sodium, potassium, chloride, triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol using standard methods. The eGFR was estimated using the updated CKD-EPI formula.<sup>15</sup> Both the blood in the plain tube and urine specimen were analyzed on the AU480 Beckman Coulter chemistry analyzer using commercial reagent kits as per the manufacturer's guidelines.

## Identification of ACE Alleles and Genotypes

### Red Blood Cell Lysis

Blood samples collected in ethylenediamine tetraacetic acid (EDTA) tubes were used to harvest the red blood cells. The red blood cells were lysed by lysis buffer (2M Tris pH 7.6, 1M MgCl<sub>2</sub>, 3M NaCl) by adding lysis buffer to 1 mL of blood, incubated for 5 minutes, centrifuged, and the supernatant poured off. The above process was repeated until the red blood cells were completely lysed by forming a colourless solution.



**Figure 1** Flow diagram for study participants selection.

## DNA Extraction

Exactly 500  $\mu\text{L}$  of cell lysis buffer (0.1M Tris pH 8.0, 50 mM EDTA, 1% SDS) was mixed with the lysed red blood cells and vortexed for 1 minute. To the mixture, 275  $\mu\text{L}$  of 7M ammonium acetate pH 7.0 was added. The mixture was incubated for 5 minutes at 65°C (dry bath) and 5 minutes on ice. To this, 500  $\mu\text{L}$  of chloroform was added, vortexed for 1 minute, and spun at 15,000 revolutions per minute for 5 minutes. The supernatant was transferred to a new tube and one mL of isopropanol was added. The mixture was incubated for 5 minutes at room temperature (with mixing by inverting the tube several times) before being spun at 15,000 revolutions per minute for 7 minutes. Isopropanol was removed, and the pellet was suspended in 500  $\mu\text{L}$  of 70% ethanol. Spinning at 15,000 revolutions per minute for 3 minutes, ethanol was removed by decanting. The remaining ethanol was removed with a pipette tip after a hard spin in a microcentrifuge. The DNA pellet was dried at room temperature in open microcentrifuge tubes. The dry DNA pellet was suspended in 50  $\mu\text{L}$  of nuclease free water. The extracted DNA was stored at  $-20^{\circ}\text{C}$ .

## DNA Quantification

DNA purity and quantification were measured using a NanoDrop lite spectrophotometer (Thermo Scientific-168 Third Avenue Waltham, MA USA 02451).

## Detection of ACE I/D Encoding Gene

The presence of the ACE gene was established by PCR amplification using the following primer sets: forward primer 5'CTGGAGAGCCACTCCCATCCTTTCT3'; reverse primer 5'GACGTGGCCATCACATTCGTCAGAT3'; and insertion-specific primer 5'-TTTGAGACGGAGTCTCGCTC-3'.<sup>11</sup> The primers used in this study were manufactured by Eurofins Genomics. The expected product size lengths were 490 bp and 190 bp for insertion and deletion alleles, respectively. In case of insertion deletion presence from the gel, another run was set which included the previous primers and insertion-specific primers added together. The PCR master mix was prepared as follows: 2.5 $\mu\text{L}$  10x buffer, 0.5  $\mu\text{L}$  dNTPs, 0.5 Taq polymerase – New England Bio-labs, 0.5 $\mu\text{L}$  forward (10 $\mu\text{M}$ ), 0.5 $\mu\text{L}$  reverse (10 $\mu\text{M}$ ), 3.0 $\mu\text{L}$  DNA template and 17.5 $\mu\text{L}$  RNAase-Free- $\text{H}_2\text{O}$  making up to 25.0 $\mu\text{L}$  final reaction volume.

## PCR Conditions

The PCR DNA amplification was carried out in a conventional PCR Thermocycler (CLASSIC K960 Thermal Cycler) following an initial denaturation step at 95°C for 5 minutes. This was followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 58°C for 1 and a half minutes and elongation at 72°C for 2 minutes, and the final extension cycle at 72°C for 2 minutes.

## Gel Electrophoresis

DNA amplicons were electrophoresed using 1.5% agarose gel in 1X Tris-Borate EDTA buffer (TBE), 5 $\mu\text{L}$  Safe View Classic<sup>TM</sup> DNA stain (cat # G108), 6X loading dye (Thermo Scientific #R0611), and DNA ladder/marker 1kb (NEB-Biolabs #N3231L). Electrophoresis was run at 200V and 80mA for 1 hour. Bands were visualized using the Gene-Flash Trans-illuminator called Dark Reader<sup>®</sup> trans-illuminator manufactured by Clare Chemical Research.

## Statistical Analyses

Raw data were transferred into STATA version 17 for further analysis. Continuous variables were expressed as medians with interquartile ranges (IQR) after checking for normality. Frequencies and percentages were used for categorical variables. Associations of binary variables were done using chi-square ( $\chi^2$ ) for categorical variables. A non-parametric Mann-Witney test (Wilcoxon-rank-sum test) was used to compare the median levels of the continuous variables (metabolic biomarkers of nephropathy) in the two groups (present or absent) of the outcome genotypic variables (ACE I/D alleles or genotypes). This is because all the continuous variables were not normally distributed according to the Shapiro-Wilk normality test. The relationship between ACE I/D alleles or genotypes (binary variables; dependent variables) and other variables (metabolic biomarkers of nephropathy; independent variables) was further explored using binary and multivariate logistic regression analysis to identify those independent variables that could be significantly associated with the binary genotypic variables. A p-value less than 0.05 after multivariate analysis was taken to be

significant. All the variables in the bivariate analysis, whether statistically significant or not at  $p$ -value  $< 0.05$ , were included in the multivariate analysis because of their biological association with the dependent variable.

## Results

The study comprised mostly older people and females dominated. Majority of the participants resided within the same geographical location as the study site which is within greater Mbarara as shown in Table 1.

Each of the independent variables (age and gender) was compared with each of the dependent binary variable (ACE I/D alleles or genotypes). There were no statistically significant associations between the distribution of the allelic genotypes and the two groups of the binary variables indicated in Table 2.

In Table 3, the presence of the ACE insertion allele was significantly associated with increasing glycated hemoglobin and decreasing serum glucose levels, whereas the presence of the ACE D allele was associated with increasing serum glucose and chloride levels and decreasing glycated hemoglobin.

As shown in Table 4, ACE II genotype was associated with decreasing serum glucose and chloride levels, whereas the ACE ID genotype was associated with increasing glycated hemoglobin, serum triglyceride levels and decreasing serum glucose levels. ACE DD genotype was associated with decreasing glycated hemoglobin and increasing serum glucose levels.

## Discussion

In this study, the majority of the participants were older persons and female. This finding is not surprising, as the occurrence of type 2 diabetes mellitus is mostly in older people. Although the diabetic clinic at Mbarara Regional

**Table 1** Participants' Sociodemographic Characteristics

Variable	Level	n (%)
Age (median, IQR)		57 (49–64)
Age groups	Younger People	42 (17.7)
	Older People	195 (82.3)
Sex	Male	66 (27.8)
	Female	171 (72.2)
Region of residence	Within greater Mbarara	166 (70.0)
	Outside greater Mbarara	71 (30.0)
Marital Status	Single	46 (19.4)
	Married / Cohabiting	144 (60.8)
	Separated	47 (19.8)
Formal Employment status	Unemployed	77 (32.5)
	Employed	160 (67.5)
Highest level of education	None	44 (18.6)
	Primary	121 (51.1)
	Secondary	48 (20.3)
	Tertiary	24 (10.1)

**Notes:** Data are presented as median (IQR) for continuous measures; n (%) for categorical measures. Younger people, 18–44 years, and older people, 45–90 years.  
**Abbreviations:** IQR, interquartile range, n, number of participants in each sub category, %, percentage.

**Table 2** Distribution of ACE Insertion or Deletion Alleles and Genotypes Categorized by Age and Gender

Variable	Level	Age (N=237)					Gender (N=237)				
		Younger People		Older People		P value	Males		Females		P value
		Present, n (%)	Absent, n (%)	Present, n (%)	Absent, n (%)		Present, n (%)	Absent, n (%)	Present, n (%)	Absent, n (%)	
ACE alleles											
	I	21 (17.21)	21 (18.26)	101 (82.79)	94 (81.74)	0.833	34 (27.87)	32 (27.83)	88 (72.13)	83 (72.17)	0.994
	D	33 (16.67)	9 (23.08)	165 (83.33)	30 (76.92)	0.338	51 (25.76)	15 (38.46)	147 (74.24)	24 (61.54)	0.106
ACE genotypes											
	II	5 (22.73)	37 (17.21)	17 (77.27)	178 (82.79)	0.519	10 (45.45)	56 (26.05)	12 (54.55)	159 (73.95)	0.053
	ID	16 (16.00)	26 (18.98)	84 (84.00)	111 (81.02)	0.553	24 (24.00)	42 (30.66)	76 (76.00)	95 (69.34)	0.259
	DD	21 (18.10)	21 (17.36)	95 (81.90)	100 (82.64)	0.880	32 (27.59)	34 (28.10)	84 (72.41)	87 (71.90)	0.930

**Notes:** P value  $\leq 0.05$  is statistically significant. Younger people, 18–44 years, and older people, 45–90 years.

**Abbreviations:** ACE, Angiotensin Converting Enzyme; I, insertion; D, Deletion; n, number of participants in each sub category; N, number of participants in the study; %, percentage.

**Table 3** Multivariate Logistic Regression Analysis of ACE I and D Alleles with Metabolic Biomarkers of Diabetic Nephropathy

Variable	ACE I			ACE D		
	Odds Ratio	95% CI	P value	Odds Ratio	95% CI	P value
Microalbuminuria	1.093	0.480–2.483	0.833	1.504	0.541–4.184	0.434
Glycated hemoglobin	1.082	1.013–1.155	0.019*	0.924	0.855–0.999	0.047*
Serum Glucose	0.891	0.832–0.954	0.001*	1.208	1.087–1.342	0.001*
Urea	0.964	0.722–1.287	0.804	0.791	0.531–1.179	0.249
Creatinine	0.997	0.987–1.007	0.597	1.007	0.994–1.012	0.304
eGFR	0.996	0.979–1.013	0.622	0.991	0.969–1.012	0.391
Triglycerides	1.204	0.936–1.550	0.149	1.131	0.790–1.620	0.500
Total Cholesterol	1.140	0.856–1.556	0.408	1.068	0.710–1.608	0.752
HDL-Cholesterol	0.896	0.253–3.173	0.865	1.583	0.241–10.409	0.632
LDL-Cholesterol	0.635	0.364–1.106	0.109	1.288	0.562–2.951	0.549
Potassium	1.181	0.794–1.755	0.411	0.884	0.599–1.305	0.536
Chloride	0.953	0.868–1.047	0.318	1.146	1.018–1.291	0.024*
Sodium	1.009	0.951–1.070	0.756	0.953	0.882–1.029	0.221

**Notes:** P value  $\leq 0.05$  is statistically significant. \* denotes variables with significant P values.

**Abbreviations:** ACE, Angiotensin Converting Enzyme; I, insertion; D, Deletion; eGFR, estimated glomerular filtration rate; CI, confidence interval; HDL, high density lipoprotein; LDL, low density lipoprotein; %, percentage.

Referral Hospital receives more female than male patients in routine care probably due to the differences in health-seeking behavior, previous studies have reported an overrepresentation of females in research studies.<sup>16,17</sup>

Most of our study participants had the DD genotype (116/237), and a few of them had the II genotype (22/237). Majority had at least a D allele (198/237), and there was an equal distribution of the I allele. These findings are in

**Table 4** Multivariate Logistic Regression Analysis of ACE II, ID and DD Genotypes with Metabolic Biomarkers of Diabetic Nephropathy

Variable	ACE II			ACE ID			ACE DD		
	Odds Ratio	95% CI	P value	Odds Ratio	95% CI	P value	Odds Ratio	95% CI	P value
Microalbuminuria	1.046	0.264–4.134	0.949	1.027	0.450–2.346	0.950	0.912	0.398–2.087	0.827
Glycated hemoglobin	0.990	0.895–1.096	0.849	1.077	1.011–1.147	0.022*	0.917	0.858–0.980	0.010*
Serum Glucose	0.873	0.773–0.986	0.029*	0.933	0.874–0.996	0.038*	1.132	1.055–1.214	0.001*
Urea	0.997	0.599–1.660	0.992	0.974	0.724–1.312	0.865	1.056	0.790–1.412	0.712
Creatinine	1.000	0.987–1.014	0.956	0.995	0.983–1.008	0.477	1.003	0.993–1.013	0.608
eGFR	1.003	0.978–1.029	0.806	0.989	0.970–1.009	0.295	1.004	0.986–1.022	0.642
Triglycerides	0.723	0.430–1.215	0.221	1.316	1.025–1.691	0.031*	0.834	0.648–1.072	0.157
Total Cholesterol	0.938	0.545–1.613	0.816	1.177	0.859–1.613	0.311	0.891	0.652–1.216	0.466
HDL-Cholesterol	0.344	0.366–3.213	0.351	1.317	0.358–4.842	0.679	1.020	0.287–3.621	0.975
LDL-Cholesterol	1.207	0.453–3.214	0.706	0.589	0.332–1.045	0.070	1.599	0.918–2.786	0.097
Potassium	1.186	0.782–1.800	0.423	1.074	0.766–1.504	0.680	0.672	0.387–1.167	0.158
Chloride	0.850	0.735–0.982	0.028*	1.017	0.924–1.119	0.728	1.044	0.950–1.148	0.369
Sodium	1.093	0.993–1.202	0.069	0.978	0.921–1.039	0.479	0.999	0.941–1.060	0.967

**Notes:** P value  $\leq 0.05$  is statistically significant. \*denotes variables with significant P values.

**Abbreviations:** ACE, Angiotensin Converting Enzyme; I, insertion; D, Deletion; eGFR, estimated glomerular filtration rate; CI, confidence interval; HDL, high density lipoprotein; LDL, low density lipoprotein; %, percentage.

contrast to what Sapkota et al observed that the II genotype was common among patients with T2D.<sup>18</sup> The reason why most type 2 diabetes mellitus (T2D) patients have the ACE DD genotype compared to the ACE II genotype is not fully understood, although it may be due to a combination of genetic and environmental factors.<sup>19</sup> Additionally, several studies have suggested that the ACE DD genotype may be associated with an increased risk of T2D and its complications, such as hypertension<sup>20</sup> and diabetic nephropathy.<sup>21</sup> This could perhaps explain why we had more ACE DD or ID genotypes present in our T2D study participants. However, the evidence for this association is not consistent across all populations, and more research is needed to fully understand the role of the ACE I/D polymorphism in the development and progression of T2D with associated complications such as nephropathy. Moreover, other factors that may contribute to the higher prevalence of the ACE DD genotype in T2D patients include lifestyle factors such as diet, physical activity, and obesity, as well as other genetic and environmental factors that we did not fully characterize in our current study.

There was no statistically significant difference in the distribution of ACE I/D alleles and genotypes by age and sex. This finding is similar to what was observed by Jayapalan et al. In their study, they found that neither gender nor ethnicity exerted any influence on the ACE I/D gene polymorphisms<sup>22</sup>. ACE I allele was associated with increasing glycated hemoglobin and decreasing serum glucose levels, while the D allele was associated with decreasing glycated hemoglobin, and increasing serum glucose and chloride levels. ACE II genotype was associated with decreasing serum glucose and chloride levels, whereas the ID genotype was associated with increasing glycated hemoglobin and triglyceride levels and decreasing serum glucose levels. The DD genotype was associated with decreasing glycated hemoglobin and increasing serum glucose levels. Our findings partly agree with a study done in a Chinese T2D population that found a significant association between dyslipidemia and the ACE genotypes.<sup>23</sup> Unlike this previous study, we specified the dyslipidemia fraction to be triglyceride levels, and the ACE genotype implicated was the ID genotype. A recent study on the ACE I/D gene polymorphisms in Hyderabad, India, implicated the glycated hemoglobin, triglyceride levels, and DD genotype, among other factors, as independent putative mediators of diabetic nephropathy.<sup>24</sup>

We did not find any association between the ACE I/D alleles and genotypes, with microalbuminuria or significant changes in the eGFR both of which are established biomarker markers of diabetic nephropathy and renal function, respectively. Several studies have indicated that the ACE D allele and ACE ID and DD genotypes are predisposing factors to diabetic nephropathy and that the II genotype confers a protective effect.<sup>25</sup> On the contrary, a previous study found a significant association between the DD genotype (homozygous for the deletion allele) and an increased risk of developing diabetic nephropathy.<sup>26</sup>

Our study has categorically elucidated the relationship between ACE I/D gene polymorphisms and metabolic indicators and biomarkers used in assessing diabetic nephropathy. As such, we have evaluated the critical biomarker tests from the pool of tests that are usually carried out among type 2 diabetes mellitus patients. Due to the cross-sectional design of our study, we were unable to further explore the actual relationship between the ACE I/D gene polymorphisms and nephropathy in type 2 diabetes mellitus patients because we found no significant statistical association when we regressed microalbuminuria and eGFR with other metabolic indicators and biomarkers of nephropathy. We therefore recommend case-control and cohort studies to further explore this relationship.

A limitation to this study is that we did not collect information on T2D disease duration as most of the participants could not recall when they were first diagnosed with the disease. Also, the clinical disease is usually diagnosed after several years since symptoms can be mild at the on-set, therefore determining the exact disease duration is not always easy.

## Conclusion

The presence or absence of the ACE I/D alleles and genotypes affects the ultimate increase or decrease in serum glucose, glycated hemoglobin, triglycerides, and serum chloride levels. Although there was no significant association between the biomarkers of nephropathy and the ACE I/D alleles or genotypes, the above implicated metabolic indicators should be included in healthcare guidelines used when attending to type 2 diabetic patients. Therefore, genetic testing of the angiotensin converting enzyme insertion/deletion gene polymorphisms should be encouraged among patients to facilitate personalized health care.

## Data Sharing Statement

The datasets used to analyze the data during the current study are available from the corresponding author on reasonable request.

## Ethics Approval and Consent to Participate

This study complies with the Declaration of Helsinki.<sup>27</sup> Ethics approval was obtained from the Mbarara University of Science and Technology-Research Ethics Committee (study number: MUST-2021-132). It was registered by the national research regulatory body, the Uganda National Council of Science and Technology (study number: HS1941ES). Administrative clearance was obtained from the administration of Mbarara Regional Referral Hospital (MRRH) and the Head of Department of the Diabetic Clinic. Informed written consent (in English and the local dialect) was obtained from each study participant prior to recruitment into the study. We observed the ethical requirements related to conducting research involving human participants.

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## Disclosure

The authors declare that they have no competing interests in this work.

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