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ORIGINAL RESEARCH

Association Between *Angiotensin-Converting Enzyme* Insertion/Deletion Polymorphism and Diabetic Kidney Disease in Patients with Type 2 Diabetes Mellitus: A Multicenter Case-Control Study in Vietnam

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Purpose: Diabetic kidney disease (DKD) is one of the major complications of type 2 diabetes mellitus (T2DM), which is common worldwide. Its pathogenesis involves various mechanisms: Notably, overactivation of the renin-angiotensin-aldosterone system (RAAS) and genetic susceptibility. Although RAAS gene polymorphisms have been widely studied, the results vary across various ethnicities. In this study, we investigated the association between the *angiotensin-converting enzyme* (*ACE*) I/D polymorphism and DKD in the Vietnamese population with T2DM.

Patients and Methods: A case-control study was conducted from November 2022 to November 2024 at the University Medical Center at Ho Chi Minh City and Nhan Dan Gia Dinh Hospital. The case group included patients with T2DM and confirmed DKD, while the control group comprised patients with T2DM without DKD. The participants were matched by age and sex. Clinical, biochemical, and genetic data were collected, including those on ACE I/D polymorphisms.

Results: A total of 578 patients were enrolled, with 289 in the case group and 289 in the control group. The participants had a mean age of 63.4 ± 10.8 years, of which 41.3% were male. Compared with the controls, patients with DKD experienced longer T2DM durations and higher rates of hypertension, heart failure, and chronic anemia, higher HbA1c levels, and lipid profiles ($p < 0.05$). The *ACE* I/D polymorphism significantly differed between the groups, with the DD genotype being more frequent in the case group ($p < 0.001$). This genotype was associated with an increased DKD risk (odds ratio = 2.26; 95% confidence interval: 1.34–3.81; $p = 0.002$). Multivariate analysis identified that duration of T2DM, hypertension, triglyceride levels, LDL-C, and the DD genotype were independent risk factors for DKD in patients with T2DM.

Conclusion: Among the Vietnamese Kinh population, a significant association was found between the ACE I/D polymorphism and DKD in patients with T2DM.

Plain Language Summary: Type 2 diabetes mellitus (T2DM) is a common disease that affects millions of people around the world. One of its most serious complications is diabetic kidney disease (DKD), which can lead to kidney failure and the need for dialysis. Both lifestyle and genetic factors may contribute to who develops DKD, but the specific genetic causes can differ between ethnic groups. In this study, we wanted to find out whether a variation in the angiotensin-converting enzyme (ACE) gene, called the insertion/deletion (I/D) polymorphism, is linked to DKD in Vietnamese people living with T2DM. The ACE gene is part of the body's renin-angiotensin-aldosterone system (RAAS), which helps regulate blood pressure and kidney function.

We studied 578 Vietnamese patients with T2DM from two major hospitals in Ho Chi Minh City. Half of them had DKD, and half did not. We compared their medical information, blood test results, and ACE gene types. We found that people with the DD genotype

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of the ACE I/D polymorphism were more likely to have DKD. This genetic type, along with factors such as high blood pressure, elevated LDL-C, high triglyceride levels, and longer T2DM duration, increased the risk of DKD.

Our findings show that genetics may play an important role in DKD among Vietnamese people with T2DM. Understanding these genetic factors could help doctors identify high-risk patients earlier and personalize treatment to protect kidney health.

Keywords: type 2 diabetes mellitus, diabetic kidney disease, I/D variant of the *ACE* gene, case-control study

Introduction

Type 2 diabetes mellitus (T2DM) is a common non-communicable disease with increased comorbidity burden worldwide. By 2040, its prevalence is expected to reach 10.4%, affecting approximately 642 million people. Among the complications of T2DM, diabetic kidney disease (DKD) is notable, developing in 20–40% of this patient population.[1] DKD might progress to end-stage renal disease, requiring renal replacement therapy (RRT), and is associated with increased cardiovascular risks and mortality.[2] Furthermore, DKD also imposes significant economic burdens and reduces the quality of life.

The pathogenesis of DKD is multifactorial and involves overlapping mechanisms, including intrarenal hemodynamic alterations, ischemia, inflammation, mitochondrial dysfunction, podocyte autophagy, overactivation of the renin-angiotensin-aldosterone system (RAAS), and genetic factors.[3] Although the current treatments target multiple pathways, they are not yet able to halt disease progression or reverse existing renal damage. Therefore, the genetic factors are considered a potential contributor to improving the prediction and management of DKD. Numerous studies have explored the genetic basis of DKD, focusing on genes involved in lipid and glucose metabolism, kidney structure and function, and particularly, the RAAS pathway.[4] Genes related to RAAS, such as angiotensinogen, angiotensin-converting enzyme (ACE), and angiotensin II type 1 receptor, are the most studied due to the fundamental role of RAAS in DKD pathophysiology.

ACE is a key component of the RAAS, responsible for converting angiotensin I into angiotensin II.[5] Angiotensin II plays a role in targeting organ damage by inducing mesangial and tubular epithelial cell hypertrophy in the kidney and promoting profibrotic cytokines production, such as transforming growth factor-β, thereby accelerating glomerular fibrosis.[6] Among the genetic variants of the *ACE* gene, the insertion/deletion (I/D) polymorphism is the most investigated. Recent meta-analyses have confirmed that the ACE I/D polymorphism is linked to DKD, with the DD genotype and D allele increasing risk, particularly in Asian populations and patients with T2DM. On the other hand, the II genotype may have a protective effect.[7,8] However, inconsistent associations have been reported among the studies. For instance, Tomino et al,[9] Osawa et al,[10] and Ahluwalia et al[11] found a significant relationship between *ACE* I/D polymorphism and DKD in patients with T2DM, whereas Prasad et al reported no such association.[12] These discrepancies suggest that genetic susceptibility may be population-specific. Therefore, the results from one ethnic group cannot be generalized to others. Moreover, although some meta-analyses have focused on Asian populations, no data are currently available on the relationship between the *ACE* I/D polymorphism and DKD in Vietnamese patients with T2DM. In this study, we conducted to investigate this relationship in Vietnamese patients with T2DM.

Materials and Methods

Study Design

This case-control study was conducted at the University Medical Center at Ho Chi Minh City (UMC) and Nhan Dan Gia Dinh Hospital between November 2022 and November 2024. UMC and Nhan Dan Gia Dinh Hospital are two major general hospitals in southern Vietnam, with capacities of approximately 1,000 and 1,500 beds, respectively. Both hospitals are affiliated teaching institutions of the University of Medicine and Pharmacy at Ho Chi Minh City. The Endocrinology and Nephrology outpatient clinics specialize in the management of endocrine and renal disorders, including DKD. The case group included patients with T2DM diagnosed with DKD; patients with T2DM without DKD complications were allocated into the control group. To ensure comparability, the case and control groups were matched by age and sex, with the age difference between each matched pair not exceeding ±2 years.

Inclusion and Exclusion Criteria of the Case Group

The inclusion criteria were: (i) age ≥18 years and Kinh ethnicity; (ii) diagnosis of T2DM for ≥5 years; (iii) diagnosis of DKD, based on estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, persistent urine albumin-to-creatinine ratio (uACR) ≥300 mg/g, and presence of diabetic retinopathy; and (iv) receiving no RRT.

Patients with primary glomerular disease, secondary glomerular disease (eg, systemic lupus erythematosus, hepatitis B or C), chronic kidney disease of unknown etiology, or DKD coexisting with non-DKD were excluded.

Inclusion and Exclusion Criteria of the Control Group

Patients: aged ≥18 years and of Kinh ethnicity; diagnosed with T2DM for ≥5 years; and without DKD, defined by eGFR >60 mL/min/1.73 m^2 and uACR <30 mg/g were enrolled.

Patients with primary glomerular disease or secondary glomerular disease (eg, systemic lupus erythematosus, hepatitis B or C) were excluded.

Sample Size

The sample size was estimated using the formula for case-control studies as follows:[13]

$$N = \frac{(r+1)[1+(\lambda-1)P]^2}{rP^2(P-1)^2(\lambda-1)^2}\left[z_{\alpha/2}\sqrt{(r+1)P_c(1-P_c)} + z_{\beta}\sqrt{\frac{\lambda P(1-P)}{[1+(\lambda-1)P]^2} + rP(1-P)}\right]^2$$

$$P_c = \frac{P}{1+r}\left[\frac{r\lambda}{1+(\lambda-1)P} + 1\right]$$

N is the total number of patients in both case and control groups. r is the ratio between the case and control groups; we selected r = 1. $z_{\alpha/2}$ and z_{β} are constant, with a type I error $\alpha = 0.05$ and study power of 0.8, therefore $z_{\alpha/2} = 1.96$ and $z_{\beta} = 0.842$. λ is the odds ratio (OR); we selected OR = 1.65 according to the study by Ahluwalia.[11] P is the prevalence of the DD genotype of the *ACE* I/D gene (the exposure factor) in the control group; we selected $P = 0.35$ based on the study by Ahluwalia.[11] P_c is the estimated prevalence of the exposure factor in the case group. Therefore, we calculated that a minimum of 524 participants was required, including 262 patients in the case group and 262 patients in the control group. Our study enrolled a total of 578 patients.

Study Procedure

Data on: age, sex, duration of T2DM, comorbidities (hypertension, dyslipidemia, diabetic peripheral neuropathy, chronic coronary syndrome, heart failure, chronic anemia, and prior myocardial infarction or stroke),[14] family history of T2DM, smoking status, and current use of antidiabetic and antihypertensive medications were collected. Clinical assessments involved blood pressure, heart rate, and body mass index (BMI). Laboratory parameters, including fasting plasma glucose, HbA1c, total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, serum creatinine, and uACR, were collected. All laboratory tests were performed at the Departments of Biochemistry and Hematology at UMC and Nhan Dan Gia Dinh Hospitals. Genetic variables were documented at the time of recruitment into the study, comprising genotypes and the allelic frequencies of the I/D variant of the *ACE* gene.

Definition of Variables

T2DM was defined as "either a known history of T2DM under treatment (dietary management or antidiabetic medications) or a new diagnosis according to the American Diabetes Association 2022 criteria".[15] Hypertension was defined as "systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or a known history of hypertension and/or current use of antihypertensive medications". [16] BMI was classified based on the World Health Organization criteria for the Asian population: "underweight (<18.5 kg/m^2), normal weight (18.5–22.9 kg/m^2), overweight (23.0–24.9 kg/m^2), obesity class I (25.0–29.9 kg/m^2), and obesity class II (≥30.0 kg/m^2)".[17] Anemia was defined as "hemoglobin <13 g/dL in men and <12 g/dL in women".[18] Diagnosis of dyslipidemia, chronic coronary syndrome, and

heart failure was established based on the current clinical guidelines.[16,19,20] A history of myocardial infarction or stroke was identified from medical records. Diabetic peripheral neuropathy was diagnosed based on "typical symptoms and clinical signs consistent with diabetic nerve damage".[21]

Serum creatinine was measured using the Kinetic Jaffe method; eGFR was calculated by the CKD-EPI equation.[22] uACR was assessed under clinically stable conditions. In the DKD group, macroalbuminuria was defined as "uACR ≥300 mg/g on at least two occasions within a 3–6 month period".[23] Serum glucose was determined by the hexokinase method. HbA1c was analyzed by capillary electrophoresis in an alkaline buffer (pH 9.4). Total cholesterol was measured using an enzymatic colorimetric method, with the red quinoneimine complex quantified spectrophotometrically at 540/600 nm. Triglycerides were determined by an enzymatic colorimetric method, with absorbance measured at 500 nm. LDL-C was assessed by an enzymatic colorimetric method, with the red quinoneimine complex measured at 540/660 nm, while HDL-C was measured similarly at 600/700 nm. Urinary albumin was measured by a colorimetric method, and urinary creatinine was determined using the Kinetic Jaffe method.

Genetic Analysis

Genotyping of *ACE* I/D polymorphisms was performed using the polymerase chain reaction (PCR) method at the Center for Molecular Biomedicine of the University of Medicine and Pharmacy (Ho Chi Minh City, Vietnam). Genomic DNA was extracted from whole blood samples using the GeneJET^{TM} Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions. The primer sequences used for genotyping the *ACE* I/D polymorphisms are presented (Supplementary Tables 1 and 2). Representative Sanger sequencing chromatograms and agarose gel electrophoresis results illustrating the ACE insertion/deletion (I/D) polymorphism and corresponding genotypes (DD, ID, and II) (Supplementary Figures 1–3).

Regarding the *ACE* I/D polymorphism, PCR primers were designed using CLC Main Workbench software with the human *ACE* gene sequence (NCBI accession number NG_011648). Each 15 µL of PCR mixture contained: 1.5 µL of 10X PCR buffer, 1.5 µL of 2.5 mM dNTPs, 0.75 µL of forward and reverse primers (10 µM/µL), 0.1 µL of TaKaRa Taq^{TM} HotStart Polymerase (Takara Bio, Japan), 2 µL of genomic DNA (20–50 ng/µL), and 8.4 µL of deionized water. Negative controls (ddH_2O) and positive controls (DNA samples with known genotypes confirmed by Sanger sequencing) were included in each run. SimpliAmp Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) was used for performing PCR. The PCR reaction consisted of an initial denaturation at 98°C for 3 minutes, followed by 40 cycles of 98°C for 15 seconds, 56°C for 20 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 2 minutes. PCR products were stored at 4°C and analyzed by electrophoresis on 2% agarose gels stained with GelRed and imaged using a GelDoc-It^{TM} Imaging System (UVP, UK).

Statistical Analysis

Categorical variables are presented as frequencies and percentages. Continuous variables that followed a normal distribution are reported as mean ± standard deviation, whereas those that did not follow a normal distribution are presented as median [interquartile range]. Differences between categorical variables were assessed by the Chi-square test or Fisher's exact test. Considering continuous variables that did not follow a normal distribution, non-parametric tests were performed (Mann–Whitney *U*-test for two groups and Kruskal–Wallis *H*-test for three groups). Regarding normally distributed continuous variables, parametric tests were employed (Student's *t*-test for two groups and analysis of variance for three groups). Clinically relevant and commonly reported risk factors for DKD were selected, including duration of T2DM, glycemic control (HbA1c), hypertension, dyslipidemia (each lipid component), obesity, smoking status, and the *ACE* I/D polymorphism.[24–26] Univariate logistic regression was performed to assess associations with DKD. Variables with p-value < 0.05 were evaluated for multicollinearity using the variance inflation factor (VIF), with a threshold of ≥ 10 indicating severe collinearity. Variables without significant collinearity were subsequently entered into a multivariate logistic regression model. A p-value of < 0.05 was considered statistically significant. All analyses were performed using SPSS software version 22.0.

Medical Ethics

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City (No. 724/HĐĐĐ-ĐHYD, dated October 6, 2022; IRB-VN01002/IORG0008603/FWA00023448) and the Ethics Committee of Nhan Dan Gia Dinh Hospital (No. 59/NDGĐ-HĐĐĐ, dated May 12, 2023). All procedures were conducted following the ethical standards of the 1964 Declaration of Helsinki. Informed consent was obtained from all participants.

Results

A total of 289 patients were enrolled in the case group, and 289 in the control group; 423 of the 578 participants were recruited from the UMC, while the remaining 155 were from Nhan Dan Gia Dinh Hospital.

Patient Characteristics

The mean age of the study population was 63.4 ± 10.8 years; male participants accounted for 41.3%. All patients in the case group were diagnosed with diabetic retinopathy. Compared with the control group, the case group experienced longer durations of T2DM; a higher prevalence of hypertension, heart failure, and chronic anemia; more frequent use of insulin, diuretics, calcium channel blockers, beta-blockers, and alpha-blockers; and higher HbA1c levels and lipid profiles (all, $p < 0.05$). However, the control group exhibited higher rates of dyslipidemia, diabetic peripheral neuropathy, a family history of T2DM, greater use of oral antidiabetic agents, and more frequent use of RAAS inhibitors compared with the case group (all, $p < 0.05$). Full details are presented in Table 1.

Table 1 Baseline and Clinical Characteristics of the Study Population (N = 578)

Variables	Total (N = 578)	Case (n = 289)	Control (n = 289)	p[a]
Baseline characteristics				
Age (year)	63.4 ± 10.8	63.8 ± 11.0	62.9 ± 10.4	0.307[b]
Male	239 (41.3%)	123 (42.6%)	116 (40.1%)	0.554[c]
Duration of diabetes (years)	12 [8–17]	14 [9–20]	10 [7–15]	**<0.001**[d]
Hypertension	500 (86.5%)	276 (95.5%)	224 (77.5%)	**<0.001**[c]
Dyslipidemia	520 (90.0%)	248 (85.8%)	272 (94.1%)	**0.001**[c]
Diabetic peripheral neuropathy	54 (9.3%)	16 (5.5%)	38 (13.1%)	**0.002**[c]
Chronic coronary syndrome	135 (23.4%)	73 (25.3%)	62 (21.5%)	0.280[c]
Prior myocardial infarction	4 (0.7%)	1 (0.3%)	3 (1.0%)	0.624[c]
Heart failure	16 (2.8%)	14 (4.8%)	2 (0.7%)	**0.002**[c]
Prior stroke	38 (6.6%)	23 (8.0%)	15 (5.2%)	0.179[c]
Chronic anemia	98 (17.0%)	93 (32.2%)	5 (1.7%)	**<0.001**[c]
Family history of T2DM	207 (35.8%)	77 (26.6%)	130 (45.0%)	**<0.001**[c]
Smoking	120 (20.8%)	54 (18.7%)	66 (22.8%)	0.218[c]
Insulin	281 (48.6%)	198 (68.5%)	83 (28.7%)	**<0.001**[c]
Metformin	326 (56.4%)	67 (23.2%)	259 (89.6%)	**<0.001**[c]
Sulfonylureas	232 (40.1%)	59 (20.4%)	173 (59.9%)	**<0.001**[c]
DPP-4 inhibitors	296 (51.2%)	157 (54.3%)	139 (48.1%)	0.134[c]
SGLT2 inhibitors	149 (25.8%)	60 (20.8%)	89 (30.8%)	**0.006**[c]
Alpha-glucosidase inhibitors	49 (8.5%)	16 (5.5%)	33 (11.4%)	**0.011**[c]
ACE inhibitors	35 (6.1%)	4 (1.4%)	31 (10.7%)	**<0.001**[c]
ARBs	249 (43.1%)	94 (32.5%)	155 (53.6%)	**<0.001**[c]
Calcium channel blockers	306 (52.9%)	216 (74.7%)	90 (31.1%)	**<0.001**[c]
Beta-blockers	212 (36.7%)	118 (40.8%)	94 (32.5%)	**0.038**[c]
Alpha-blockers	63 (10.9%)	60 (20.8%)	3 (1.0%)	**<0.001**[c]
Diuretics	112 (19.4%)	103 (35.6%)	9 (3.1%)	**<0.001**[c]

(*Continued*)

Table 1 (Continued).

Variables	Total (N = 578)	Case (n = 289)	Control (n = 289)	*p*[a]
Clinical characteristics				
Systolic blood pressure (mmHg)	134 [123–150]	140 [130–150]	130 [120–137.5]	**<0.001**[d]
Diastolic blood pressure (mmHg)	80 [70–80]	80 [70–87]	77 [70–80]	**0.009**[d]
Heart rate (bpm)	82 [74–91]	84 [75–93]	81 [73–90]	0.080[d]
Height (cm)	158 [152–165]	158 [152–165]	158 [154–163]	0.730[d]
Weight (kg)	59.0 [53.0–67.0]	60.0 [53.0–67.0]	59.0 [53.0–66.0]	0.618[d]
Body mass index (kg/m^2)	23.8 [21.7–25.6]	23.6 [21.5–25.6]	23.9 [21.9–25.8]	0.234[d]
Underweight	19 (3.3%)	14 (4.8%)	5 (1.7%)	
Normal weight	212 (36.7%)	105 (36.3%)	107 (37.0%)	
Overweight	162 (28.0%)	87 (30.1%)	75 (26.0%)	0.103[c]
Obesity class I	160 (27.7%)	70 (24.2%)	90 (31.1%)	
Obesity class II	25 (4.3%)	13 (4.5%)	12 (4.2%)	
Laboratory parameters				
Fasting glucose (mmol/L)	7.6 [6.3–10.0]	8.2 [6.1–10.9]	7.4 [6.5–9.2]	0.204[d]
HbA1c (%)	7.6 [6.7–8.8]	7.9 [6.7–9.3]	7.4 [6.7–8.4]	**0.041**[d]
Total cholesterol (mmol/L)	4.3 [3.4–5.6]	4.7 [3.7–6.1]	4.0 [3.2–5.0]	**<0.001**[d]
Triglycerides (mmol/L)	2.0 [1.4–2.9]	2.3 [1.7–3.6]	1.7 [1.2–2.4]	**<0.001**[d]
LDL-C (mmol/L)	2.5 [1.9–3.5]	2.8 [2.2–3.8]	2.3 [1.8–3.1]	**<0.001**[d]
HDL-C (mmol/L)	1.2 [1.0–1.3]	1.1 [0.9–1.3]	1.2 [1.0–1.3]	**0.014**[d]
Serum creatinine (μmol/L)	101 [74–159]	158 [122–220]	74 [66–87]	**<0.001**[d]
eGFR (mL/min/1.73 m^2)	59.5 [33.8–78.0]	34.0 [23.0–45.0]	78.0 [68.0–89.0]	**<0.001**[d]
uACR (mg/g)	186 [8.9–1934]	1932 [815–3748]	9.0 [5.5–15.1]	**<0.001**[d]

Notes: Data are presented as n (%), mean ± SD, or median [IQR]. [a]Comparison between case and control groups. [b]Student's *t*-test. [c]Chi-square test. [d]mann–Whitney *U*-test. The *p*-value <0.05 is highlighted in bold.

Abbreviations: eGFR, estimated glomerular filtration rate; SD, standard deviation; IQR, interquartile range; DPP-4, dipeptidyl peptidase-4; SGLT2, sodium-glucose cotransporter 2; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus; uACR, urinary albumin-to-creatinine ratio; bpm, beats per minute; cm, centimeter; kg, kilogram; m, meter.

Abbreviations: eGFR, estimated glomerular filtration rate; SD, standard deviation; IQR, interquartile range; DPP-4, dipeptidyl peptidase-4; SGLT2, sodium-glucose cotransporter 2; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus; uACR, urinary albumin-to-creatinine ratio; bpm, beats per minute; cm, centimeter; kg, kilogram; m, meter.

Genotype and Allele Profiles

Among the 578 participants, the frequencies of DD, ID, and II genotypes were 13.8%, 37.7%, and 48.4%, respectively (Table 2). Hardy-Weinberg equilibrium analysis showed statistically significant deviation in the total sample and in the case group ($p < 0.05$), but not in the control group.

Association Between Genetic Variants and Diabetic Kidney Disease

The DD genotype was significantly associated with DKD (OR = 2.26, 95% confidence interval:[24] 1.34–3.81, $p = 0.002$) (Table 3). Similarly, compared with ID + II genotypes, the DD genotype remained significantly associated with increased risk (OR = 2.32, 95% CI: 1.41–3.83, $p = 0.001$). However, the ID genotype and the combined ID + DD group showed no significant association. Regarding allelic distribution, the D allele was more frequent in cases and significantly associated with disease susceptibility (OR = 1.37, 95% CI: 1.07–1.75, $p = 0.012$).

Tables 4 and 5 presents that duration of T2DM, hypertension, triglyceride levels, LDL-C, and the DD genotype are independent risk factors for DKD in patients with T2DM ($p < 0.05$).

Table 2 Genotypes and Alleles of the Genetic Variants (N = 578)

Genetic Variants		Total (N = 578)	Case (n = 289)	Control (n = 289)
I/D variant of the *ACE* gene	DD genotype	80 (13.8%)	54 (18.7%)	26 (9.0%)
	ID genotype	218 (37.7%)	101 (34.9%)	117 (40.5%)
	II genotype	280 (48.4%)	134 (46.4%)	146 (50.5%)
	HWE	**0.003**	**<0.001**	0.905
	D allele	378 (32.7%)	209 (36.2%)	169 (29.2%)
	I allele	778 (67.3%)	369 (63.8%)	409 (70.8%)

Notes: Data are presented as n (%). The *p*-value <0.05 is highlighted in bold.
Abbreviation: HWE, Hardy-Weinberg equilibrium.

Table 3 Univariate Analysis of the Association Between I/D Polymorphism of the *ACE* Gene and Diabetic Kidney Disease (N = 578)

	Case (n = 289)	Control (n = 289)	OR (95% CI)	*p*
Genotypes of the *ACE* I/D variant				
II	134 (46.4%)	146 (50.5%)	1	
ID	101 (34.9%)	117 (40.5%)	0.94 (0.66–1.34)	0.735
DD	54 (18.7%)	26 (9.0%)	2.26 (1.34–3.81)	**0.002**
DD genotype vs ID + II genotypes				
ID + II	235 (81.3%)	263 (91.0%)	1	
DD	54 (18.7%)	26 (9.0%)	2.32 (1.41–3.83)	**0.001**
II genotype vs ID + DD genotypes				
II	134 (46.4%)	146 (50.5%)	1	
ID + DD	155 (53.6%)	143 (49.5%)	1.18 (0.85–1.64)	0.318
Allele types of the *ACE* I/D variant				
I allele	369 (63.8%)	409 (70.8%)	1	
D allele	209 (36.2%)	169 (29.2%)	1.37 (1.07–1.75)	**0.012**

Notes: Data are presented as n (%). The *p*-value <0.05 is highlighted in bold.
Abbreviations: OR, odds ratio; CI, confidence interval; ACE, angiotensin-converting enzyme.

Discussion

Our analysis of the *ACE* I/D polymorphism revealed that the II genotype occurred most frequently, followed by the ID and DD genotypes, in both the case and control groups (Table 2). We observed a significant association between the *ACE* I/D polymorphism and DKD in patients with T2DM (Table 3). Using multivariate logistic regression analysis, adjusted for other risk factors, the *ACE* I/D polymorphism was identified as an independent risk factor for DKD in patients with T2DM (Table 5). Previous studies have shown that the *ACE* I/D polymorphism affects plasma ACE concentrations. In a study by Susilo et al, the DD genotype was found to be associated with significantly higher ACE concentrations compared with the II and ID genotypes.[27] Moreover, the I/D polymorphism accounts for approximately 40–50% of the variability in *ACE* activity in plasma and tissue.[28,29] Patients carrying the DD homozygous genotype exhibit the highest ACE activity, whereas those with the II homozygous genotype demonstrate the lowest. Consequently, individuals with the DD genotype tend to have elevated plasma ACE levels and enhanced enzymatic activity, thereby predisposing them to target organ complications.

Our findings are consistent with those in previous studies. A 2005 meta-analysis assessed the association between the *ACE* I/D polymorphism and DKD. The analysis included 47 studies of both T1DM and T2DM in Caucasian and Asian

Table 4 Univariate Logistic Regression Analysis of Associated Factors for Diabetic Kidney Disease

Risk Factors for Diabetic Kidney Disease	Risk		
	OR	95% CI	*p*
Duration of T2DM	1.07	1.04–1.10	**<0.001**
Hypertension	6.16	3.31–11.46	**<0.001**
HbA1C	1.00	0.97–1.04	0.921
Total cholesterol	1.39	1.23–1.59	**<0.001**
Triglycerides	1.45	1.27–1.65	**<0.001**
LDL-C	1.53	1.31–1.80	**<0.001**
HDL-C	0.78	0.44–1.38	0.397
Obesity	0.74	0.52–1.05	0.091
Smoking	0.78	0.52–1.16	0.219

Notes: The p-value <0.05 is highlighted in bold.
Abbreviations: T2DM, type 2 diabetes mellitus; OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 5 Multivariate Logistic Regression Analysis of Associated Factors and the *ACE* I/D Gene for Diabetic Kidney Disease

Risk Factors for Diabetic Kidney Disease	Risk		
	OR	95% CI	*p*
Duration of T2DM	1.08	1.05–1.11	**<0.001**
Hypertension	5.95	2.84–12.46	**<0.001**
Triglycerides	1.35	1.18–1.56	**<0.001**
LDL-C	1.42	1.18–1.71	**<0.001**
Genotypes of the *ACE* I/D variant			
II	Control		
ID	0.98	0.65–1.48	0.913
DD	2.68	1.47–4.88	**0.001**

Notes: The *p*-value <0.05 is highlighted in bold.
Abbreviations: T2DM, type 2 diabetes mellitus; OR, odds ratio; CI, confidence interval; ACE, angiotensin-converting enzyme.

populations, with a total of 8,663 cases and 6,064 controls.[30] The pooled results demonstrated that individuals with the II genotype have a lower risk of DKD compared with carriers of the D allele (OR = 0.78; 95% CI, 0.69–0.88). Subgroup analysis revealed that Asian patients with T2DM and the II genotype have up to a 35% reduced risk of DKD compared with those carrying the D allele (OR = 0.65; 95% CI, 0.51–0.83). However, among Caucasians, the protective effect of the II genotype was observed only in T2DM, whereas no significant reduction in risk was found in patients with T1DM. The authors concluded that the *ACE* I/D polymorphism is more strongly associated with DKD in Asians and in patients with T2DM than in Caucasians and T1DM cases.[30] Similarly, Wang et al performed a meta-analysis of 63 studies (32 case-control, 24 cross-sectional, and 7 cohort studies) with a total of 14,108 cases and 12,472 controls across both diabetes types and different ethnic groups.[31] The results indicated that both DD and ID genotypes increase the risk of DKD compared with the II genotype, with ORs of 1.27 (95% CI, 1.13–1.44) and 1.12 (95% CI, 1.02–1.24), respectively. Subgroup analysis confirmed a stronger association in Asian populations with T2DM, where the ID genotype has an OR of 1.25 (95% CI, 1.07–1.47) and the DD genotype an OR of 1.57 (95% CI, 1.24–1.98) compared with the II genotype. The findings of this study also indicate that the D allele is associated with an increased risk of DKD, particularly among Asian populations with T2DM.[31] In 2019, Silveira et al conducted a meta-analysis to evaluate the association between the *ACE* I/D polymorphism and DKD. Case-control studies published between 1995 and 2018 were included. Eligible

studies were required to involve adult participants of different ethnicities; patients diagnosed with T2DM according to WHO criteria; assessment of renal function and urinary albumin excretion; a minimum duration of T2DM of 3 years; and *ACE* I/D genotyping performed by PCR. A total of 30 studies met the inclusion criteria, comprising 9,131 patients, including 4,774 with DKD and 4,357 without DKD. In the DKD group, the frequencies of the II, ID, and DD genotypes were 23.6%, 45.8%, and 30.6%, respectively, compared with 28.6%, 46.4%, and 25.0% in the non-DKD group. The DD genotype was significantly more frequent in patients with DKD than in controls, with an OR of 1.54 (95% CI, 1.27–1.86).[7] Another meta-analysis, by Zeng et al, also comprising 63 studies, further supports these findings.[8] The DD genotype significantly increases the risk of DKD compared with ID + II genotypes (OR = 1.41; 95% CI, 1.25–1.59). Subgroup analyses revealed particularly high risks among Asians (OR = 1.81; 95% CI, 1.55–2.12) and Chinese patients (OR = 1.92; 95% CI, 1.66–2.23). At the allelic level, the D allele is associated with a higher risk of DKD compared with the I allele across all populations (OR = 1.31; 95% CI, 1.21–1.42). This association is even stronger in Asian (OR = 1.51; 95% CI, 1.36–1.67) and Chinese patients (OR = 1.55; 95% CI, 1.36–1.76), and in patients with T2DM (OR = 1.36; 95% CI, 1.24–1.49). No significant associations were observed in Caucasians or in T1DM.[8] Osawa investigated the association between genes of the RAAS and DKD in a Japanese population. This case-control study included patients with T2DM, comprising 747 cases and 557 controls. The cases were patients with diabetic retinopathy and uACR ≥ 300 mg/g or those receiving RRT, while controls were patients with diabetic retinopathy and uACR < 30 mg/g. The genes analyzed included AGT, *ACE*, and AGTR1. The results demonstrated that eight single-nucleotide polymorphisms (SNPs) in the *ACE* gene were significantly associated with DKD. Notably, the *ACE* I/D polymorphism was associated with DKD, with an OR of 1.34 (95% CI, 1.07–1.69).[10] Another study by Ahluwalia investigating the impact of RAAS genes on DKD in patients with T2DM reported similar findings. The study included 440 patients with T2DM, comprising 240 patients with DKD and 200 without DKD. DKD was defined as T2DM duration ≥ 10 years with kidney involvement indicated by an albumin excretion rate > 200 μg/min, uACR > 300 mg/g, or ongoing RRT. Controls were patients with T2DM duration ≥ 10 years and normal urinary albumin excretion. The results showed that the *ACE* I/D polymorphism was associated with DKD. A significant difference in D and I allele frequencies was observed between patients with and without DKD. The DD genotype was significantly more frequent in the DKD group (55%) than in controls (35%), with an OR of 1.65 (95% CI, 1.01–2.69).[11]

Our findings, in conjunction with robust evidence from previous meta-analyses and population-based studies, consistently demonstrate that the *ACE* I/D polymorphism, particularly the D allele and DD genotype, is a significant genetic risk factor for DKD, with a more pronounced effect in Asian populations and in patients with T2DM. In the present multicenter case-control study, we further confirm a significant association between the *ACE* I/D polymorphism and DKD in patients with T2DM within the Vietnamese Kinh population, reinforcing the biological relevance of the RAAS in the pathogenesis of DKD. From a clinical perspective, identification of this genetic association has important implications. Genetic screening for the *ACE* I/D polymorphism may help identify patients with T2DM who are at increased risk of developing DKD, thereby enabling earlier risk stratification, closer renal monitoring, and more aggressive management of modifiable risk factors, including glycemic control, blood pressure, and dyslipidemia, to prevent or delay DKD progression. Moreover, these findings provide a rationale for future pharmacogenetic studies investigating genotype-specific responses to RAAS blockade, such as angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, particularly in patients carrying the DD genotype.

Our study has several limitations. First, the diagnosis of DKD in our study was not confirmed by renal histopathological examination. However, we included only patients in the case group who also had diabetic retinopathy, which increases the likelihood of accurately diagnosing DKD. Second, the combination of macroalbuminuria, eGFR < 60 mL/min, and the presence of diabetic retinopathy is typically used to diagnose DKD associated with macroalbuminuria. Consequently, this study excluded patients with microalbuminuric and non-albuminuric DKD. As a result, the ability to generalize our findings across the entire population of DKD is limited. Third, in the control group, the uACR was assessed only once, without repeat testing. Given that uACR values may fluctuate over time, this may have led to potential misclassification of patients with early-stage DKD as having no DKD. Fourth, other key components of the RAAS, such as the AGT and AGTR1 genes, were not

comprehensively investigated. Studies have also shown that polymorphisms in the AGT and AGTR1 genes are associated with the risk of DKD.[32,33] Future studies, including a broader panel of RAAS gene variants, may allow the development of more comprehensive and accurate predictive models for DKD in patients with T2DM. Finally, laboratory analyses were conducted at two different hospitals without standardization in a central laboratory. Therefore, inter-laboratory variability might have occurred between the two centers. However, both hospitals are large, well-established institutions with high laboratory quality standards, ensuring that the test results remain highly reliable and valid.

Conclusion

DKD is a common complication of T2DM, contributing to increased morbidity, mortality, and a socioeconomic burden. This study in the Vietnamese Kinh population found a significant association between the *ACE* I/D polymorphism and DKD in T2DM.

Abbreviations

ACE, angiotensin-converting enzyme; BMI, body mass index; CI, confidence interval; DKD, Diabetic kidney disease; eGFR, estimated glomerular filtration rate; OR, odds ratio; PCR, polymerase chain reaction; RAAS, renin-angiotensin-aldosterone system; RRT, renal replacement therapy; T2DM, type2 diabetes mellitus; uACR, urine albumin-to-creatinine ratio; UMC, University Medical Center Ho Chi Minh City.

Data Sharing Statement

The original contributions of this study are contained within the article and data supporting the findings are available upon reasonable request. For further inquiries, please contact the corresponding author.

Ethical Approval

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City (No. 724/HĐĐĐ-ĐHYD, dated October 6, 2022; IRB-VN01002/IORG0008603/FWA00023448) and the Ethics Committee of Nhan Dan Gia Dinh Hospital (No. 59/NDGĐ-HĐĐĐ, dated May 12, 2023). All procedures were conducted following the ethical standards of the 1964 Declaration of Helsinki. Informed consent was obtained from all participants.

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Author Contributions

Xuan-Dien Le-Nguyen: Investigation, Formal analysis, Data curation, Conceptualization, Writing – original draft. Sy Van Hoang: Investigation, Formal analysis, Data curation, Conceptualization, Writing – original draft. Nam Quang Tran: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – review and editing, Writing – original draft, Investigation. All authors took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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