PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
			Section; page in main manuscript
Title	1	**Association Between Angiotensin Converting Enzyme Insertion / Deletion Genotypes and Diabetic Nephropathy Defined by Urinary Albumin-to-Creatinine Ratio: A Systematic Review and Meta-Analysis**	Title page, 1
ABSTRACT			
Abstract	2	**Background** Diabetic nephropathy (DN) is a major complication of type 2 diabetes mellitus (T2DM) and a leading cause of kidney failure. Evidence on the influence of ACE I/D polymorphisms in DN risk is inconsistent across populations. **Methods** A systematic review and meta-analysis was conducted following the PRISMA 2020 guidelines. Studies published between January 1990 to February 2025 were retrieved from PubMed, EMBASE and Web of Science. Eligible observational studies reported the frequency of ACE genotypes with DN in T2DM. Independent reviewers screened studies using Rayyan software, extracted data, and assessed risk of bias using the ROBINS-E tool. Reporting on the quality of studies was determined using the STREGA guidelines. Pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated using random-effects models in R version 4.4.2. Subgroup, meta-regression, and sensitivity analyses addressed heterogeneity; Egger's test assessed publication bias. **Results** Of the 46 studies included in this review, the combined sample size was 16,322 participants. The majority of studies (29 out of 46) were conducted in Asia. Only 5 studies reported DN–related comorbidities by ACE genotypes and one assessed mortality. Twenty-five of the included 46 studies contributed data to the meta-analysis. The ACE II genotype was protective against DN; II vs. ID [OR= 0.70 (CI: 0.63–0.77)] and II vs. DD [OR= 0.68 (CI: 0.55–0.84)]; Heterogeneity was (I^2 = 71.7%, τ^2 = 0.1776, p < 0.0001). Stronger associations were observed in studies using urinary Albumin-Creatinine-Ratio over Albumin-Excretion-Rate. Egger's test showed no publication bias (p = 0.55). **Discussion** The ACE II genotype is significantly protective against DN risk in T2DM. Standardization of urinary albumin measurement and further genotype-phenotype studies are needed to strengthen clinical utility of the ACE I/D polymorphisms. **Protocol registration** Registered in PROSPERO (CRD42024577680). **Funding** Fogarty International Center of the National Institutes of Health (D43TWO11632).	Abstract section; 1, 2
INTRODUCTION			
Rationale	3	• Despite substantial research on diabetic nephropathy (DN), critical gaps remain in our understanding of the interplay between angiotensin-converting enzyme (ACE) insertion/deletion (I/D) genotypes and DN progression in type 2 diabetes mellitus (T2DM). • While numerous studies have reported associations between ACE I/D polymorphisms and DN risk, the findings are inconsistent owing to variations in study design, population, and methods of microalbuminuria (UACR) measurement (Ezzidi et al., 2009; Jayapalan et al., 2010; Rahimi, 2012; Yu et al., 2012). • The heterogeneity of these results has hindered the development of standardized clinical guidelines for the genetic risk assessment of DN.	Introduction; 5
Objectives	4	• This systematic review and meta-analysis evaluated the association between Angiotensin Converting Enzyme (ACE) genotypes and Diabetic Nephropathy (DN) defined by Urine Albumin-to-Creatinine Ratio (UACR) in adults with Type 2 Diabetes Mellitus (T2DM). • Secondary outcomes included DN-related mortality and comorbidities.	Introduction; 5
METHODS			
Eligibility criteria	5	• Studies were eligible for inclusion if they enrolled male and female participants aged 18 years or older with a confirmed diagnosis of type 2 diabetes mellitus (T2DM), and investigated angiotensin-converting enzyme (ACE) insertion/deletion (I/D) genotypes (II, ID, DD) as the primary exposure. • Diabetic nephropathy (DN) was the primary outcome, commonly defined using microalbuminuria markers such as the albumin excretion rate (AER), urine albumin-to-creatinine ratio (UACR), or albumin-to-creatinine ratio (ACR). • Diagnostic thresholds for microalbuminuria were standardized across studies at 30–300 mg/day for AER and 30–300 mg/g for UACR/ACR. One study that defined DN using traditional renal function tests, including estimated glomerular filtration rate (eGFR) and serum creatinine, was also included to capture broader clinical definitions of nephropathy. This deviation from the protocol was noted but deemed valuable for completeness.	Eligibility criteria; 6

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The comparator across studies was typically T2DM patients with normal UACR levels, enabling evaluation of the association between ACE genotypes and DN risk.
Secondary outcomes included nephropathy-related mortality and comorbidities such as cardiovascular disease, hypertension, neuropathy, and retinopathy.
Eligible studies employed observational designs (cohort, case-control, or cross-sectional) and were published between January 1990 and February 2025. Articles with missing data on key study variables or those that did not explore the ACE I/D-DN relationship were excluded.
Non-English articles whose abstracts were available in English were included in the initial screening; however, those whose full texts were not available for retrieval and were eventually excluded.
Abstracts, unpublished studies, and grey literature were not included in this review, and no direct contact was made with study authors for clarification or additional data.
Discrepancies were resolved through discussion, ensuring consistency and minimizing bias, although formal blinding and interrater reliability statistics were not applied.

</td><td></td></tr>
<tr><td>Information sources</td><td>6</td><td>

We conducted a comprehensive search on February 28, 2025 across multiple electronic databases (PubMed, Web of Science, and EMBASE) to identify studies relevant to the research objectives.
Additional studies were retrieved from Google Scholar and reference lists of the included articles to ensure broad coverage.

</td><td>Information sources; 7</td></tr>
<tr><td>Search strategy</td><td>7</td><td>
<table>
<tr><th>Database</th><th>Search Date</th><th>Search String</th><th>#Hits</th></tr>
<tr><td>PubMed</td><td>28th February, 2025</td><td>(("Type 2 Diabetes Mellitus" OR "T2DM" OR "Diabetes Mellitus, Type 2"[tiab]) AND ("Diabetic Nephropathies" OR "Diabetic Nephropathy" OR "DN" OR "Nephropathy" OR "Kidney Disease") AND ("Angiotensin-Converting Enzyme" OR "ACE" OR "ACE I/D" OR "ACE Insertion/Deletion Polymorphism" OR "ACE Genotypes") AND ("Urine Albumin-to-Creatinine Ratio" OR "UACR" OR "Microalbuminuria" OR "Albuminuria") AND ("Mortality" OR "Comorbidities" OR "Complications") AND ("Genetic Markers" OR "Polymorphism, Genetic" OR "Biomarkers"))</td><td>48</td></tr>
<tr><td>EMBASE</td><td>28th February, 2025</td><td>(("Type 2 Diabetes Mellitus" or T2DM or "Diabetes Mellitus Type 2") and ("Diabetic Nephropathies" or "Diabetic Nephropathy" or DN or Nephropathy or "Kidney Disease") and ("Angiotensin-Converting Enzyme" or ACE or "ACE I/D" or "ACE Insertion/Deletion Polymorphism" or "ACE Genotypes") and ("Urine Albumin-to-Creatinine Ratio" or UACR or Microalbuminuria or Albuminuria) and (Mortality or Comorbidities or Complications) and ("Genetic Markers" or Polymorphism or Genetic or Biomarkers)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]</td><td>15</td></tr>
<tr><td>Web of Science</td><td>28th February, 2025</td><td>TS=(("Type 2 Diabetes Mellitus" OR T2DM OR "Diabetes Mellitus Type 2") AND
("Diabetic Nephropathy" OR "Diabetic Nephropathies" OR DN OR Nephropathy OR "Kidney Disease") AND
("Angiotensin-Converting Enzyme" OR ACE OR "ACE I/D" OR "ACE Insertion/Deletion Polymorphism" OR "ACE Genotypes") AND
("Urine Albumin-to-Creatinine Ratio" OR UACR OR Microalbuminuria OR Albuminuria) AND
(Mortality OR Comorbidities OR "Hypertension" OR "Cardiovascular Disease") AND
("Genetic Markers" OR Polymorphism OR Genetic OR Biomarkers)
AND TS=(Cohort OR "Case-Control" OR "Cross-Sectional" OR "Observational Study" OR "Randomized Controlled Trial" OR RCT)
AND TS=(Adult OR "18 years and above")
NOT TS=("Type 1 Diabetes" OR "Gestational Diabetes" OR Prediabetes OR "Case Report" OR Review OR Editorial OR "Animal Study")</td><td>13</td></tr>
</table>
In addition to the above, we searched Google scholar (February 28, 2025) and reference lists of retrieved articles
</td><td>Search Strategy; 7</td></tr>
</table>

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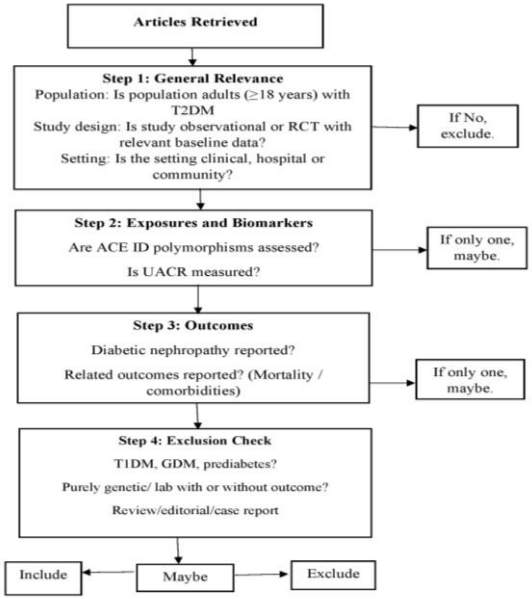
Selection process	8	 • Two reviewers, RK and JT, independently assessed the titles and abstracts of the studies according to the inclusion criteria using Rayyan software https://www.rayyan.ai/ • Full-text articles were retrieved from studies that met the eligibility requirements. Any disagreements regarding inclusion were resolved through a consensus between the two reviewers. • Full-text articles were retrieved from eligible studies, and the selection process is shown in Figure above.	Selection process; 8
Data collection process	9	• Data collection was performed independently by two authors, RK and JT, using a standardized data extraction form in Microsoft excel. • The extracted data included study characteristics (author, year, population, and design, age, sex and clinical characteristics such as glycated hemoglobin levels, blood pressure, T2DM duration, body mass index), exposure details (ACE I/D genotypes), comparators (UACR measurement methods), outcomes (DN, mortality, and comorbidities), and statistical measures such as the frequency counts were also retrieved and computed in to Odds ratios with confidence intervals. • Discrepancies were resolved through discussion with GNK and RA.	Data collection process; 8
Data items	10a	• The data extraction captured a broad range of information including details on study design such as case control, cross-sectional or cohort, sample size and distribution between males and females, age, genotyping methods such as PCR, UACR or other DN diagnostic approaches, and outcome reporting. • Genotype frequencies were entered in Excel, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using R software version 4.4.2. • Secondary outcomes such as comorbidities and mortality were recorded as binary variables (Yes/No) based on whether a particular study explicitly reported on them.	Data items; 8
	10b	• No missing data were encountered during the meta-analysis, as only studies with complete and extractable data were included in the quantitative synthesis. • Studies lacking sufficient data, as identified during visual inspection of the compiled Excel sheet, were excluded from meta-analysis and instead included in the narrative synthesis.	Data items; 9
Study risk of bias assessment	11	• The risk of bias in individual studies was assessed using the ROBINS-E tool, which evaluates bias across key domains: confounding, selection bias, classification of interventions, and outcome assessment. • Confounding was assessed as part of the ROBINS-E risk of bias evaluation, where considerations were made on whether studies appropriately controlled for potential confounders such as age, sex, duration of diabetes, and comorbidities. • Where studies reported such adjustments or stratifications, these were documented and factored into the overall bias assessment. • Studies were assigned an overall remark of either a low, moderate, or high risk of bias. The results of the bias assessment are available as Extended data. • The ROBINS-E risk of bias assessment was conducted independently by CNB and SPR..	Risk of bias assessment; 9

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Effect measures	12	• We included 25 studies in the meta-analysis to assess the association between ACE I/D genotypes and diabetic nephropathy risk. • For the primary outcome, diabetic nephropathy (DN) assessed using urine albumin to creatinine ratio or renal function tests, odds ratios (ORs) with 95% confidence intervals (CIs) were used as the effect measure to compare the risk of DN across ACE I/D genotypes (II vs. ID and II vs. DD). • This binary outcome metric was selected due to the categorical nature of the genotype data and the case or non-case definition of DN. odds ratios ORs were calculated using genotype frequency data. • Secondary outcomes such as comorbidities (hypertension, cardiovascular disease) and mortality were synthesized narratively, and their presence (Yes) or absence (No) was recorded per study.	Effect Measures; 9
Synthesis methods	13a	• Studies were deemed eligible for synthesis if they included adult patients with T2DM, assessed ACE I/D polymorphisms and reported DN outcomes using laboratory-based criteria. • The review included studies that assessed DN using albumin excretion rate (AER) or microalbuminuria, albumin-to-creatinine ratio (ACR), or renal function markers such as serum creatinine and estimated glomerular filtration rate (eGFR). • These criteria allowed for flexible yet clinically relevant definitions of nephropathy. • Study characteristics and outcome definitions were tabulated to determine synthesis eligibility. Studies without sufficient genotype data or outcome definitions were excluded.	Study Selection and Eligibility for Synthesis; 10
	13b	• Data preparation involved standardizing genotype frequency data across included studies. • Frequencies for ACE I/D genotypes were extracted and used to calculate odds ratios (ORs) with corresponding 95% confidence intervals (CIs). • For meta-analysis, all effect sizes were transformed into log odds ratios and back-transformed for interpretation. • Secondary outcomes such as comorbidities and mortality were recorded as binary variables (reported vs. not reported) in Excel spreadsheets.	Data Preparation and Conversion ; 10
	13c	• Forest plots were generated to visually present pooled ORs and 95% CIs. Individual study estimates and weights were shown. • Funnel plots and Egger's regression tests assessed publication bias. • All statistical visualizations and analyses were produced in R software. • Reporting bias was examined via funnel plots and Egger's test; no significant asymmetry was detected.	Presentation of Results; 11
	13d	• The primary effect measure used was the odds ratio (OR) for the association between ACE I/D genotypes and DN. • Random-effects models were used for all meta-analyses to account for between-study heterogeneity in population, study design, and UACR diagnostic methods. • Statistical significance was determined at $p < 0.05$. • Heterogeneity was assessed using the I^2 and τ^2 statistics. • All statistical estimates were generated using R software (version 4.4.2).	Statistical Models; 11
	13e	• Meta-regression was performed to explore heterogeneity sources, particularly differences in urinary albumin measurement methods such as (ACR vs. AER).	Statistical Models; 11
	13f	• To explore sources of heterogeneity, subgroup analyses were planned based on urinary albumin measurement methods (ACR vs. AER) of the included studies. • Meta-regression was used to assess the potential influence of these study-level characteristics on effect size estimates. • Sensitivity analyses were conducted using a leave-one-out approach, in which each study was sequentially removed from the meta-analysis to evaluate the robustness and stability of the pooled effect estimates.	Subgroup and Sensitivity Analyses; 12
Reporting bias assessment	14	**Selective Outcome Reporting and Qualitative Assessment** • Quality appraisal of the studies was conducted using the STREGA reporting guidelines. • Studies were rated as fair, good, very good, or excellent. • Excellent studies provided thorough methodological and genotyping details; very good ones had minor gaps; good studies had moderate reporting deficiencies; and fair studies lacked core STREGA elements. • KR and DT assessed adherence, with discrepancies resolved by ENO and BBA. **Funnel Plot Asymmetry and Egger's Test** • A funnel plot and Egger's regression test were used to assess publication bias. • This was done using R software.	Reporting bias assessment;12
Certainty assessment	15	• We appraised the certainty of evidence by triangulating risk of bias (via ROBINS-E), reporting on the quality of studies (via STREGA), consistency, precision of pooled estimates, and the direction of effects across studies.	Certainty Assessment; 12
RESULTS			

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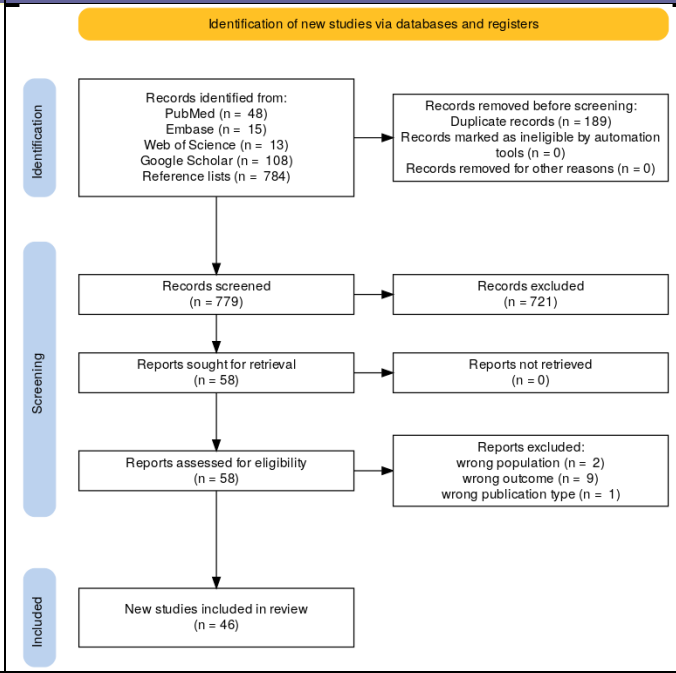
Study selection	16a	 • This PRISMA flow diagram outlines the process of identifying, screening, and including studies in the review. • A total of 968 records were identified through database searches and reference lists: PubMed (n = 48), EMBASE (n = 15), Web of Science (n = 13), Google Scholar (n = 108), and reference lists (n = 784). • After removing 189 duplicates, 779 records were screened. • Of these, 721 were excluded based on title and abstract screening, and 58 full-text reports were assessed for eligibility. • Eleven reports were excluded due to wrong population (n = 2), wrong outcome (n = 9), or wrong publication type (n = 1). • Ultimately, 46 studies were included in the final review.	Study Selection; 12 and 13
	16b	After full-text review, 12 studies that initially appeared to meet inclusion criteria were excluded for the following reasons: a) **Wrong publication type (n = 1):** • Panagiotopoulos et al., (Panagiotopoulos et al., 1995) was a narrative review rather than a primary research study. b) **Wrong outcome (n = 9):** These studies, including • Wong et al., (Wong TY et al., 2001), investigated ACE polymorphisms interest was with macroangiopathy and not type 2 diabetes mellitus associated nephropathy; • Ahluwalia et al., (Ahluwalia et al., 2009) and Pai et al., (Pai et al., 2024) assessed ACE polymorphisms not as insertion / deletion SNPs. • Fujisawa et al., (Fujisawa et al., 1995) presented ACE I/D polymorphisms data in relation to retinopathy and myocardial infarction but not diabetic nephropathy (DN). • Taha et al., (Taha et al., 2024) assessed ACE polymorphisms in type 2 diabetes mellitus patients but did not stratify them into nephropathy cases and controls. • Araz et al., (Araz et al., 2002) ACE ID, DD, II were not distributed across UACR/albuminuric groups. • Canani et al., (Canani et al., 2005) ACE DD and ID genotypes were not reported independently. • Wong et al., (Wong et al., 1999) distribution of ACE I/D across different DN categories determined by UACR/Microalbuminuria/AER/AEI was not done. • Draman et al., (Draman et al., 2008) microalbuminuria / UACR was not measured. c) **Wrong Population (n=2):** • Pontremoli et al., (Pontremoli et al., 1996) focused on essential Hypertension and Al-Harbi et al., (Al-Harbi et al., 2012) in the methods section of the full text did not specify the population as type 2 diabetes mellitus (T2DM) but unrelated adult patients. These studies were excluded to ensure consistency with our predefined PICO criteria and focus on clinically relevant DN outcomes in adults with T2DM.	Exclusion of ineligible studies; 13
Study characteristics	17	**Summary of Included Studies Examining ACE I/D Polymorphism in Relation to Diabetic Nephropathy and Comorbidities**	Examining ACE I/D Polymorphism in

Section and Topic	Item #	Checklist item						Location where item is reported
		First author, year	**Study design**	**Method of ACE I/D genotyping**	**Diabetic Nephropathy assessment**	**Results / conclusion**	**Studies Reported on ACE I/D polymorphism and mortality/ comorbidities in DN**	Relation to Diabetic Nephropathy and Comorbidities; 15
		Aggarwal et al., 2017	Cohort	Polymerase chain reaction RFLP	Albumin-creatinine ratio	ACE inhibitor therapy reduced ACR in 50% of DN patients, with response independent of ACE I/D and AGT M235T polymorphisms.	No	
		Araz et al., 2001	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	No link found between ACE I/D polymorphism and DN or DR in Turkish T2DM patients.	Yes	
		Arfa et al., 2008	Case-control	Polymerase chain reaction	Albumin Excretion rate	Preliminary findings show no association between ACE I/D polymorphism, T2DM, or DN in Tunisians.	No	
		Degirmenci et al., 2005	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	DD genotype linked to highest ACE activity; ID poses neuropathy risk, II protective; DD/ID may predict DR with longer diabetes duration.	Yes	
		Doi et al., 1996	Case-control	Polymerase chain reaction	Albumin-creatinine ratio	D allele linked to DN but not DR in Japanese NIDDM patients; no difference in genotype between controls and uncomplicated diabetics.	Yes	
		Ergen et al., 2004	Case-control	Polymerase chain reaction	Albumin Excretion rate	No link between ACE polymorphism and DN, but DD genotype more frequent in T2DM vs. controls.	No	
		Ezzidi et al	Case-control	Polymerase chain reaction	Albumin-creatinine ratio	Specific ACE variants and DN-related haplotypes linked to DN pathogenesis in Tunisian T2DM patients.	No	
		Fathi et al., 2015	Case-control	Polymerase chain reaction and RFLP	Albumin Excretion rate	ACE-D and VEGF-G alleles identified as independent risk factors for microalbuminuria in T2DM patients.	No	
		Felehgari et al., 2011	Case-control	Polymerase chain reaction	Albumin-creatinine ratio	Findings highlight ethnic influence on DN development and genotype-specific therapy responses in Kurdish T2DM patients.	No	
		Grzeszczszak et al., 1998	Case-control	Polymerase chain reaction	Albumin-creatinine ratio	No association found between ACE I/D, PstI polymorphisms and nephropathy in NIDDM.	No	
		Gupta et al., 2023	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	VEGF D allele linked to DR in DN patients, highlighting VEGF's role in retinopathy.	Yes	
		Ha et al., 2000	Case-control	Polymerase chain reaction	Albumin-creatinine ratio	ACE gene polymorphisms might have a role in determining ACE inhibitor responsiveness among proteinuric NIDDM patients.	No	
		Ha et al., 2003	Cohort	Polymerase chain reaction	Albumin-creatinine ratio	ACE DD genotype identified as a potential risk factor for DN progression in Korean T2DM patients.	No	

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		Hadjadj et al., 2003	Cohort	Polymerase chain reaction	Albumin Excretion rate	No association found between ACE I/D polymorphism and albuminuria in French T2DM patients.	No	
		Hsieh et al., 2000	Cohort	Polymerase chain reaction	Albumin Excretion rate	ACE DD genotype is markedly high in T2DM and it is significantly associated with DN	No	
		Huang et al., 1998	Cohort	Polymerase chain reaction	Albumin Excretion rate	9-year follow-up found no evidence that ACE I/D polymorphism is a major genetic marker for DN in NIDDM patients.	No	
		Ismail et al., 2017	Case-control	Polymerase chain reaction	Albumin-creatinine ratio	ACE DD, TCF7L2 T allele, and PPARGC1A A allele linked to DN risk; these polymorphisms may serve as susceptibility markers in T2DM.	No	
		Jayapalan et al., 2010	Case-control	Polymerase chain reaction with sequencing	Albumin-creatinine ratio	ACE I/D polymorphism not linked to T2DM or DN in Malaysian population, irrespective of ethnicity or gender.	No	
		Kiconco et al., 2024	Cross-sectional	Polymerase chain reaction	Microalbuminuria	ACE I/D affects metabolic markers, not nephropathy; recommends guideline inclusion.	No	
		Kimura et al., 1998	Cohort	Polymerase chain reaction with sequencing	Albumin Excretion rate	ACE DD and PAI-1 4G4G genotypes are independent risk factors for macroangiopathy, not DN progression, in NIDDM; combined genotyping may aid in predicting vascular events.	No	
		Kuramoto et al., 1999	Cross-sectional	Polymerase chain reaction	Albumin Excretion rate	ACE D allele linked to increased DN risk in NIDDM patients with insulin resistance.	No	
		Lee YJ and Tsai JC 2002	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	ACE I/D polymorphism linked to metabolic syndrome in Chinese T2DM, implicating RAS in metabolic dysfunction.	Yes	
		Luo et al., 2019	Case-control	Polymerase chain reaction with sequencing	Albumin Excretion rate	ACE gene at the rs4646994 locus may increase the risk of developing T2DN	No	
		Mahwish et al., 2020	Case-control	Polymerase chain reaction	Albumin Excretion rate	ACE DD genotype linked to higher DN risk, while ID genotype showed protective effect in dyslipidemic T2DM patients.	No	
		Mansoor et al., 2010	Case-control	Polymerase chain reaction	Albumin Excretion rate	ACE I allele linked to DN progression in Pakistani men, not women; neuropathy and family history also key factors.	No	
		Mansouri et al., 2017	Case-control	Polymerase chain reaction	Albumin-creatinine ratio	D allele most common in Moroccans; ACE I/D polymorphism not linked to DN risk.	No	
		Movva et al., 2007	Case-control	Polymerase chain reaction	Albumin Excretion rate	D allele linked to increased DN risk in Asian Indians with T2DM.	No	
		Nikzamir et al., 2009	Cross-sectional	Polymerase chain reaction	Albumin Excretion rate	In Iranian T2DM patients, D allele is linked to albuminuria progression, not its onset.	No	

Section and Topic	Item #	Checklist item						Location where item is reported
		Ohno et al.,1996	Cross-sectional	Polymerase chain reaction and RFLP	Albumin Excretion rate	ACE D allele linked to higher ACE activity and abnormal albuminuria; ACE I/D, not AGN M235T, is a DN risk factor in Japanese NIDDM.	No	
		Okuno et al., 2003	Cohort	Polymerase chain reaction	Albumin Excretion rate	ACE D allele identified as a strong independent risk factor for microalbuminuria in T2DM patients.	No	
		Oue et al., 1999	Cohort	Polymerase chain reaction	Albumin-creatinine ratio	ACE DD genotype, poor glycemic control, and older age identified as key risk factors for microalbuminuria progression in Japanese T2DM patients.	No	
		Parchwani et al., 2013	Case-control	Polymerase chain reaction	Albumin Excretion rate	ACE DD variant linked to higher DN risk, not severity, in T2DM.	No	
		Pawar et al., 2024	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	ACE DD and AGT Met235Thr genotypes significantly associated with increased risk of DN and DR in T2DM patients, highlighting RAAS gene variants as potential genetic markers.	No	
		Rahimi et al., 2012	Case-control	polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP)	Albumin-creatinine ratio	ACE D and eNOS T alleles individually showed no significant DN risk, but their combined presence was associated with a fivefold increased risk of macroalbuminuria, suggesting a synergistic effect.	No	
		Schmidt et al., 1995	Cross-sectional	Enhanced primers with DMSO	Albumin Excretion rate	No significant association found between ACE I/D polymorphism and nephropathy in either type 1 or type 2 diabetes, despite large sample size and long disease duration.	No	
		Shaihk et al., 2014	Cross-sectional / case-control study.	Polymerase chain reaction and ARMS	RFTs	ACE D allele and AGT T allele identified as genetic risk factors for diabetic nephropathy.	No	
		Solini et al., 1999	Observational	Polymerase chain reaction and RFLP	Albumin Excretion rate	No link found between ACE/AGN polymorphisms and albuminuria in T2DM families.	No	
		Tseng et al., 2010	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	ACE DD genotype, combined with hypertension, smoking, dyslipidemia, and obesity, significantly contributes to albuminuria in Taiwanese T2DM patients.	No	
		Uddin et al., 2007	Case-control	Polymerase chain reaction	Albumin Excretion rate	Percentage of DD genotype and D allele was significantly higher in DN compared to controls.	No	
		Viswanathan et al., 2001	Case-control	Polymerase chain reaction	Albumin-creatinine ratio	D allele linked to nephropathy; no ID/DD distribution difference.	No	
		Wang et al., 2016	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	ACE I/D polymorphism linked to DKD onset in T2DM; DD genotype showed greatest renoprotective response to valsartan.	No	

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Wyawahare et al., 2017	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	ID genotype most common; no link to albuminuria levels.	No
Yahya et al., 2024	Cross-sectional	Polymerase chain reaction	Albumin-creatinine ratio	DD genotype linked to 2× DN risk; AGT1R A1166C not associated with DN in Iraqi T2DM	No
Yahya et al., 2024	Cross-sectional	HRM-RT and PCR-RFLP.	Albumin-creatinine ratio	ID and AC genotypes linked to lower ACE1/2, lower ACR, better ACEI response.	No
Yoshida et al., 1996	Cohort	Polymerase chain reaction	Albumin Excretion rate	ACE DD genotype strongly predicts progression to ESRD in NIDDM patients with albuminuria and is linked to higher mortality after dialysis initiation.	Yes
Young et al., 1998	Case-control	Polymerase chain reaction	Albumin Excretion rate	High AGT TT genotype and T allele frequencies may underlie elevated albuminuria rates in Chinese T2DM; potential synergism with ACE D allele warrants further study.	No

This table provides a summary of included studies that investigated the association between angiotensin-converting enzyme (ACE) I/D polymorphism and diabetic nephropathy (DN) among patients with type 2 diabetes mellitus (T2DM). It includes details on study design, methods of ACE I/D genotyping, approaches used to assess DN (albumin-to-creatinine ratio, albumin excretion rate, or microalbuminuria), main findings or conclusions, and whether mortality or comorbid conditions (such as retinopathy, cardiovascular disease) related to ACE I/D polymorphism were reported. Most studies employed polymerase chain reaction (PCR) techniques for genotyping and utilized albumin-based measures to assess nephropathy. While several studies reported a significant association between the D allele or DD genotype and DN, comorbidity and mortality data were less frequently examined or reported.

Abbreviations: ACE, angiotensin-converting enzyme; ACEI, angiotensin-converting enzyme inhibitor; ACR, albumin-to-creatinine ratio; AGT, angiotensinogen; AGT1R, angiotensin II type 1 receptor; ALR2, aldose reductase 2; DD, homozygous deletion genotype; DN, diabetic nephropathy; DR, diabetic retinopathy; HRM-RT, high-resolution melt real-time; ID, heterozygous insertion/deletion genotype; I/D, insertion/deletion; NIDDM, non-insulin-dependent diabetes mellitus; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; T2DM, type 2 diabetes mellitus; VEGF, vascular endothelial growth factor.

Risk of bias in studies — 18

ROBINS-E assessment of included studies;

Author(s)	Year	Country	Confounding	Participants	Exposures	Deviations	Missing Data	Outcomes	Results	Overall
Viswanathan et al.	2001	India	Moderate	Low	Low	Low	Low	Low	Moderate	Low
Yahya et al	2024	Iraq	Moderate	Low	Low	Low	Low	Low	Moderate	Low
Kiconco et al.	2024	Uganda	High	Low	Low	Low	Moderate	Low	Moderate	High
Luo et al	2019	China	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate
Uddin et al	2007	Bangladesh	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate
Yahya et al	2024	Iraq	Moderate	Moderate	Low	Low	Moderate	Low	Low	Moderate
Parchwani et al	2013	India	Low	Low	Low	Low	Low	Low	Moderate	Low
Gupta et al	2023	India	Moderate	Low	Low	Low	Low	Low	Moderate	Low
Grzeszczak et al	1998	Poland	High	Low	Low	Low	Moderate	Low	Moderate	High
Araz et al	2001	Turkey	Low	Low	Low	Low	Low	Low	Moderate	Low
Arfa et al	2008	Tunisia	Low	Low	Low	Low	Low	Low	Moderate	Low
Mansouri et al	2017	Morocco	Low	Low	Low	Low	Low	Low	Moderate	Low
Mansoor et al	2010	Pakistan	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate
Lee YJ and Tsai JC	2002	China	Low	Low	Low	Low	Low	Low	Moderate	Low
Degirmenci et al	2005	Turkey	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate
Doi et al	1996	Japan	Moderate	Low	Low	Low	Low	Low	Low	Low
Ismail et al	2017	Egypt	Low	Low	Low	Low	Moderate	Low	Moderate	Moderate
Ergen et al	2004	Turkey	Low	Low	Low	Low	Moderate	Low	Moderate	Moderate
Ezzidi et al	2009	Tunisia	Moderate	Low	Low	Low	Low	Low	Moderate	Low

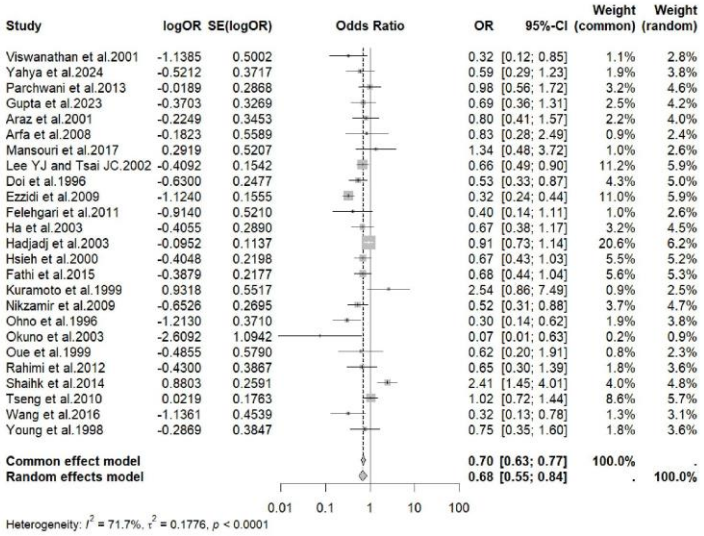
Mentioned as extended data; 2,9,12.18, 22,24,28

Section and Topic	Item #	Checklist item										Location where item is reported
		Felehgari et al	2011	Iran	Moderate	Low	Low	Low	Low	Low	Moderate	Low	
		Ha et al	2000	Korea	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate	
		Ha et al	2003	Korea	Moderate	Low	Low	Low	Low	Low	Moderate	Low	
		Hadjadj et al	2003	France	Low	Low	Low	Low	Low	Low	Moderate	Low	
		Hsieh et al	2000	Taiwan	Moderate	Low	Low	Low	Low	Low	Moderate	Low	
		Huang et al	1998	Finland	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate	
		Jayapalan et al	2010	Malaysia	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate	
		Mahwish et al	2020	India	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate	
		Fathi et al	2015	Iran	Low	Low	Low	Low	Low	Low	Low	Low	
		Kuramoto et al	1999	Japan	Moderate	Low	Low	Low	Low	Low	Moderate	Low	
		Movva et al	2007	India	High	Low	Low	Low	Moderate	Low	Moderate	High	
		Nikzamir	2009	Iran	Moderate	Low	Low	Low	Low	Low	Moderate	Low	
		Ohno et al	1996	Japan	Moderate	Low	Low	Low	Low	Low	Low	Low	
		Okuno et al	2003	Japan	Low	Low	Low	Low	Low	Low	Low	Low	
		Oue et al	1999	Japan	Low	Low	Low	Low	Low	Low	Low	Low	
		Kimura et al	1998	Japan	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate	
		Rahimi et al	2012	Iran	Low	Low	Low	Low	Low	Low	Moderate	Low	
		Pawar et al	2024	India	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate	
		Shaihk et al	2014	Pakistan	Low	Low	Low	Low	Moderate	Low	Low	Low	
		Solini et al	1999	Italy	Moderate	Low	Low	Low	Moderate	Low	High	High	
		Tseng et al	2010	Taiwan	Low	Low	Low	Low	Moderate	Low	Moderate	Low	
		Wang et al	2016	China	Moderate	Low	Low	Low	Low	Low	Low	Low	
		Aggarwal et al	2017	India	Moderate	Low	Low	Low	Moderate	Low	High	High	
		Yoshida et al	1996	Japan	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate	
		Young et al	1998	China	Low	Low	Low	Low	Low	Low	Low	Low	
		Wyawahare et al	2017	India	Moderate	Low	Low	Low	Moderate	Moderate	Moderate	Moderate	
		Schmidt et al	1995	Germany	Moderate	Low	Low	Low	High	Low	Low	High	

- The risk of bias in individual studies was assessed using the ROBINS-E tool (Higgins et al., 2024), which evaluates bias across key domains: confounding, selection bias, classification of interventions, and outcome assessment.
- Confounding was assessed as part of the ROBINS-E risk of bias evaluation, where considerations were made on whether studies appropriately controlled for potential confounders such as age, sex, duration of diabetes, and comorbidities.
- Where studies reported such adjustments or stratifications, these were documented and factored into the overall bias assessment. Studies were assigned an overall remark of either a low, moderate, or high risk of bias.
- The ROBINS-E risk of bias assessment was conducted independently by CNB and SPR.

Results of individual studies	19	We followed the Centre for Reviews and Dissemination (CRD) approach (Booth et al., 2010) and the Synthesis Without Meta-analysis (SWiM) guidelines (Campbell et al., 2020) due to their detailed and structured framework. • Forty-six (46) observational studies investigating the association between ACE I/D polymorphisms and DN in patients with T2DM. • The total sample size across all studies was 16,322 participants, with individual study populations ranging from 50 to 3744 participants. • The combined mean of mean age in the studies was 58.11 (±4.30). • The combined median of mean numbers of males was 90.5 (IQR: 62.75 - 171.5) and that of females was 105.5 (IQR: 57.0 - 183.75). • Most studies were conducted in Asia (29/46), Europe (8/46), Middle East (6/46) and Africa (5/46) reflecting a diverse geographical representation. • Studies were grouped based on outcome assessment methods (ACR vs. AER), study design (case-control, cross-sectional, or cohort), and genotype comparisons (II vs. DD, II vs. ID), to account for methodological and clinical heterogeneity and to enable meaningful synthesis of effect estimates across similar contexts Most studies assessed the primary outcome of DN using either ACR, AER or RFTs • ACR include: (Aggarwal et al., 2017; Araz et al., 2001; Degirmenci et al., 2005; Doi et al., 1996; Ergen et al., 2004; Ezzidi et al., 2009; Felehgari et al., 2011; Grzeszczak et al., 1998; Gupta P. et al., 2023; Ha et al., 2000, 2003; Ismail et al., 2017; Jayapalan et al., 2010; Lee and Tsai, 2002a; Mansouri et al., 2017; Oue et al., 1999; Pawar et al., 2024; Rahimi et al., 2012; Tseng et al., 2010; Viswanathan V. et al., 2001; Wang et

Section and Topic	Item #	Checklist item	Location where item is reported
		al., 2016; Wyawahare et al., 2017; Yahya A.A. et al., 2024; Yahya et al., 2024) • AER include: (Arfa et al., 2008; Fathi et al., 2015; Hadjadj et al., 2003; Hsieh et al., 2000; Huang et al., 1998; Kiconco et al., 2024; Kimura et al., 1998; Kuramoto et al., 1999; Luo et al., 2016; Mahwish et al., 2020; Mansoor et al., 2019; Movva et al., 2007; Nikzamir et al., 2009; Ohno et al., 1996; Okuno et al., 2003; Parchwani et al., 2015; Schmidt et al., 1995; Solini et al., 1999; Uddin et al., 2007; Yoshida et al., 1996; Young et al., 1998), • RFTs (renal function tests) include; (Shaikh et al., 2014) (serum creatinine, eGFR, and urea levels). • Genotyping for ACE I/D polymorphism was primarily conducted using polymerase chain reaction (PCR) methods, including conventional PCR, PCR-RFLP, high-resolution melting, and sequencing approaches.	
Results of syntheses	20a	• For summary of all included studies characteristics, refer to item 17 and for the risk of bias assessment, refer to item 18 of this PRISMA 2020 checklist. • From the 46 studies, only those with a low overall score on the ROBINS E assessment were included in the quantitative synthesis.	Quantitative Synthesis; 15
	20b	We conducted a meta-analysis, meta-regression, subgroup analysis, sensitivity analysis, and publication bias assessment on 25 studies to evaluate the association between ACE I/D polymorphisms and diabetic nephropathy (DN) in the study population.	Meta-analysis; 15,16 Meta-regression; 18 Subgroup analyses: 18



Meta-analysis

- Quantitative synthesis of results was conducted in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (Brooke et al., 2021), and the corresponding checklist was completed to ensure comprehensive and transparent reporting.
- The included studies were deemed appropriate for assessing the hypothesis as they reported on ACE I/D genotypes and diabetic nephropathy outcomes in adult T2DM populations.
- Data were selected and coded based on sound clinical principles, prioritizing studies that clearly defined diabetic nephropathy using established albuminuria or renal function markers and reported ACE I/D genotype distributions relevant to the study objectives. Data classification and coding were performed independently by two reviewers using a standardized extraction template.
- Most included studies used standardized measures of albuminuria to define nephropathy, ensuring relevance to the primary outcome and alignment with the review objective. Data classification and coding were performed independently by two reviewers (RK and JT) using a standardized extraction template.
- Discrepancies were resolved through discussion with another reviewer; EAO, ensuring consistency and minimizing bias, although formal blinding and interrater reliability statistics were not applied.
- Random-effects models using the DerSimonian–Laird method(Jackson et al., 2010) were used to estimate the pooled odds ratios (ORs) and 95% confidence intervals (CIs) across genotype comparisons to account for expected heterogeneity in study populations and methodologies across the included observational studies.
- ACE genotype II was associated with a lower risk of developing DN.
- The random-effects models showed a significant protective effect of the homozygous insertion (II) genotype, with pooled odds ratios (ORs) of 0.70 (95% CI: 0.63–0.77) versus heterozygous insertion/deletion (ID) and 0.68 (95% CI: 0.55–0.84) versus homozygous deletion (DD).
- Heterogeneity across studies was moderate to substantial (I^2 = 71.7%, τ^2 = 0.1776, $p < 0.0001$), suggesting variability in the effect estimates, possibly due to methodological or population differences.
- A forest plot summarizing the pooled estimates and heterogeneity is shown in Figure above.

<table>
<tr><th>Section and Topic</th><th>Item #</th><th>Checklist item</th><th>Location where item is reported</th></tr>
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Meta-regression

Meta-regression was performed to explore the sources of heterogeneity in the association between ACE I/D genotypes and diabetic nephropathy (DN) risk.
In the crude model (k = 25; DerSimonian–Laird estimator), the residual heterogeneity was $\tau^2 = 0.1100$ (SE = 0.0917; $\tau = 0.3317$), with $I^2 = 52.3\%$ ($p = 0.0141$) and $R^2 = 38.06\%$.
The test for residual heterogeneity was significant (QE(12) = 25.16, p = 0.0141), while the test for moderators was not (QM(12) = 16.56, p = 0.17), indicating limited explanatory power overall. However, the form of UACR measurement was a significant individual predictor ($\beta = 0.57$, SE = 0.19, p = 0.0033; 95% CI: 0.19 - 0.95), suggesting that variations in measurement methods contributed meaningfully to heterogeneity in DN risk estimates.
In the adjusted model, which included additional moderators, the residual heterogeneity increased ($\tau^2 = 0.1253$, SE = 0.0692; $\tau = 0.3540$), with $I^2 = 63.0\%$ ($p < 0.0001$) and $R^2 = 29.45\%$.
The overall test for residual heterogeneity remained significant (QE(23) = 62.21, $p < 0.0001$).
The test of moderators was significant (QM(1) = 5.79, p = 0.016), confirming that the form of UACR measurement remained a key source of heterogeneity ($\beta = 0.40$, SE = 0.17, p = 0.016; 95% CI: 0.07 - 0.72).

Subgroup Analysis

Subgroup analyses were based on the methods used to assess the primary outcome which is DN and these included; ACR, AER and RFTs. They revealed differences in the strength of associations between ACE I/D genotypes and diabetic nephropathy (DN) risk.
Studies using albumin-to-creatinine ratio (ACR) showed the strongest protective effect of the II genotype, with pooled odds ratios (ORs) of 0.59 (95% CI: 0.47–0.75) under the random-effects model and 0.59 (95% CI: 0.51–0.67) under the common-effect model.
For studies using the albumin excretion rate (AER), the association was weaker (random-effects OR = 0.71, 95% CI: 0.55–0.92; common-effect OR = 0.77, 95% CI: 0.66–0.90).
The weakest association was observed in the study using renal function tests (RFTs), with a random-effects OR of 0.68 (95% CI: 0.55–0.84) and a common-effect OR of 0.70 (95% CI: 0.63–0.77).
Heterogeneity varied across subgroups: $I^2 = 60.3\%$ ($p = 0.0019$) for ACR and $I^2 = 58.3\%$ ($p = 0.0102$) for AER; no heterogeneity was detected in the RFT-based subgroup.
These results suggested that methods used to assess DN may influence the estimated association between ACE I/D polymorphisms with DN risk.

</td><td></td></tr>
<tr><td></td><td>20c</td><td>
Heterogeneity among study results was assessed.

The funnel plot and Egger's regression test were used to assess the publication bias.
The plot showed a relatively symmetrical distribution around the pooled effect size, with no substantial asymmetry.
Egger's test (t = -0.61, df = 23, p = 0.55; bias = -0.50, SE = 0.82) indicated no significant small study effects.
The residual heterogeneity variance was $\tau^2 = 3.62$, using standard error as the predictor and inverse-variance weights.
These results suggest that the pooled estimates are unlikely to be affected by publication bias.

</td><td>Publication bias; 18</td></tr>
<tr><td></td><td>20d</td><td>
Sensitivity analyses were conducted to assess the robustness of the synthesized results.

Leave-one-out sensitivity analysis was conducted to assess the robustness of the findings and the influence of individual studies on the pooled effect estimates. One study was excluded at a time, and the overall odds ratio (OR) and 95% confidence interval (CI) were calculated.
The pooled log ORs ranged from -0.34 to -0.45, with minimal variation across iterations.
The confidence intervals remained narrow and did not cross the null, suggesting consistent associations.
The exclusion of Shaihk et al.(Shaikh et al., 2014) produced the most marked change (log OR = -0.4474; 95% CI: -0.6255 to -0.2692), followed by Kuramoto et al.(Kuramoto et al., 1999) and Tseng et al.(Tseng et al., 2010), though none altered the overall interpretation.
These results indicated that no single study had a disproportionate impact on the association between ACE I/D genotypes and diabetic nephropathy in the meta-analysis.
The full results are shown in the Table below.

Sensitivity Analysis of Individual Studies Included in the Meta-Analysis of ACE I/D Genotypes and Diabetic Nephropathy
<table>
<tr><th>Study</th><th>OR</th><th>95% CI lower</th><th>95% CI upper</th></tr>
<tr><td>Araz et al.2001</td><td>-0.3966089</td><td>-0.6166201</td><td>-0.1765978</td></tr>
<tr><td>Arfa et al.2008</td><td>-0.3945578</td><td>-0.6110598</td><td>-0.1780557</td></tr>
<tr><td>Doi et al.1996</td><td>-0.3771501</td><td>-0.5979375</td><td>-0.1563626</td></tr>
<tr><td>Ezzidi et al.2009</td><td>-0.3353554</td><td>-0.5341380</td><td>-0.1365728</td></tr>
<tr><td>Fathi et al.2015</td><td>-0.3901966</td><td>-0.6134694</td><td>-0.1669238</td></tr>
<tr><td>Felehgari et al.2011</td><td>-0.3749063</td><td>-0.5898228</td><td>-0.1599899</td></tr>
</table>
</td><td>Sensitivity analyses; 18</td></tr>
</table>

Section and Topic	Item #	Checklist item	Location where item is reported
		Gupta et al.2023 -0.3906916 -0.6114558 -0.1699273 Ha et al.2003 -0.3891762 -0.6107695 -0.1675828 Hadjadj et al.2003 -0.4090621 -0.6311435 -0.1869808 Hsieh et al.2000 -0.3892575 -0.6124589 -0.1660562 Kuramoto et al.1999 -0.4196006 -0.6267025 -0.2124986 Lee YJ and Tsai JC.2002 -0.3890242 -0.6136330 -0.1644155 Mansouri et al.2017 -0.4072607 -0.6214458 -0.1930757 Nikzamir et al.2009 -0.3765290 -0.5966524 -0.1564056 Ohno et al.1996 -0.3558761 -0.5648249 -0.1469272 Okuno et al.2003 -0.3697799 -0.5791523 -0.1604074 Oue et al.1999 -0.3871209 -0.6034409 -0.1708009 Parchwani et al.2013 -0.4071222 -0.6259972 -0.1882471 Rahimi et al.2012 -0.3881874 -0.6076244 -0.1687504 Shaihk et al.2014 -0.4473571 -0.6255171 -0.2691971 Tseng et al.2010 -0.4139499 -0.6325336 -0.1953663 Viswanathan et al.2001 -0.3675264 -0.5801649 -0.1548878 Wang et al.2016 -0.3649410 -0.5771711 -0.1527110 Yahya et al.2024 -0.3845316 -0.6039635 -0.1650997 Young et al.1998 -0.3936058 -0.6130589 -0.1741526 • This table summarizes the results of sensitivity analyses for individual studies included in the meta-analysis. For each study, the log-transformed odds ratio (OR) and its corresponding 95% confidence interval (CI) bounds are presented. The sensitivity analysis evaluates the stability and influence of each study on the overall pooled estimate by recalculating the log ORs after removing one study at a time. **Abbreviations:** ACE – Angiotensin-Converting Enzyme; I/D – Insertion/Deletion; DN – Diabetic Nephropathy; OR – Odds Ratio; CI – Confidence Interval.	
Reporting biases	21	• Assessment of reporting bias was informed by the "Selection of Reported Result" domain in ROBINS-E and several reporting items in STREGA. The full results of STREGA appraisal are provided in the Extended data • In ROBINS-E, most studies were rated as having a moderate risk for selective reporting, indicating that not all measured outcomes may have been reported. • In the STREGA assessment, most studies did not report sample size justification or apply multiple testing correction, and Hardy-Weinberg Equilibrium (HWE) was tested in only a few. • These findings suggest a moderate risk of bias due to missing or selectively reported results across the included studies.	Reporting bias assessment; 18
Certainty of evidence	22	• Certainty in the body of evidence was evaluated using the ROBINS-E tool for risk of bias and STREGA for reporting quality. Out of 46 studies, 25 were rated as having low risk of bias, 15 as moderate, and 6 as high according to ROBINS-E. • STREGA compliance ratings showed that 19 studies were rated as very good, 13 as fair, 9 as good, and 5 as excellent. • Based on this distribution, the overall confidence in the evidence is moderate, reflecting generally sound methodology and reporting, though limitations in transparency and bias remain in a subset of studies. • Heterogeneity was assessed using I^2 and τ^2 statistics and explored further through subgroup and meta-regression analyses. • Publication bias was evaluated through funnel plots and Egger's regression test. • Together, these assessments provide a moderate to high level of confidence in the observed association between ACE I/D genotypes and diabetic nephropathy, particularly the protective effect of the II genotype. However, the certainty of evidence for secondary outcomes, such as mortality and comorbidities, remains low due to limited data.	Certainty of evidence; 19
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence. • We evaluated the association between ACE insertion/deletion (I/D) polymorphisms and DN in T2DM patients, with a focus on differences in UACR assessments, study populations, and clinical outcomes such as hypertension, cardiovascular disease, stroke and mortality from DN. • Narrative synthesis demonstrated a generally consistent association between ACE I/D variants and DN risk, although the effect sizes varied across studies. The extent to which ACE I/D genotypes influence DN outcomes likely varies depending on several intersecting factors. Population characteristics, such as geographical location, age, sex, and prevalence of hypertension or other metabolic comorbidities, which modify the genetic effect of the ACE I/D polymorphism. For example, the distribution of I/D alleles and their association with DN may differ among Asian, African, and European populations due to underlying genetic diversity and environmental exposure.	Discussion ; 20

Section and Topic	Item #	Checklist item	Location where item is reported
		• The meta-analysis revealed that individuals with the ACE II genotype had significantly lower odds of developing diabetic nephropathy compared to those with ID or DD genotypes, highlighting a protective genetic association as the primary outcome of this systematic review. Previous studies that encompassed ethnicity on this topic found that there was a reduced risk of diabetic nephropathy associated with genotype II among Europeans (Caucasians) with type 2 diabetes, although this observation was more applicable to Asians (Chinese, Japanese, Koreans)(Ng et al., 2005; Zeng et al., 2022). Moreover, the meta-analysis confirmed a statistically significant protective association of the II genotype compared to both ID and DD genotypes, consistent with prior research implicating the renin–angiotensin–aldosterone system (RAAS) in DN pathogenesis (Degirmenci et al., 2005; Ng et al., 2005; Zeng et al., 2022). These findings reinforce the hypothesis that genetic variation at the ACE locus contributes to an individual's susceptibility to DN. However, the modest magnitude of the effect suggests that this polymorphism is likely one of multiple genetic contributors within a polygenic framework, as noted from the heterogeneity in the method used to assess diabetic nephropathy in primary studies. • We observed that the identification of DN cases varied considerably across studies. Most studies assessed DN using either ACR or AER, while only one study relied on traditional renal function tests, such as serum creatinine, estimated glomerular filtration rate (eGFR), or urea levels. This inconsistency in diagnostic criteria significantly affects the comparability and generalizability of reported genotype-phenotype associations. Moreover, in our meta-regression analysis, the UACR measurement method emerged as a critical moderator of heterogeneity, underscoring the methodological influence on the pooled effect sizes. • Although these biochemical markers are practical for large-scale research and clinical use, they are surrogates rather than definitive diagnostic tools. Notably, kidney histology remains the gold standard for diagnosing DN, offering detailed insight into glomerular and tubular changes(Schnuelle, 2023). However, renal biopsy is rarely performed due to its invasive nature, associated risks, and ethical limitations in asymptomatic populations(Peces et al., 2011). • Subgroup analyses revealed stronger genotype-phenotype associations in studies using urinary albumin-to-creatinine ratio (UACR) compared to albumin excretion rate (AER) or serum-based renal function tests. These findings align with prior recommendations advocating standardized albuminuria assessment in DN research to improve reproducibility and comparability across studies (Mooyaart et al., 2011; Persson and Rossing, 2018). Methodological inconsistency in DN diagnosis remains a major source of heterogeneity in the genetic literature (Palmer and Freedman, 2012). These methodological differences may obscure or exaggerate the true effects of ACE I/D polymorphism (Crisan and Carr, 2000; Gómez-Montañez et al., 2024). • Sensitivity analyses confirmed the robustness of the pooled estimates, with no individual study unduly influencing the overall results. While studies by Shaikh et al.(Shaikh et al., 2014), Kuramoto et al.(Kuramoto et al., 1999), and Tseng et al.(Tseng et al., 2010) showed relatively greater influence when excluded, the overall direction and significance of the associations remained unchanged. This stability across leave-one-out analyses supports the internal validity of the meta-analysis. Assessment of publication bias using funnel plot symmetry and Egger's regression test indicated no evidence of small-study effects(Egger et al., 1997). The non-significant test and symmetrical funnel distribution suggest that selective reporting did not materially influence the meta-analysis findings.	
	23b	Limitations of the evidence included in the review. • Despite the breadth of studies evaluating the genetic associations with DN, a major gap exists in the reporting of clinical outcomes. • From the narrative synthesis of the included studies in this review, only one study addressed DN-related mortality(Yoshida et al., 1996), and five reported comorbidities such as metabolic disease(Lee and Tsai, 2002b), neuropathy (Degirmenci et al., 2005) and retinopathy(Araz et al., 2001; Doi et al., 1996; Gupta P. et al., 2023). Most studies have been conducted in broader T2DM populations without disaggregating the outcomes for DN-specific cohorts. • Consequently, the translational relevance of ACE I/D polymorphisms to clinical endpoints in DN remains poorly defined. Future studies should prioritize DN-specific outcomes and adopt standardized reporting frameworks to improve clinical interpretability.	Discussion ; 21
	23c	Limitations of the review processes used. • One limitation in this study is the presence of moderate between-study heterogeneity. This heterogeneity persisted even after subgroup and meta-regression analyses were conducted. A likely explanation for this is residual confounding. This may be due to variations in ethnicity, environmental exposure, and gene–environment interactions. Unfortunately, these variables were not captured in the data-extraction process. For instance, although the review predominantly included studies defining diabetic nephropathy based on urinary albuminuria markers (ACR or AER), one study using serum creatinine and eGFR was retained to incorporate relevant genetic data. This slight deviation was carefully considered and does not substantially compromise the consistency or validity of the overall findings, as the included outcome remains clinically relevant to nephropathy assessment. • In addition, the primary diagnostic marker for DN in our systematic review was urinary albumin-to-creatinine ratio (UACR), as reported in the original studies. However, we acknowledge that there are differences between the quantitative and semi-quantitative methods for measuring urinary albumin levels. To address this, we used the ROBINS-E tool to assess the risk of bias related to outcome measurements. Studies that lacked clarity or likely used semi-quantitative methods were rated as moderate-to-high risk, and only those with low overall risk (25 studies) were	Discussion ; 22

Section and Topic	Item #	Checklist item	Location where item is reported
		included in the meta-analysis. This helped ensure a more accurate DN classification in our pooled estimates. Moreover, in our subgroup/meta-regression analyses, the measurement; ACR vs. AER was statistically significant, reinforcing the impact of methodology on DN diagnosis. • This review did not include unpublished studies or abstracts, and no author contact was made for missing data, which may have limited access to additional insights; however, efforts were made to ensure comprehensive coverage through rigorous database and reference list searches.	
	23d	Recommendations • We suggest that future studies prioritize standardized methods for assessing microalbuminuria, particularly the use of ACR over AER, to reduce heterogeneity in outcome reporting. • There is also a clear need for well-designed, large-scale genetic association studies that specifically examine ACE I/D polymorphisms in relation to DN-related mortality and comorbidities, such as hypertension and cardiovascular disease, which are underrepresented in the current literature. • Additionally, future research should explore gene-environment and gene-gene interactions, as well as the utility of ACE genotyping in clinical risk prediction models for DN progression within diverse populations, especially in regions such as sub-Saharan Africa, where such data are scarce.	Discussion; 23
OTHER INFORMATION			
Registration and protocol	24a	The protocol was registered with PROSPERO("PROSPERO," 2025) under registration number CRD42024577680.	Registration and Protocol; 5
	24b	The protocol is published as a Preprint in Open science Framework (OSF) preprints(Kiconco et al., 2025).	Registration and Protocol; 5
	24c	One study that defined DN using traditional renal function tests, including estimated glomerular filtration rate (eGFR) and serum creatinine, was also included to capture broader clinical definitions of nephropathy. This deviation from the protocol was noted but deemed valuable for completeness.	Eligibility criteria; 6
Support	25	The research reported in this publication was supported by the Fogarty International Center of the National Institutes of Health under Award Number D43TWO11632. The content is the sole responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.	Grant information; 25
Competing interests	26	No competing interests were disclosed.	Conflict of interest; 25
Availability of data, code and other materials	27	The materials used in this review are available in Zenodo. The project title is "ACE I/D gene mutations in diabetic nephropathy". The project contains Microsoft excel files of two raw datasets used in this review, ROBINS-E and STREGA assessments. It also contains PDF files of MOOSE, SWiM, and 2020 PRISMA completed checklists. Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0). **REFERENCES** Aggarwal, N., Kare, P.K., Varshney, P., Kalra, O.P., Madhu, S.V., Banerjee, B.D., Yadav, A., Raizada, A., Tripathi, A.K., 2017. Role of angiotensin converting enzyme and angiotensinogen gene polymorphisms in angiotensin converting enzyme inhibitor-mediated antiproteinuric action in type 2 diabetic nephropathy patients. World J Diabetes 8, 112–119. https://doi.org/10.4239/wjd.v8.i3.112 Ahluwalia, T.S., Ahuja, M., Rai, T.S., Kohli, H.S., Bhansali, A., Sud, K., Khullar, M., 2009. ACE Variants Interact with the RAS Pathway to Confer Risk and Protection against Type 2 Diabetic Nephropathy. DNA and Cell Biology 28, 141–150. https://doi.org/10.1089/dna.2008.0810 Al-Harbi, E.M., Farid, E.M., Gumaa, K.A., Singh, J., 2012. Genotypes and allele frequencies of angiotensin-converting enzyme (ACE) insertion/deletion polymorphism among Bahraini population with type 2 diabetes mellitus and related diseases. Mol Cell Biochem 362, 219–223. https://doi.org/10.1007/s11010-011-1146-1 Araz, M., Okan, V., Celen, Z., Aynacioglu, S., 2002. Angiotensin converting enzyme gene polymorphism and glomerular filtration rate changes in type 2 diabetic patients. Int J Clin Pract 56, 416–418. Araz, M., Yilmaz, N., Güngör, K., Okan, V., Kepekci, Y., Aynacioglu, A.S., 2001. Angiotensin-converting enzyme gene polymorphism and microvascular complications in Turkish type 2 diabetic patients. Diabetes Research and Clinical Practice 54, 95–104. https://doi.org/10.1016/S0168-8227(01)00257-1 Arfa, I., Abid, A., Nouira, S., Elloumi-Zghal, H., Malouche, D., Mannai, I., Zorgati, M.M., Ben Alaya, N., Rebai, A., Zouari, B., Ben Ammar, S., Ben Rayana, M.C., Hmida, S., Blousa-Chabchoub, S., Abdelhak, S., 2008. Lack of association between the angiotensin-converting enzyme gene (I/D) polymorphism and diabetic nephropathy in Tunisian type 2 diabetic patients. J Renin Angiotensin Aldosterone Syst 9, 32–36. https://doi.org/10.3317/jraas.2008.002	Data availability; 25

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