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Original article

The impact of angiotensin converting enzyme insertion/deletion gene polymorphism on diabetic kidney disease: A debatable issue

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ABSTRACT

Objective: The objective of this study was to evaluate the influence of ACE I/D gene polymorphisms on diabetic kidney disease (DKD) risk.
Methods: All eligible investigations were identified, the number of various genotype in the case and control group were reviewed. The pooled analysis was performed using Stata software.
Results: In overall subjects, 24,321 participants with 12,961 cases and 11,360 controls were included. the pooled analysis showed a significant link between D allele, DD or II genotype and DKD risk (D versus I: OR = 1.316, 95% CI: 1.213–1.427, *P* = 0.000; DD versus ID + II: OR = 1.414, 95% CI: 1.253–1.595, *P* = 0.000; II versus DD + ID: OR = 0.750, 95% CI: 0.647–0.869, *P* = 0.000). The subgroup pooled analysis showed that ACE I/D gene polymorphism was correlated with DKD both in Asian and in Chinese population. In addition, ACE I/D gene polymorphism was correlated with type 2 DKD (D versus I: OR = 1.361, 95% CI: 1.243–1.490, *P* = 0.000; DD versus ID + II: OR = 1.503, 95% CI: 1.310–1.726, *P* = 0.000; II versus DD + ID: OR = 0.738, 95% CI: 0.626 –0.870, *P* = 0.000). However, there was no obvious correlation in Caucasian subjects and type 1 diabetic patients.
Conclusion: ACE I/D polymorphisms were correlated with DKD in Asian and type 2 diabetic populations. ACE D allele/DD genotype might be a risk factor, while ACE II genotype might be a protective factor for DKD.

Abbreviations: SNPs, single nucleotide polymorphisms; HWE, Hardy–Weinberg equilibrium; OR, odds ratios; CIs, confidence intervals.

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El impacto del polimorfismo del gen de inserción/deleción de la enzima convertidora de la angiotensina en la enfermedad renal diabética: una cuestión discutible

Palabras clave:
AS
Polimorfismo del gen
Enfermedad diabética del riñón
Análisis combinado

RESUMEN

Objetivo: El objetivo de este estudio fue evaluar la influencia de los polimorfismos del gen I/D de la ECA en el riesgo de enfermedad renal diabética (ERD).
Métodos: Se identificaron todas las investigaciones elegibles, se revisó el número de varios genotipos en el grupo de casos y controles. El análisis combinado se realizó con el software Stata.
Resultados: En el conjunto de los sujetos, se incluyeron 24.321 participantes con 12.961 casos y 11.360 controles. El análisis combinado mostró una relación significativa entre el alelo D, el genotipo DD o II y el riesgo de DKD (D frente a I: OR = 1,316, IC del 95%: 1,213–1,427, P = 0,000; DD frente a ID + II: OR = 1,414, IC del 95%: 1,253-1,595, P = 0,000; II frente a DD + ID: OR = 0,750, 95% CI: 0,647-0,869, P = 0,000). El análisis de subgrupos mostró que el polimorfismo del gen I/D de la ECA se correlacionaba con la DMD tanto en la población asiática como en la china. Además, el polimorfismo del gen I/D de la ECA se correlacionó con la DKD de tipo 2 (D frente a I: OR = 1,361, IC del 95%: 1,243-1,490, P = 0,000; DD frente a ID + II: OR = 1,503, IC del 95%: 1,310-1,726, P = 0,000; II frente a DD + ID: OR = 0,738, 95% CI: 0,626 -0,870, P = 0,000). Sin embargo, no hubo una correlación evidente en los sujetos caucásicos y en los pacientes diabéticos de tipo 1.
Conclusión: Los polimorfismos I/D de la ECA se correlacionaron con la DKD en poblaciones asiáticas y diabéticas de tipo 2. El alelo D de la ECA/genotipo DD podría ser un factor de riesgo, mientras que el genotipo II de la ECA podría ser un factor de protección para la DKD.

Introduction

Diabetic kidney disease (DKD) is a severe and common complications in diabetic patients, it brings serious economic burden on society both in Western and Eastern countries.[1] Recent studies indicated that chronic kidney disease (CKD) induced by diabetes was more common than primary glomerulonephritis in China.[2] It has been demonstrated that albuminuria, elevated blood pressure, metabolic abnormalities, excessive oxidative stress and mitochondrial dysfunction were vital pathogenic factors in DKD.[3,4] Unfortunately, the detailed pathogenesis of DKD is still not fully understood, and the mainstay of current treatment for DKD including controlling blood glucose and blood pressure are not fully effective. Hence a better understanding of the DKD pathogenesis is urgently needed.

Recent studies showed that genetic factors damage was involved in the onset of DKD.[5] Additionally, the susceptibility of DKD was associated with some single genes polymorphism (e.g. methylenetetrahydrofolate reductase and angiotensin converting enzyme).[6] Angiotensin converting enzyme (ACE) gene contained 21 kb base, it was located on 17q23 including 26 exons and 25 introns. Single nucleotide polymorphisms (SNPs) frequently occurs in the ACE gene, it has been identified 6 polymorphism markers of ACE, and Alu insertion/deletion (I/D) fragment in the 16th intron is the most investigated, ACE gene polymorphism could be divided into DD, ID, II genotype based on this I/D polymorphic marker locus.[7] Some previous studies has found that ACE I/D polymorphism could influence the occurrence of diabetes-related renal damage.[8] In addition, some pooled analysis as regards the impacting of ACE I/D gene polymorphism on DKD susceptibility has been completed.[9,10] However, the pooled results were controversial and inconsistent. In this study, we further assess the potential impact of ACE I/D gene polymorphism on DKD through analyzing much more trials.

Methods

Search strategy

The eligible trials were carefully searched form various databases (e.g. PubMed, Cochrane databases, Embase and China National Knowledge Infrastructure Database). Various search terms were used as follows: angiotensin-converting enzyme, ACE, ACE insertion/deletion, ACE I/D, diabetic nephropathy, diabetic kidney disease, DN, DKD, diabetes mellitus, kidney, renal, gene, gene polymorphism.

Study inclusion criteria

The inclusion criteria were used as follows: (1) the study including two comparison group (DKD patients vs control patients), (2) the association between ACE I/D gene polymorphism and DKD has been reported, (3) the detailed number of ACE genotypes has been provided, (4) the ACE I/D genotype

distributions of control group was conformed to Hardy–Weinberg equilibrium (HWE) testing.

Data extraction and analysis

Each study characteristics was extracted, the pooled analysis was performed using the Stata software (version 12.0). An odds ratio (OR) with a 95% confidence interval (CI) was calculated. It was considered statistically significant for the pooled OR when a *P*-value < 0.05. The impact of ACE I/D gene polymorphism on DKD risk was analyzed using different four models: Method 1, D allele versus I allele; Method 2, DD genotype versus ID genotype + II genotype; Method 3, II genotype versus DD genotype + ID genotype; Method 4, ID genotype versus DD genotype + II genotype. The heterogeneity was assessed using Q and I^2 statistics. In addition, Begg's adjusted rank correction test was performed to evaluate the publication bias, there was potential publication bias when a *P* value < 0.05.[11]

Results

Study characteristics

After carefully searching and checking in various databases, we finally included 77 studies in this research.[12–88] The principal characteristics of included trials are described in Table 1. 24,321 participants with 12,961 cases and 11,360 controls were included, 31 studies were published in Chinese and 46 in English, from a total 22 countries. In this studies, both type 1 and type 2 diabetic patients were analyzed. The average age of participants ranged from 4 to 74 years. According to the Newcastle-Ottawa Scale (NOS), the quality of included studies was generally at the medium level. As shown in Table 2, 10 studies were not included in this pooled analysis due to they failing to meet the HWE testing.[24,39,56,57,64,65,69,74,82,85] In addition, we have extracted the number of various genotype in the case and control group (Table 2).

Correlation between ACE I/D gene polymorphism and DKD in overall diabetic patients

The forest plot concerned the impact of ACE I/D gene polymorphism on the risk of DKD in 63 trials. The pooled analysis indicated that ACE I/D gene polymorphism was correlated with the risk of DKD in the overall populations (D allele vs I allele: OR = 1.316, 95% CI: 1.213–1.42, *P* = 0.000; DD genotype vs ID + II genotype: OR = 1.414, 95% CI: 1.253–1.595, *P* = 0.000; II genotype vs DD + ID genotype: OR = 0.750, 95% CI: 0.647–0.869, *P* = 0.000) (shown in Table 3).

Correlation between ACE I/D gene polymorphism and DKD in Asian diabetic patients

41 included studies analyzed the correlation between ACE I/D gene polymorphism and DKD risk. A significant correlation was observed between ACE D allele/DD genotype and DKD risk in the Asian diabetic patients (D allele vs I allele: OR = 1.513, 95% CI: 1.363–1.679, *P* = 0.000; DD genotype vs ID + II genotype: OR = 1.819, 95% CI: 1.559–2.122, *P* = 0.016, Table 3). On the contrary, our pooled analysis indicated that the II genotype might be a protective factor against the DKD risk (II genotype vs DD + ID genotype: OR = 0.678, 95% CI: 0.547–0.840, *P* = 0.000, Table 3).

Correlation between ACE I/D gene polymorphism and DKD in Caucasian diabetic patients

There were 20 trials evaluating the impact of ACE I/D gene polymorphism on DKD susceptibility in Caucasian diabetic patients. The pooled-analysis indicated no significant correlation between ACE I/D gene polymorphism and DKD (D allele vs I allele: OR = 1.058, 95% CI: 0.975–1.149, *P* = 0.176; DD genotype vs ID + II genotype: OR = 1.023, 95% CI: 0.920–1.127, *P* = 0.755; II genotype vs DD + ID genotype: OR = 0.858, 95% CI: 0.719–1.025, *P* = 0.092; ID genotype vs DD + II genotype: OR = 1.075, 95% CI: 0.981–1.178, *P* = 0.121, Shown in Table 3).

Correlation between ACE I/D gene polymorphism and DKD risk in Chinese diabetic patients

27 studies analyzed the correlation between ACE I/D gene polymorphism and DKD risk in Chinese subjects. It showed a significant correlation between the ACE D allele/DD genotype and DKD in the Chinese population (D allele vs I allele: OR = 1.552, 95% CI: 1.368–1.760, *P* = 0.002; DD genotype vs ID + II genotype: OR = 1.929, 95% CI: 1.666–2.234, *P* = 0.000, Table 3). On the contrary, our pooled analysis showed that the II genotype might have or induce a protective role against DKD in Chinese diabetic patients (II genotype vs DD + ID genotype: OR = 0.650, 95% CI: 0.548–0.771, *P* = 0.000, shown in Table 3).

Correlation between ACE I/D gene polymorphism and DKD susceptibility in type 1 diabetic patients

There were 13 studies exploring the impact of ACE I/D gene polymorphism on DKD susceptibility in type 1 diabetic subjects, our pooled analysis showed that there was no association between ACE I/D gene polymorphism and DKD susceptibility in type 1 diabetic patients (D allele vs I allele: OR = 1.139, 95% CI: 0.952–1.364, *P* = 0.155; DD genotype vs ID + II genotype: OR = 1.103, 95% CI: 0.884–1.377, *P* = 0.153; II genotype vs DD + ID genotype: OR = 0.803, 95% CI: 0.568–1.134, *P* = 0.212; ID genotype vs DD + II genotype: OR = 1.048, 95% CI: 0.870–1.263, *P* = 0.622, Table 3).

Correlation between ACE I/D gene polymorphism and DKD susceptibility in type 2 diabetic patients

There were 50 studies exploring the correlation between ACE I/D gene polymorphism and DKD susceptibility in type 2 diabetic subjects, our pooled analysis indicated that the ACE D allele/DD genotype might increase the risk of DKD in type 2 diabetic subjects (D allele vs I allele: OR = 1.361, 95% CI: 1.243–1.490, *P* = 0.000; DD genotype vs ID + II genotype: OR = 1.503, 95% CI: 1.310–1.726, *P* = 0.000, Table 3). On the contrary, this pooled analysis showed that the ACE II genotype might be a protective factor for DKD in type 2 diabetic

Table 1 – Characteristics of studies included in the meta-analysis.

Trials	Design	Country Ethnicity	Year	Sex (M/F)	Case	Control	Source of control	Diabetes type	Genotyping method	Control type	NOS scores
Ahluwalia 2009	Case–control	India Asian	C:58.4 ± 5.8 D:54.9 ± 7.6	C:159/81 D:94/106	240	200	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
An 2015 in Chinese	Case–control	China Asian	C:56.6 ± 15.1 D:62.1 ± 13.1	C:70/75 D:37/49	86	145	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Araz 2001	Case–control	Turkey Asian/Europe	C:57 ± 7 D:51 ± 9	C:49/67 D:39/84	116	123	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	7
Arzu 2004	Case–control	Turkey Asian/Europe	C:59.6 ± 13.5 D:57.1 ± 14.5	C:20/5 D:33/17	25	50	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Azar 2001	Case–control	Lebanon Asian	C:22.8 ± 5.2 D:26 ± 9	C:24/28 D:5/5	52	10	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Bai 2012 in Chinese	Case–control	China Asian	C:64.3 ± 9.7 D:62.2 ± 11.2	NR	69	75	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Barnas 1997	Case–control	Austria Europe	C:47 ± 11 D:47 ± 12	C:35/15 D:22/18	50	40	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Bu 2008 in Chinese	Case–control	China Asian	C:57.9 ± 10.0 D:56.8 + 8.2	C:33/32 D:46/46	65	92	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Chen 2010 in Chinese	Case–control	China Asian	C:60.1 ± 12.2 D:60.0 ± 11.7	C:49/71 D:30/44	120	74	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Cheng 2005 in Chinese	Case–control	China Asian	C:53.1 ± 17.7 D:52.0 ± 15.2	C:17/20 D:22/50	37	72	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Chowdhury 1996	Case–control	Britain Europe	C:39.3 ± 7.6 D:37.9 ± 6.3	C:132/110 D:79/87	242	166	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
De Cosmo 1999	Case–control	Italy Europe	C:43 ± 11 D:43 ± 13	C:107/68 D:70/66	175	136	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	7
Ding 2012 in Chinese	Case–control	China Asian	C:50.1 ± 16.2 D:48.0 ± 14.1	C:21/29 D:20/36	50	56	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Doi 1996	Case–control	Japan Asian	C:62 ± 12 D:61 ± 13	C:28/36 D:50/74	64	124	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6

Table 1 (*Continued*)

Trials	Design	Country Ethnicity	Year	Sex (M/F)	Case	Control	Source of control	Diabetes type	Genotyping method	Control type	NOS scores
Dudley 1995	Case–control	Britain Europe	NR	NR	163	267	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Eroglu 2008	Case–control	Turkey Asian/Europe	C:58.3 ± 10.5 D:52.3 ± 9.5	C:19/27 D:22/34	46	56	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	7
Freire 1998	Case–control	Israel Asian	C:10 ± 6 D:11 ± 7	C:48/29 D:39/50	77	89	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Fu 2002 in Chinese	Case–control	China Asian	NR	NR	44	47	PB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Gallego 2008	Case–control	Australia Australia	C:4.0–10.6 D:5.9–11.9	C:16/25 D:199/213	41	412	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Gao 2014 in Chinese	Case–control	China Asian	C:57.6 ± 11.3 D:54.6 ± 16.8	C:19/9 D:21/9	28	30	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Grzeszczak 1998	Case–control	Poland Europe	C:61.8 ± 9.4 D:62.7 ± 8.3	NR	462	254	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Gu 2010 in Chinese	Case–control	China Asian	NR	NR	75	100	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Guo 2007 in Chinese	Case–control	China Asian	27–83	NR	27	33	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Gutiérrez 1997	Case–control	Spain Europe	C:60.1 ± 10.6 D:64.2 ± 9.2	C:28/32 D:47/53	60	100	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Hadjadj 2003	Case–control	France Europe	C:65.7 ± 8.3 D:65.0 ± 7.3	C:2285/854 D:292/313	3139	605	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	8
Hadjadj 2007	Case–control	Denmark, Finland, France. Europe	C:42.0 ± 10.2 D:44.8 ± 11.0	C:757/544 D:671/744	1301	1415	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	8
Hibberd 1997	Case–control	Britain Europe	C:43.0 ± 11.6 D:50.9 ± 13.6	C:34/38 D:45/41	72	86	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Hsieh 2000	Case–control	Taiwan Asian	C:59.6 ± 9.5 D:59.5 ± 10.4	C:87/92 D:68/89	179	157	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	7

Table 1 (*Continued*)

Trials	Design	Country Ethnicity	Year	Sex (M/F)	Case	Control	Source of control	Diabetes type	Genotyping method	Control type	NOS scores
Huang 1998	Case–control	Finland Europe	56.2 ± 7.2	NR	13	46	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Huang 2004 in Chinese	Case–control	China Asian	C:59.8 ± 7.5 D:57.3 ± 6.4	C:44/49 D:46/48	93	94	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Ilić 2014	Case–control	Serbia Europe	C:25.8 ± 6.8 D:28.1 ± 5.8	NR	46	33	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Jayapalan 2010	Case–control	Malaysia Asian	C:59.8 ± 10.2 D:57.0 ± 10.2	C:79/96 D:31/50	175	81	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Jeffers 1997	Case–control	USA America	NR	NR	50	459	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Lee 2002	Case–control	Taiwan Asian	NR	NR	294	417	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Li 2003 in Chinese	Case–control	China Asian	C:64.2 ± 1.2 D:63.5 ± 1.0	NR	97	105	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Li 2004 in Chinese	Case–control	China Asian	C:63.6 ± 12.6 D:64.2 ± 10.3	C:116/102 D:35/45	218	80	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Li 2005 in Chinese	Case–control	China Asian	NR	NR	38	21	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Liao 2002 in Chinese	Case–control	China Asian	NR	C:20/14 D:31/21	34	52	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Liu 2015 in Chinese	Case–control	China Asian	NR	NR	100	100	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Liu 2018 in Chinese	Case–control	China Asian	C:45.9 ± 9.0 D:46.0 ± 9.4	C:126/110 D:98/93	236	191	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Liu 2019 in Chinese	Case–control	China Asian	C:61.4 ± 10.8 D:62.3 ± 11.2	C:216/84 D:215/85	300	300	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Mansouri 2017	Case–control	Morocco Africa	C:63.7 ± 9.2 D:60.1 ± 8.9	C:50/80 D:15/70	130	85	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6

Table 1 (*Continued*)

Trials	Design	Country Ethnicity	Year	Sex (M/F)	Case	Control	Source of control	Diabetes type	Genotyping method	Control type	NOS scores
Marre 1994	Case–control	France Europe	C:39 ± 14 D:43 ± 18	C:37/25 D:30/32	62	62	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Marre 1997	Case–control	France Europe	C:43 ± 13 D:46 ± 13	C:193/144 D:84/73	337	157	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	7
Miura 1999	Case–control	Japan Asian	C:34.8 ± 7.3 D:33.5 ± 8.6	C:33/65 D:51/47	98	103	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Möllsten 2008	Case–control	Sweden Europe	C:47.0 ± 10.7 D:43.9 ± 11.3	C:30/43 D:88/109	73	197	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Movva 2007	Case–control	India Asian	C:57.2 ± 10.5 D:55.4 ± 10.8	C:122/52 D:133/42	174	175	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Nakajima 1996	Case–control	Japan Asian	C:57.0 ± 7.9 D:55.0 ± 6.0	C:65/36 D:24/17	101	41	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Oh 1996	Case–control	Korea Asian	C:34.6 ± 12.6 D:35.7 ± 9.8	C:13/18 D:16/12	31	28	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Ohno 1996	Case–control	Japan Asian	C:60.5 ± 7.2 D:60.3 ± 8.6	C:42/37 D:30/23	79	53	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Okuno 2003	Case–control	Japan Asian	C:68.6 ± 8.1 D:67.6 ± 10.0	C:6/6 D:18/20	12	38	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Oue 1999	Case–control	Japan Asian	C:61 ± 12 D:51 ± 10	C:15/12 D:20/20	21	30	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Panagiotopoulos 1995	Case–control	Australia Australia	C:61.9 ± 1.8 D:64.4 ± 0.9	C:33/17 D:49/66	50	115	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Park 2005	Case–control	Korea Asian	C:60.3 ± 10.1 D:60.1 ± 11.0	NR	103	88	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Pong 2001 in chines	Case–control	China Asian	C:74.6 ± 7.7 D:73.9 ± 7.5	NR	62	78	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Powrie 1994	Case–control	Britain Europe	NR	NR	19	85	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6

Table 1 (*Continued*)

Trials	Design	Country Ethnicity	Year	Sex (M/F)	Case	Control	Source of control	Diabetes type	Genotyping method	Control type	NOS scores
Prasad 2006	Case–control	India Asian	C:57 ± 12.8 D:60.6 ± 11.5	C:65/131 D:76/149	196	225	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Ringel 1997#	Case–control	Germany Europe	C:38.9 ± 13.1 D:35.7 ± 11.4	C:76/58 D:130/96	134	226	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	7
Ringel 1997#	Case–control	Germany Europe	C:61.4 ± 10.6 D:58.6 ± 9.6	C:84/77 D:69/71	161	140	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	7
Schmidt 1995#	Case–control	Germany Europe	C:45 ± 15.5 D:44 ± 15.4	C:71/43 D:75/58	114	133	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	7
Schmidt 1995#	Case–control	Germany Europe	C:65 ± 9.3 D:63 ± 9.7	C:119/128 D:81/127	247	208	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	7
Schmidt 1997	Case–control	Germany Europe	C:65 ± 9 D:63 ± 9	C:153/158 D:158/189	311	347	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	7
Seruga 2017	Case–control	Slovenia Europe	C:64.7 ± 9.2 D:63.7 ± 8.0	C:163/143 D:196/179	276	375	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Sun 2006 in Chinese	Case–control	China Asian	C:54.9 ± 7.8 D:47.4 ± 6.6	C:26/14 D:19/11	40	30	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Tarnow 1995	Case–control	Denmark Europe	C:40.9 ± 9.6 D:42.7 ± 10.2	C:121/77 D:118/72	198	190	HB	Type 1	PCR-RFLP	Type 1 diabetic patients	6
Tien 2009	Prospective observational	Taiwan Asian	C:61.0 ± 14.4 D:59.5 ± 10.9	NR	47	202	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Viswanathan 2001	Case–control	India Asian	C:56.7 ± 8.9 D:56.7 ± 9.3	C:57/29 D:15/8	86	23	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Wang 1999 in Chinese	Case–control	China Asian	C:63.3 ± 8.5 D:59.1 ± 9.1	C:16/33 D;26/28	49	54	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Wang 2007 in Chinese	Case–control	China Asian	46–69	74/70	80	64	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5

Table 1 (*Continued*)

Trials	Design	Country Ethnicity	Year	Sex (M/F)	Case	Control	Source of control	Diabetes type	Genotyping method	Control type	NOS scores
Wyawahare 2017	Case–control	India Asian	C:55.4 ± 9.4 D:56.2 ± 8.5	NR	129	50	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Xu 2001 in Chinese	Case–control	China Asian	C:59.5 ± 7.4 D:57.5 ± 8.2	C:55/56 D:68/70	111	138	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Xue 2000 in Chinese	Case–control	China Asian	C:60.1 ± 10 D:60.9 ± 11.6	C:76/64 D:48/33	140	81	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Yan 2008 in Chinese	Case–control	China Asian	C:57.7 ± 8.9 D:60.2 ± 8.1	C:66/59 D:56/36	125	92	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Yang 2003 in Chinese	Case–control	China Asian	C:59.1 ± 10.8 D:55.2 ± 11.3	C:19/42 D:31/40	61	71	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Young 1998	Case–control	China Asian	C:57.4 ± 11.5 D:53.5 ± 9.0	C:19/37 D:20/34	56	54	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	6
Zhang 2011 in Chinese	Case–control	China Asian	38–71	72/96	42	126	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Zhao 2001 in Chinese	Case–control	China Asian	NR	NR	61	47	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Zhong 2005 in Chinese	Case–control	China Asian	C:52.7 ± 9.6 D:51.6 ± 8.9	C:52/41 D:53/49	93	102	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5
Zhong 2008 in Chinese	Case–control	China Asian	NR	C:22/31 D:30/24	53	54	HB	Type 2	PCR-RFLP	Type 2 diabetic patients	5

C: case subjects; H: diabetic subjects; HB: hospital-based; PB: population-based; NR: not reported; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism. * Months.

Table 2 – Characteristics of the studies evaluating the effects of ACE I/D gene polymorphisms on DKD risk.

Author (year)	Gene sites	Case				Control				HWE(p)
	ACE I/D	DD	ID	II	Total	DD	ID	II	Total	
Ahluwalia 2009		132	64	44	240	89	117	49	255	0.3445
An 2015 in Chinese		23	37	26	86	15	73	57	145	0.2327
Araz 2001		34	64	18	116	43	57	23	123	0.5945
Arzu 2004		9	11	5	25	24	21	5	50	0.8974
Azar 2001		23	27	2	52	1	7	2	10	0.1903
Bai 2012 in Chinese		23	28	18	69	14	34	27	75	0.5720
Barnas 1997		14	27	9	50	4	21	15	40	0.3901
Bu 2008 in Chinese		26	25	14	65	21	42	29	92	0.4429
Chen 2010 in Chinese		62	43	15	120	17	34	23	74	0.5188
Cheng 2005 in Chinese		28	9	0	37	40	31	1	72	0.0635
Chowdhury 1996		78	124	40	242	55	79	32	166	0.7033
De Cosmo 1999		73	79	23	175	65	53	18	136	0.1803
Ding 2012 in Chinese		12	18	20	50	15	20	21	56	0.0379
Doi 1996		14	30	20	64	12	56	56	124	0.7105
Dudley 1995		47	85	31	163	70	148	49	267	0.0591
Eroglu 2008		16	17	13	46	19	24	13	56	0.3200
Freire 1998		33	32	12	77	34	45	10	89	0.3930
Fu 2002 in Chinese		17	22	5	44	8	16	23	47	0.0972
Gallego 2008		15	17	9	41	102	204	103	409	0.9607
Gao 2014 in Chinese		17	6	5	28	9	13	8	30	0.4684
Grzeszczak 1998		129	230	103	462	73	118	63	254	0.2685
Gu 2010 in Chinese		25	34	16	75	30	48	22	100	0.7352
Guo 2007 in Chinese		7	14	6	27	6	13	14	33	0.3493
Hadjadj 2003		1119	1468	552	3139	208	282	115	605	0.2662
Hibberd 1997		21	42	9	72	36	43	7	86	0.2341
Hsieh 2000		40	59	80	179	21	50	86	157	0.0038
Huang 1998		4	9	0	13	19	20	7	46	0.6498
Huang 2004 in Chinese		32	37	24	93	18	40	36	94	0.2585
Ilić 2014		10	23	13	46	10	12	11	33	0.1181
Jayapalan 2010		21	77	77	175	19	31	31	81	0.0504
Lee 2002		40	137	117	294	39	170	208	417	0.6181
Li 2003 in Chinese		19	43	35	97	10	42	53	105	0.6910
Li 2004 in Chinese		50	93	75	218	22	35	23	80	0.2641
Li 2005 in Chinese		38	47	16	101	21	42	38	101	0.1477
Liao 2002 in Chinese		16	14	4	34	13	22	17	52	0.2832
Liu 2015 in Chinese		17	58	25	100	16	54	30	100	0.3097
Liu 2019 in Chinese		45	129	126	300	22	124	154	300	0.6633
Mansouri 2017		76	42	12	130	47	32	6	85	0.8627
Marre 1994		23	35	4	62	19	28	15	62	0.4640
Marre 1997		119	168	50	337	48	69	40	157	0.1368
Miura 1999		13	49	36	98	10	58	35	103	0.0459
Möllsten 2008		16	45	12	73	48	113	36	197	0.0335
Movva 2007		39	88	47	174	27	74	74	175	0.2415
Nakajima 1996		14	50	37	101	4	19	18	41	0.7529
Oh 1996		10	9	12	31	7	10	11	28	0.1518
Ohno 1996		15	38	26	79	5	15	33	53	0.1178
Okuno 2003		3	8	1	12	5	12	21	38	0.1521
Oue 1999		5	8	8	21	0	15	15	30	0.0679
Panagiotopoulos 1995		15	25	10	50	43	44	28	115	0.0175
Park 2005		27	49	27	103	7	51	30	88	0.0220
Pong 2001 in chines		14	23	25	62	7	33	38	78	0.9656
Powrie 1994		7	8	4	19	24	37	24	85	0.2328
Ringel 1997#		35	68	31	134	57	130	39	226	0.0177
Ringel 1997#		44	84	33	161	35	69	36	140	0.8662
Schmidt 1995#		52	38	24	114	55	55	12	122	0.7442
Schmidt 1995#		101	105	41	247	83	91	34	208	0.2886
Schmidt 1997		121	129	61	311	131	154	62	347	0.1577
Seruga 2017		90	143	43	276	115	169	91	375	0.0659
Sun 2006 in Chinese		15	17	8	40	6	10	14	30	0.1221
Tarnow 1995		63	95	40	198	67	77	46	190	0.0134
Viswanathan 2001		24	45	17	86	5	8	10	23	0.1956
Wang 1999 in Chinese		15	20	14	49	9	27	18	54	0.8337

Table 2 (*Continued*)

Author (year)	Gene sites	Case				Control				HWE(p)
	ACE I/D	DD	ID	II	Total	DD	ID	II	Total	
Wang 2007 in Chinese		19	27	34	80	7	35	22	64	0.2082
Wyawahare 2017		21	56	52	129	6	26	18	50	0.4640
Xu 2001 in Chinese		42	48	21	111	30	72	36	138	0.5934
Xue 2000 in Chinese		42	45	53	140	19	35	27	81	0.2520
Yan 2008 in Chinese		40	64	21	125	12	22	58	92	0.0005
Yang 2003 in Chinese		19	24	18	61	14	27	30	71	0.0940
Young 1998		3	30	24	57	8	20	26	54	0.2207
Zhang 2011 in Chinese		12	22	8	42	24	42	60	126	0.0021
Zhao 2001 in Chinese		15	23	23	61	5	17	25	47	0.4239
Zhong 2005 in Chinese		16	54	23	93	15	56	31	102	0.2041
Zhong 2008 in Chinese		10	31	12	53	8	30	16	54	0.3174

Table 3 – Meta analysis of the association of ACE I/D gene polymorphisms on DKD risk.

Genetic contrasts	Group and subgroups	Studies number	Q test P value	Model selected	OR (95% CI)	P value	Begg's test
D versus I	Overall	63	0.000	Random	1.316 (1.213–1.427)	0.000	0.006
	Asian	41	0.000	Random	1.513 (1.363–1.679)	0.000	–
	Caucasian	20	0.167	Random	1.058 (0.975–1.149)	0.176	–
	Chinese	27	0.002	Random	1.552 (1.368–1.760)	0.002	–
	Non-Chinese	36	0.000	Random	1.169 (1.066–1.281)	0.000	–
	Type 1 diabetic	13	0.022	Random	1.139 (0.952–1.364)	0.155	–
	Type 2 diabetic	50	0.000	Random	1.361 (1.243–1.490)	0.000	–
DD versus ID + II	Overall	63	0.000	Random	1.414 (1.253–1.595)	0.000	0.016
	Asian	41	0.016	Random	1.819 (1.559–2.122)	0.016	–
	Caucasian	20	0.755	Random	1.023 (0.92–1.127)	0.755	–
	Chinese	27	0.112	Fixed	1.929 (1.666–2.234)	0.000	–
	Non-Chinese	36	0.008	Random	1.137 (1.045–1.237)	0.003	–
	Type 1 diabetic	13	0.153	Fixed	1.103 (0.884–1.377)	0.153	–
	Type 2 diabetic	50	0.000	Random	1.503 (1.310–1.726)	0.000	–
II versus DD + ID	Overall	63	0.000	Random	0.750 (0.647–0.869)	0.000	0.107
	Asian	41	0.000	Random	0.678 (0.547–0.840)	0.000	–
	Caucasian	20	0.021	Random	0.858 (0.719–1.025)	0.092	–
	Chinese	27	0.040	Random	0.650 (0.548–0.771)	0.000	–
	Non-Chinese	36	0.000	Random	0.845 (0.683–1.046)	0.123	–
	Type 1 diabetic	13	0.011	Random	0.803 (0.568–1.134)	0.212	–
	Type 2 diabetic	50	0.000	Random	0.738 (0.626–0.870)	0.000	–
ID versus DD + II	Overall	63	0.005	Random	0.999 (0.914–1.091)	0.981	0.822
	Asian	41	0.002	Random	0.949 (0.829–1.085)	0.443	–
	Caucasian	20	0.516	Fixed	1.075 (0.981–1.178)	0.121	–
	Chinese	27	0.276	Fixed	0.915 (0.803–1.043)	0.186	–
	Non-Chinese	36	0.003	Random	1.055 (0.939–1.185)	0.369	–
	Type 1 diabetic	13	0.299	Fixed	1.048 (0.870–1.263)	0.622	–
	Type 2 diabetic	50	0.003	Random	0.990 (0.896–1.095)	0.845	–

patients (II genotype vs DD + ID genotype: OR = 0.738, 95% CI: 0.626–0.870, $P = 0.000$, Table 3).

Publication bias

In this study, we used funnel plots and Begg's test to evaluate the publication bias. In the analysis for the association of ACE D allele/DD genotype with DKD susceptibility in overall diabetic patients, there was potential publication bias noted by Begg's test (D vs. I: Begg' s test $P = 0.006$; DD vs. ID + II: Begg's test $P = 0.016$). In line with this, the funnel plots were asymmetrical (Table 3, Fig. 1).

Discussion

This pooled-analysis showed that the ACE I/D polymorphism was statistically associated with DKD susceptibility, it indicated that ACE D allele/DD genotype might be a risk factor for DKD. On the contrary, ACE II genotype might be a protective factor for DKD.

Genome-wide association studies (GWAS) research was frequently carried out to explore the relationship between various gene single nucleotide polymorphisms (SNPs) and an array of diseases. In such studies, HWE testing for each SNPs was often the first and quality control step. Those SNPs that

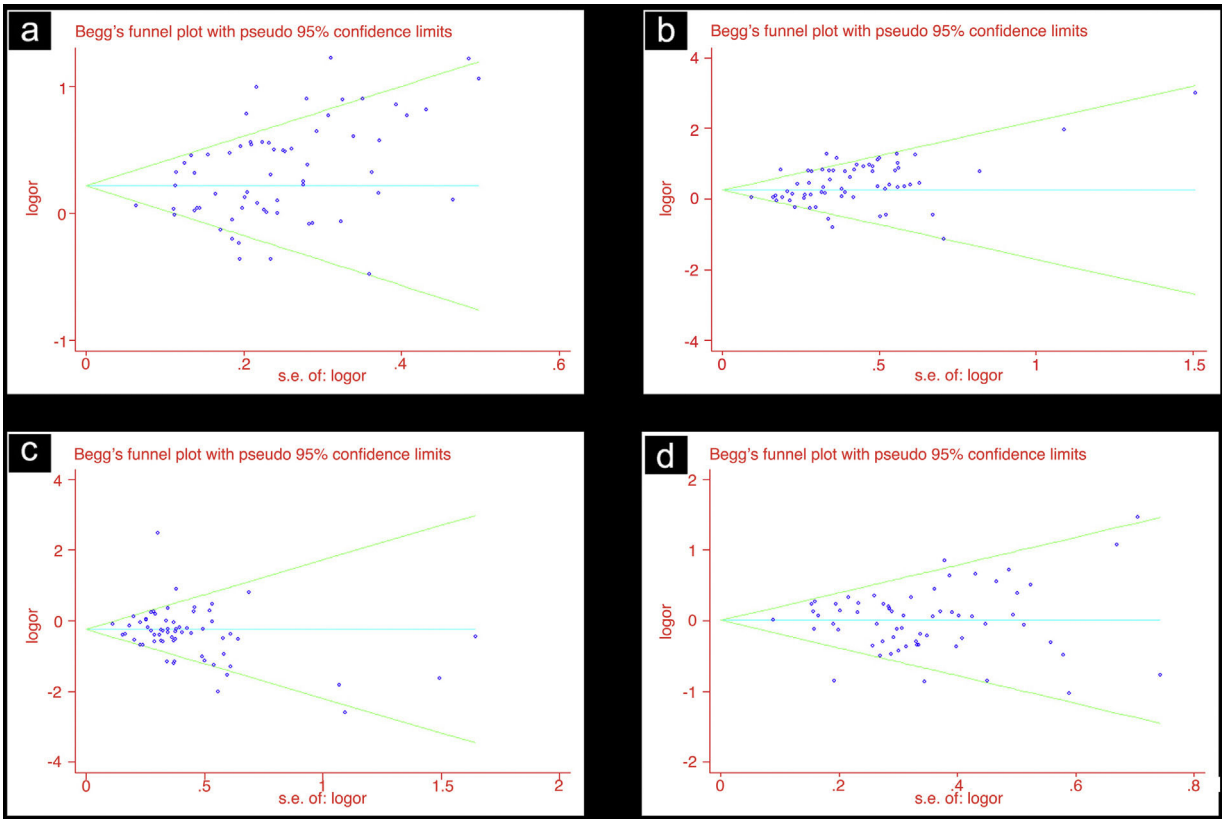

Fig. 1 – The funnel plot of different model for pooled analysis. (a): D vs I; (b): DD vs ID + II; (c): II vs DD + ID; (d): ID vs DD + II.

did not pass the HWE tests were eliminated before moving on to the next step.[89] On the other hand, in case-control genetic association studies, departures from HWE in controls have been associated with problems in the design, genotyping error or selection bias.[90] In pooled analysis, checking HWE among controls was a good idea for included trials. Trikalinos et al. has demonstrated that exclusion of trials with departures from HWE may sometimes change the estimate pooled analysis result, they advocated that studies with departures from HWE should be excluded in pooled analysis.[91] For this reason, in this pooled analysis, we carefully checked all selected trials and excluded these studies failing to meet the HWE test. In addition, the quality of included studies were generally at the medium level according to the Newcastle-Ottawa Scale (NOS), it indicated that the included studies met the criteria accepted for valid SNP-association studies.

Some previous pooled analysis concerning about the impact of ACE I/D gene polymorphism on DKD risk has been completed. In 2012, a meta analysis included 14,108 DKD cases and 12,472 controls from 63 published studies has been performed, it indicated that ACE I/D polymorphism was associated with DKD development in the Asian type 2 diabetes subjects.[92] However, the genotype distributions of the control groups do not conform to HWE in some trials. And because of that, in order to gain a more credible pooled analysis result, we re-examined the related studies and included some other more high quality trials. In line with Wang et al., our study also found that the ACE I/D genotype was correlated with DKD risk in type 2 diabetes patients. Due to the fact that we included a plethora of studies in our analysis compared to the aforementioned analyses, we feel that our pooled results are more convincing.

ACE is a pivotal factor of the renin–angiotensin–aldosterone system (RAAS), it contains 26 exons and 25 introns located on 17q23. In 1990, ACE gene polymorphism was firstly described based on the insertion or deletion (I/D) of a 287 bp Alu in the 16th intron.[93] Whereafter, a series of ACE polymorphic genetic markers have been found (e.g. A240T, T93C, T594lC). Among these polymorphic marker, I/D polymorphism (rs4340) was the most investigated. On account of this I/D polymorphic marker, we could divide ACE gene polymorphism into DD homozygote, II homozygote and ID heterozygote. It has been demonstrated that ACE I/D polymorphism could affect ACE activity level both in plasma and various tissues.[94] Additionally, a great number of previous studies have been carried out and verified the impact of ACE I/D gene polymorphism on various diabetes-related diseases.

DKD is a severe complication both in type 1 and type 2 diabetic patients, it damages about 40% of all diabetic subjects and is a crucial cause of chronic renal failure both in the Eastern and Western world. The pathogenesis of DKD is very complicated, it has been verified that various signaling pathways and molecular factors are activated during DKD, such molecular events include activation of systemic and local RAAS, generation of pro-inflammatory cytokines and excessive reactive oxygen species.[3] In addition, recent GWAS studies demonstrated that DKD patients always suffer from genetic damage, and genetic factors are involved in the

development of DKD, more critically, some specific gene SNPs might be associated with DKD susceptibility, thus it could provide remarkable clinical significance for preventing and early diagnosing of DKD through detailed illuminating the genetic mechanisms involved in DKD.

RAAS activation play a vital role in the occurrence and development of DKD, the RAAS is a pivotal regulator of renal arterial blood pressure by angiotensin II. However, conversion of low activity angiotensin I to high activity angiotensin II was relying on ACE. It has been showed that the ACE level is strongly correlated with ACE I/D polymorphism. Although the ACE I/D gene polymorphism is taken place in the non-coding gene region, the base insertion or deletion itself might alter the splicing process of the ACE precursor mRNA, then influence the stability of ACE mRNA, and ultimately affect the expression or stabilization of ACE. In situ hybridization for ACE mRNA on renal biopsy studies have found that the expression of ACE mRNA was increased in those subjects with the ACE DD genotype.[95] Additionally, the serum ACE levels was also higher in the those individuals with D genotype than those with ID genotype or II genotype.[93] And because of that, it was reasonable to consider that ACE I/D genetic variation was associated with the development of DKD. ACE D allele carriers had more higher ACE levels both in serum and kidney tissue, which lead to a more efficient activation of angiotensin II, and consequently resulted in the deterioration of DKD. In line with these, our pooled study further demonstrated that DKD risk was higher in those subjects with D allele than I allele carriers. We observed that the presence of II genotype offered a significant protective effect for DKD, whereas the presence of DD genotype conferred remarkable risk for DKD. The detailed mechanistic aspects that underlie the relationship between ACE I/D gene polymorphism and DKD was not completely clear. As mentioned earlier, the impact of ACE I/D gene polymorphism on DKD could be partially attributed to the effect of the ACE I/D polymorphic variant on the expression of the ACE gene. On the other hand, a recent study performed by Mahwish et al. found that ACE I/D genotypes was associated with dyslipidemia in diabetic patients, the DD genotype subgroup subjects were characterized by a significant higher levels of plasma triglycerides and total cholesteroln.[96] In addition, the association of ACE I/D genotypes with atherosclerotic risk factors such as hypertension, dyslipidemia, and obesity in type 2 diabetic patients has been reported.[97] Taken together, ACE DD genotype might result in the formation of diabetic renal lesions through elevating angiotensin II levels and a key contributor to dyslipidemia in a hyperglycemic environment further culminating in renal complications.

In this study, we found that the impact of ACE I/D gene polymorphism on DKD susceptibility was inconsonant in different types of diabetes and races. The pooled analysis showed that ACE I/D gene polymorphism was correlated with DKD susceptibility in Asian individuals, but there was no obvious correlation in Caucasian subjects. For another, we found that there was no correlation between them among 13 studies concerning type 1 diabetic patients, while ACE I/D gene polymorphism was correlated with the onset of DKD risk in type 2 diabetic patients. It indicates that ACE I/D genetic factors contribute more in patients with type 2 diabetes mellitus. Likewise, this inconsistency was also found in previous pooled analysis, Ng et al. found that ACE gene polymorphism was associated with DKD among type 2 diabetic Asians, while there was a reduced risk of DKD associated with the ACE I/D gene polymorphism among Caucasians with either type 1 or type 2 diabetes.[98] Similarly, a pooled analysis performed by Wang et al. further found that the Asian group with T2DM showed a significant association. However, it failed to find any significant effects for different genetic models in T1DM and Caucasian subjects.[92] Conversely, another pooled analysis performed by Xu et al. included 17 case-control studies in 2016 showed that ACE I/D polymorphism was correlated with DKD in the Asian groups with type 1 diabetes.[9] While Fujisawa et al. found that the association was significant both in Asian populations and in Caucasian populations.[10] Some reasons may account for the different results between Asians and Caucasians. Firstly, different lifestyle, environmental exposure, and different socioeconomic status may modify individual DKD susceptibility in different ethnic groups. Secondly, different genetic backgrounds in different racial subjects may influence genetic phenotypes.[9] On the other hand, there are some other explanations for the predisposition to DKD in patients with type 2 DM. As mentioned above, the D allele of the ACE gene has been connected with higher ACE activity and increased level of angiotensin II. It has been found that increased angiotensin II could worsen insulin resistance and lipid metabolism disorders.[99] In addition, both muscle capillary density and endogenous hepatic glucose production also could be affected by ACE I/D gene polymorphism.[100]

This study has several potential limitations. Firstly, most included trials were limited number and size. Second, there were evidences of public bias in this pooled study, in addition, the included trials were from various countries and races, which might decrease the reliability of this pooled analysis. Finally, our research was focused on ACE I/D genetic alteration, but previous studies have indicated that gene polymorphism in many other genes including Interleukin-6 -174G/C and angiotensinogen T174M gene polymorphism were correlated with DKD susceptibility,[101,102] thus it can be argue that further pooled analysis concerning these genes SNPs are needed.

Conclusion

ACE I/D gene polymorphism is correlated with DKD risk in Asian, Chinese populations and type 2 diabetic individuals. ACE D allele and DD genotype is a risk factor for DKD. Conversely, ACE II genotype seems to be a protective factor of DKD. However, no correlation between ACE I/D gene polymorphism and the susceptibility of DKD was found in Caucasian or type 1 diabetic patients.

Authors' contribution

Shi-kun Yang, Wen-li Zeng, Fen-fen Chu analyzed the data for the manuscript and wrote the manuscript. Shi-kun Yang, Na Song, Wen-li Zeng performed the literature search. Fen-fen Chu, Shi-kun Yang edited the manuscript.

Data availability and ethics committee

The data used to support the findings of this study are available from the first author and corresponding author upon request. This is a meta analysis using previous relevant published studies. There is no Human participants and/or Animals informed consent. None of the authors is in any condition that may represent a potential conflict of interest. The experiments were carried out according to the Ethics Review Committee of The Third Xiangya Hospital, Central South University.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Lin YC, Chang YH, Yang SY, Wu KD, Chu TS. Update of pathophysiology and management of diabetic kidney disease. J Formos Med Assoc. 2018;117:662–75.
2. Zhang L, Long J, Jiang W, Shi Y, He X, Zhou Z, et al. Trends in chronic kidney disease in China. N Engl J Med. 2016;375:905–6.
3. Kanwar YS, Sun L, Xie P, Liu FY, Chen S. A glimpse of various pathogenetic mechanisms of diabetic nephropathy. Annu Rev Pathol. 2011;6:395–423.
4. Yang S, Han Y, Liu J, Song P, Xu X, Zhao L, et al. Mitochondria: a novel therapeutic target in diabetic nephropathy. Curr Med Chem. 2017;24:3185–202.
5. Jeong KH, Kim JS, Woo JT, Rhee SY, Lee YH, Kim YG, et al. Genome-wide association study identifies new susceptibility loci for diabetic nephropathy in Korean patients with type 2 diabetes mellitus. Clin Genet. 2019;96:35–42.
6. El-Baz R, Settin A, Ismaeel A, Khaleel AA, Abbas T, Tolba W, et al. MTHFR C677T A1298C and ACE I/D polymorphisms as risk factors for diabetic nephropathy among type 2 diabetic patients. J Renin Angiotensin Aldosterone Syst. 2012;13:472–7.
7. Shen W, Jiang XX, Li YW, He Q. I/D polymorphism of ACE and risk of diabetes-related end-stage renal disease: a systematic review and meta-analysis. Eur Rev Med Pharmacol Sci. 2019;23:1652–60.
8. Ha SK. ACE insertion/deletion polymorphism and diabetic nephropathy: clinical implications of genetic information. J Diabetes Res. 2014;2014:846068.
9. Xu HY, Liu MM, Wang X, He XY. Association of angiotensin-converting enzyme insertion/deletion polymorphism with type 1 diabetic nephropathy: a meta-analysis. Ren Fail. 2016;38:1320–7.
10. Fujisawa T, Ikegami H, Kawaguchi Y, Hamada Y, Ueda H, Shintani M, et al. Meta-analysis of association of insertion/deletion polymorphism of angiotensin I-converting enzyme gene with diabetic nephropathy and retinopathy. Diabetologia. 1998;41:47–53.
11. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50:1088–101.
12. AN XH CW, Song DP. The association of Concomitant presence of endothelial nitric oxide synthase and angiotensin converting enzyme polymorphism with diabetic kidney disease. J Kunming Med Univ. 2015;36:12–6.
13. Ahluwalia TS, Ahuja M, Rai TS, Kohli HS, Bhansali A, Sud K, et al. ACE variants interact with the RAS pathway to confer risk and protection against type 2 diabetic nephropathy. DNA Cell Biol. 2009;28:141–50.
14. Araz M, Yilmaz N, Gungor K, Okan V, Kepekci Y, Sukru Aynacioglu A. Angiotensin-converting enzyme gene polymorphism and microvascular complications in Turkish type 2 diabetic patients. Diabetes Res Clin Pract. 2001;54:95–104.
15. Arzu Ergen H, Hatemi H, Agachan B, Camlica H, Isbir T. Angiotensin-I converting enzyme gene polymorphism in Turkish type 2 diabetic patients. Exp Mol Med. 2004;36:345–50.
16. Azar ST, Zalloua PA, Medlej R, Halabi G. The DD genotype of the ACE gene polymorphism is associated with diabetic nephropathy in the type-1 diabetics. Endocr Res. 2001;27:99–108.
17. Bai Y, Master thesis of Shanxi Medical University Study on the relationship between Angiotensin I converting enzyme gene polymorphism and diabetic kidney disease in patients with type 2 diabetes mellitus; 2001 [article in Chinese].
18. Barnas U, Schmidt A, Illievich A, Kiener HP, Rabensteiner D, Kaider A, et al. Evaluation of risk factors for the development of nephropathy in patients with IDDM: insertion/deletion angiotensin converting enzyme gene polymorphism, hypertension and metabolic control. Diabetologia. 1997;40:327–31.
19. Bu TY, Zhou JX, Li X. The relationship between angiotensin I - converting enzyme gene insertion/deletion polymorphism and type 2 diabetics nephropathy. J Tianjing Med Univ. 2008;14:293–6 [article in Chinese].
20. Chen XL, Luo P, Zhang JW. Association of the gene polymorphisms in the renin–angiotensin system with diabetic nephropathy. Chin J Diabetes. 2010;18:434–7 [article in Chinese].
21. Cheng LJ, Shen MY, Wang JS. Study on the relationship between ACE gene polymorphism and type 2 diabetes with nephropathy. J China Mod Med. 2005;7:14–6 [article in Chinese].
22. Chowdhury TA, Dronsfield MJ, Kumar S, Gough SL, Gibson SP, Khatoon A, et al. Examination of two genetic polymorphisms within the renin–angiotensin system: no evidence for an association with nephropathy in IDDM. Diabetologia. 1996;39:1108–14.
23. De Cosmo S, Margaglione M, Tassi V, Garrubba M, Thomas S, Olivetti C, et al. ACE PAI-1, decorin and Werner helicase genes are not associated with the development of renal disease in European patients with type 1 diabetes. Diabetes Metab Res Rev. 1999;15:247–53.
24. Ding QW, Ma YN, Jiang XQ. Association relationship between type II diabetic nephropathy with angiotensin converting enzyme. J Taishan Med Coll. 2012;33:358–9 [article in Chinese].
25. Doi Y, Yoshizumi H, Yoshinari M, Iino K, Yamamoto M, Ichikawa K, et al. Association between a polymorphism in the angiotensin-converting enzyme gene and microvascular complications in Japanese patients with NIDDM. Diabetologia. 1996;39:97–102.
26. Dudley CR, Keavney B, Stratton IM, Turner RC, Ratcliffe PJ. U.K. Prospective Diabetes Study. XV: Relationship of

renin-angiotensin system gene polymorphisms with microalbuminuria in NIDDM. Kidney Int. 1995;48:1907–11.
27. Eroglu Z, Cetinkalp S, Erdogan M, Kosova B, Karadeniz M, Kutukculer A, et al. Association of the angiotensinogen M235T and angiotensin-converting enzyme insertion/deletion gene polymorphisms in Turkish type 2 diabetic patients with and without nephropathy. J Diabetes Complicat. 2008;22:186–90.
28. Freire MB, van Dijk DJ, Erman A, Boner G, Warram JH, Krolewski AS. DNA polymorphisms in the ACE gene, serum ACE activity and the risk of nephropathy in insulin-dependent diabetes mellitus. Nephrol Dial Transplant. 1998;13:2553–8.
29. Fu HM, Wu HW, Yang JP. Association between the angiotensin-converting enzyme insertion/deletion gene polymorphisms and diabetic nephropathy in Western Hunan province. Chin J Nephrol. 2002;18:228–9 [article in Chinese].
30. Gallego PH, Shephard N, Bulsara MK, van Bockxmeer FM, Powell BL, Beilby JP, et al. Angiotensinogen gene T235 variant: a marker for the development of persistent microalbuminuria in children and adolescents with type 1 diabetes mellitus. J Diabetes Complicat. 2008;22:191–8.
31. Gao YT, Li ZJ, Wang XM. Association of angiotensin concerting enzyme gene polymorphism with type 2 diabetic kidney disease. J Shaanxi Med. 2014;43:1283–6 [article in Chinese].
32. Grzeszczak W, Zychma MJ, Lacka B, Zukowska-Szczechowska E. Angiotensin I-converting enzyme gene polymorphisms: relationship to nephropathy in patients with non-insulin dependent diabetes mellitus. J Am Soc Nephrol. 1998;9:1664–9.
33. Gu MF, Deng ZX, Tang JY. Relationship between angiotensin I converting enzyme gene polymorphism and diabetic nephropathy in type 2 diabetes. Jiangsu Med J. 2000;26:169–71 [article in Chinese].
34. Guo MQ, Li L, Miao C. Relationshhip between ACE and AT1R gene polymorphism and diabetic nephropathy. Central China Med J. 2007;31:25–7 [article in Chinese].
35. Gutierrez C, Vendrell J, Pastor R, Llor C, Aguilar C, Broch M, et al. Angiotensin I-converting enzyme and angiotensinogen gene polymorphisms in non-insulin-dependent diabetes mellitus Lack of relationship with diabetic nephropathy and retinopathy in a Caucasian Mediterranean population. Metabolism. 1997;46:976–80.
36. Hadjadj S, Gallois Y, Alhenc-Gelas F, Chatellier G, Marre M, Genes N, et al. Angiotensin-I-converting enzyme insertion/deletion polymorphism and high urinary albumin concentration in French Type 2 diabetes patients. Diabet Med. 2003;20:677–82.
37. Hadjadj S, Tarnow L, Forsblom C, Kazeem G, Marre M, Groop PH, et al. Association between angiotensin-converting enzyme gene polymorphisms and diabetic nephropathy: case–control, haplotype, and family-based study in three European populations. J Am Soc Nephrol. 2007;18:1284–91.
38. Hibberd ML, Millward BA, Demaine AG. The angiotensin I-converting enzyme (ACE) locus is strongly associated with age and duration of diabetes in patients with type I diabetes. J Diabetes Complicat. 1997;11:2–8.
39. Hsieh MC, Lin SR, Hsieh TJ, Hsu CH, Chen HC, Shin SJ, et al. Increased frequency of angiotensin-converting enzyme DD genotype in patients with type 2 diabetes in Taiwan. Nephrol Dial Transplant. 2000;15:1008–13.
40. Huang XH, Rantalaiho V, Wirta O, Pasternack A, Hiltunen TP, Koivula T, et al. Angiotensin-converting enzyme insertion/deletion polymorphism and diabetic albuminuria in patients with NIDDM followed up for 9 years. Nephron. 1998;80:17–24.
41. Huang ZG, Zhang YS, Cao MY. The association between ACE AT1R gene polymorphisms and diabetic nephropathy. Shandong Med J. 2004;44:15–7 [article in Chinese].
42. Ilic V, Ilic M, Soldatovic I, Popovic S, Magic Z. Association of renin–angiotensin system genes polymorphism with progression of diabetic nephropathy in patients with type 1 diabetes mellitus. Vojnosanit Pregl. 2014;71:627–33.
43. Jayapalan JJ, Muniandy S, Chan SP. Null association between ACE gene I/D polymorphism and diabetic nephropathy among multiethnic Malaysian subjects. Indian J Hum Genet. 2010;16:78–86.
44. Jeffers BW, Estacio RO, Raynolds MV, Schrier RW. Angiotensin-converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus and its relationship with diabetic nephropathy. Kidney Int. 1997;52:473–7.
45. Lee YJ, Tsai JC. ACE gene insertion/deletion polymorphism associated with 1998 World Health Organization definition of metabolic syndrome in Chinese type 2 diabetic patients. Diabetes Care. 2002;25:1002–8.
46. Li C, Master thesis of Suzhou Medical University The role of ACE gene insertion/deletion polymorphism in the end stage renal disease of type 2 diabetes; 2003 [article in Chinese].
47. Li M, Liu LM, Zheng TS. Association between angiotensin I converting enzyme gene insertion/deletion polymorphism and Type 2 diabetes nephropathy. Shanghai Med J. 2004;27:457–9 [article in Chinese].
48. Li YM, Q XY. A paired case-control study on the association between polymorphism of angiotensin converting enzyme gene and diabetic nephropathy in type 2 diabetic patients. Chin J Prev Contr Chron Non-commun Dis. 2005;13:12–3. [article in Chinese].
49. Liao YH, Sun AY, Xian S. The relationship between ACE gene polymorphism and type 2 diabetic nephropathy in GuangXi province. Clin Assemble. 2002;17:378–9 [article in Chinese].
50. Liu PL, Chen YL. Association between ACE AGT gene polymorphism and diabetic nephropathy. Chinese J Gerontol. 2015;35:4177–9 [article in Chinese].
51. Liu XN, Wang Y, Li TT. Association between ACE gene re4353 polymorphism and diabetic microangiopathy. Int J Endocrinol Metab. 2018;38:149–53 [article in Chinese].
52. Liu YY, Ma L, Jiang YW. Relationship between insertion/deletion polymorphism of angiotensin converting enzyme gene and type 2 diabetic kidney disease. Chin J Lab Med. 2019;42:116–22 [article in Chinese].
53. Mansouri M, Zniber A, Boualla L, El Badaoui G, Benkacem M, Rifai K, et al. Associations between clinical characteristics and angiotensin-converting enzyme gene insertion/deletion polymorphism in Moroccan population with Type-2 diabetic nephropathy. Saudi J Kidney Dis Transpl. 2017;28: 261–7.
54. Marre M, Bernadet P, Gallois Y, Savagner F, Guyene TT, Hallab M, et al. Relationships between angiotensin I converting enzyme gene polymorphism, plasma levels, and diabetic retinal and renal complications. Diabetes. 1994;43:384–8.
55. Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, et al. Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes: Genetique de la Nephropathie Diabetique (GENEDIAB) study group. J Clin Invest. 1997;99:1585–95.
56. Miura J, Uchigata Y, Yokoyama H, Omori Y, Iwamoto Y. Genetic polymorphism of renin-angiotensin system is not associated with diabetic vascular complications in Japanese subjects with long-term insulin dependent diabetes mellitus. Diabetes Res Clin Pract. 1999;45:41–9.
57. Mollsten A, Kockum I, Svensson M, Rudberg S, Ugarph-Morawski A, Brismar K, et al. The effect of

polymorphisms in the renin–angiotensin–aldosterone system on diabetic nephropathy risk. J Diabetes Complicat. 2008;22:377–83.
58. Movva S, Alluri RV, Komandur S, Vattam K, Eppa K, Mukkavali KK, et al. Relationship of angiotensin-converting enzyme gene polymorphism with nephropathy associated with Type 2 diabetes mellitus in Asian Indians. J Diabetes Complicat. 2007;21:237–41.
59. Nakajima S, Baba T, Yajima Y. Is ACE gene polymorphism a useful marker for diabetic albuminuria in Japanese NIDDM patients? Diabetes Care. 1996;19:1420–2.
60. Oh TG, Shin CS, Park KS, Kim SY, Cho BY, Lee HK, et al. Relationships between angiotensin I converting enzyme gene polymorphism and renal complications in Korean IDDM patients. Korean J Intern Med. 1996;11:133–7.
61. Ohno T, Kawazu S, Tomono S. Association analyses of the polymorphisms of angiotensin-converting enzyme and angiotensinogen genes with diabetic nephropathy in Japanese non-insulin-dependent diabetics. Metabolism. 1996;45:218–22.
62. Okuno S, Utsugi T, Ohno T, Ohyama Y, Uchiyama T, Tomono S, et al. Angiotensin-converting enzyme gene polymorphism as a potent risk factor for developing microalbuminuria in Japanese patients with type 2 diabetes mellitus: a 9-year follow-up study. J Int Med Res. 2003;31:290–8.
63. Oue T, Namba M, Nakajima H, Ono A, Horikawa Y, Yamamoto K, et al. Risk factors for the progression of microalbuminuria in Japanese type 2 diabetic patients—a 10 year follow-up study. Diabetes Res Clin Pract. 1999;46: 47–55.
64. Panagiotopoulos S, Smith TJ, Aldred GP, Baker EJ, Jacklin CJ, Jerums G. Angiotensin-converting enzyme (ACE) gene polymorphism in type II diabetic patients with increased albumin excretion rate. J Diabetes Complicat. 1995;9:272–6.
65. Park HC, Choi SR, Kim BS, Lee TH, Kang BS, Choi KH, et al. Polymorphism of the ACE Gene in dialysis patients: overexpression of DD genotype in type 2 diabetic end-stage renal failure patients. Yonsei Med J. 2005;46:779–87.
66. Pong XF, Gong YX, Zhu LM. Association of polymorphism in ACE gene with diabetes and diabetic nephropathy in elderly. Geriatr Health Care. 2001;7:94–6 [article in Chinese].
67. Powrie JK, Watts GF, Ingham JN, Taub NA, Talmud PJ, Shaw KM. Role of glycaemic control in development of microalbuminuria in patients with insulin dependent diabetes. BMJ. 1994;309:1608–12.
68. Prasad P, Tiwari AK, Kumar KM, Ammini AC, Gupta A, Gupta R, et al. Chronic renal insufficiency among Asian Indians with type 2 diabetes: I. Role of RAAS gene polymorphisms. BMC Med Genet. 2006;7:42.
69. Ringel J, Beige J, Kunz R, Distler A, Sharma AM. Genetic variants of the renin–angiotensin system, diabetic nephropathy and hypertension. Diabetologia. 1997;40:193–9.
70. Schmidt S, Schone N, Ritz E. Association of ACE gene polymorphism and diabetic nephropathy? The Diabetic Nephropathy Study Group. Kidney Int. 1995;47:1176–81.
71. Schmidt S, Strojek K, Grzeszczak W, Bergis K, Ritz E. Excess of DD homozygotes in haemodialysed patients with type II diabetes. The Diabetic Nephropathy Study Group. Nephrol Dial Transplant. 1997;12:427–9.
72. Seruga M, Makuc J, Zavrsnik M, Cilensek I, Ekart R, Petrovic D. Polymorphism of angiotensin-converting enzyme (rs4340) and diabetic nephropathy in Caucasians with type 2 diabetes mellitus. Balkan J Med Genet. 2016;19:29–34.
73. Sun H, Master thesis of Inner Mongolia Medical University The association between ACE gene polymorphism and diabetic nephropathy; 2006.
74. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Lecerf L, et al. Lack of relationship between an insertion/deletion polymorphism in the angiotensin I-converting enzyme gene and diabetic nephropathy and proliferative retinopathy in IDDM patients. Diabetes. 1995;44:489–94.
75. Tien KJ, Hsiao JY, Hsu SC, Liang HT, Lin SR, Chen HC, et al. Gender-dependent effect of ACE I/D and AGT M235T polymorphisms on the progression of urinary albumin excretion in Taiwanese with type 2 diabetes. Am J Nephrol. 2009;29:299–308.
76. Viswanathan V, Zhu Y, Bala K, Dunn S, Snehalatha C, Ramachandran A, et al. Association between ACE gene polymorphism and diabetic nephropathy in South Indian patients. JOP. 2001;2:83–7.
77. Wang Y, Master thesis of Tianjing Medical University Association between ACE gene polymorphism and type 2 diabetic nephropathy in Tianjing; 2001 [Article in Chinese].
78. Wang Q, Ma ZX, Qiu SJ. Relationship between ACE gene polymorphism and type 2 diabetic nephropathy. Shandong Med J. 2007;47:71–2 [article in Chinese].
79. Wyawahare M, Neelamegam R, Vilvanathan S, Soundravally R, Das AK, Adithan C. Association of angiotensin-converting enzyme gene polymorphisms and nephropathy in diabetic patients at a tertiary care centre in South India. Clin Med Insights Endocrinol Diabetes. 2017;10, 1179551417726779.
80. Xu L, Zhang XY, Ma YY. Association between AT1R and ACE gene polymorphism and Chinese type 2 diabetes with nephropathy. Acta Academiae Medicinae Shandong. 2001;39:514–6 [article in Chinese].
81. Xue YF, Li CC, Lin HL. Association between ACE gene polymorphisms and type 2 diabetic nephropathy. J Postgrad Med. 2000;23:26–8 [article in Chinese].
82. Yan XF, Pan SZ, Yang LY. Correlation analysis of polymorphisms of ACE gene PAI-1 gene and nephropathy in type 2 diabetes. China Med. 2008;3:81–3 [article in Chinese].
83. Yang T, Chen JW, Zhou HW. A study for gene polymorphism in Chinese type 2 diabetic nephropathy. J Qiqihar Med Coll. 2003;24:1086–8 [article in Chinese].
84. Young RP, Chan JC, Critchley JA, Poon E, Nicholls G, Cockram CS. Angiotensinogen T235 and ACE insertion/deletion polymorphisms associated with albuminuria in Chinese type 2 diabetic patients. Diabetes Care. 1998;21:431–7.
85. Zhang QS, Yang QM, Li L. Association between ACE gene insertion/deletion polymorphism and carotid intima-media thickness in Hans Chinese type 2 diabetic patients from Chenzhou. J Xiangnan Univ (Med Sci). 2011;13:4–7 [article in Chinese].
86. Zhao CX, Hu DN, Xie CP. Relationship between polymorphism of ACE gene and NIDDM nephropathy. J Chinese Aerospace Ind Med. 2001;3:35–7 [article in Chinese].
87. Zhong WP, Qin JR, He SJ. Association of the polymorphisms in the RAS with type 2 diabetes mellitus and diabetic nephropathy. Basic Clin Med. 2005;25:446–50 [article in Chinese].
88. Zhong W. The analysis of ACE I/D, ATG T174M gene polymorphisms in type 2 diabetic nephropathy. J Guangxi Med Univ. 2008;25:908–10 [article in Chinese].
89. Ma VL, Lin SL. Examining the rare disease assumption used to justify HWE testing with control samples. Math Biosci Eng. 2019;17:73–91.
90. Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. How should we use information about HWE in the meta-analyses of genetic association studies? Int J Epidemiol. 2008;37:136–46.
91. Trikalinos TA, Salanti G, Khoury MJ, Ioannidis JP. Impact of violations and deviations in Hardy–Weinberg equilibrium on postulated gene-disease associations. Am J Epidemiol. 2006;163:300–9.

92. Wang F, Fang Q, Yu N, Zhao D, Zhang Y, Wang J, et al. Association between genetic polymorphism of the angiotensin-converting enzyme and diabetic nephropathy: a meta-analysis comprising 26,580 subjects. J Renin Angiotensin Aldosterone Syst. 2012;13:161–74.
93. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest. 1990;86:1343–6.
94. Crisan D, Carr J. Angiotensin I-converting enzyme: genotype and disease associations. J Mol Diagn. 2000;2:105–15.
95. Mizuiri S, Hemmi H, Kumanomidou H, Iwamoto M, Miyagi M, Sakai K, et al. Angiotensin-converting enzyme (ACE) I/D genotype and renal ACE gene expression. Kidney Int. 2001;60:1124–30.
96. Mahwish UN, Ponnaluri KC, Heera B, Alavala SR, Devi KR, Raju SB, et al. Link between ACE I/D gene polymorphism and dyslipidemia in diabetic nephropathy: a case–control study from Hyderabad, India. Indian J Nephrol. 2020;30: 77–84.
97. Tseng CH, Tseng CP, Chong CK. Joint effects of hypertension, smoking, dyslipidemia and obesity and angiotensin-converting enzyme DD genotype on albuminuria in Taiwanese patients with type 2 diabetes mellitus. Clin Biochem. 2010;43:629–34.
98. Ng DP, Tai BC, Koh D, Tan KW, Chia KS. Angiotensin-I converting enzyme insertion/deletion polymorphism and its association with diabetic nephropathy: a meta-analysis of studies reported between 1994 and 2004 and comprising 14,727 subjects. Diabetologia. 2005;48:1008–16.
99. Kajantie E, Rautanen A, Kere J, Andersson S, Ylihärsilä H, Osmond C, et al. The effects of the ACE gene insertion/deletion polymorphism on glucose tolerance and insulin secretion in elderly people are modified by birth weight. J Clin Endocrinol Metab. 2004;89:5738–41.
100. Zhang B, Shono N, Fan P, Ando S, Xu H, Jimi S, et al. Histochemical characteristics of soleus muscle in angiotensin-converting enzyme gene knockout mice. Hypertens Res. 2005;28:681–8.
101. Liu N, Wang Y. Association between angiotensinogen T174M polymorphism and the risk of diabetic nephropathy: a meta-analysis. J Renin Angiotensin Aldosterone Syst. 2019;20, 1470320318823927.
102. Cui ZH, Lu XT, Xiao KL, Chen Y, Li HQ. Association of interleukin-6-174G/C polymorphism with the risk of diabetic nephropathy in type 2 diabetes: a meta-analysis. Curr Med Sci. 2019;39:250–8.