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ACE insertion/deletion polymorphism and diabetic nephropathy: an evidence-based meta-analysis

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ABSTRACT. The angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) polymorphism could influence predisposition for diabetic nephropathy (DN) by vascular modulation in the kidney, through a direct effect on cellular hypertrophy. However, studies on the association between this polymorphism and DN report conflicting results. To help determine if this association exists, we conducted a meta-analysis. Published studies until 2018 were researched from electronic databases PubMed/NCBI and Cochrane Library. Thirty studies including 4,774 DN cases and 4,357 individuals without DN were included in this meta-analysis. Extraction of data from all eligible publications was performed by two investigators independently, according to the inclusion and exclusion criteria. We used the statistical software "R" by the overall odds ratio (OR) with a 95% confidence interval to evaluate the association of ACE I/D polymorphism with a possible risk towards DN development. We included various genetic model analyses, sensitivity analyses, and assessments of bias in our meta-

analysis. We found a significant association for ACE I/D polymorphism; the D allele is a predisposing factor for DN in diabetic patients. The risk for development of diabetes complications, such as DN, is highly complex and could be considered multifactorial. In summary, our meta-analysis shows that the ACE I/D polymorphism is associated with susceptibility to DN.

Key words: *ACE I/D* polymorphism; Diabetic nephropathy; Genetic association; Systematic reviews; Meta-analysis

INTRODUCTION

Diabetic nephropathy (DN) is a multifactorial and polygenic complication of diabetes mellitus (DM) progression. It is characterized by a microvascular complication of the kidney, weakening of the glomerular basement membrane, decreased numbers of podocytes, reduced glomerulus function, and tubulointerstitial fibrosis, resulting in an increase of proteins excreted through urine, mainly albumin, and progressive loss of kidney function. A cumulative incidence of DN in the last 10 years has been observed, mainly in patients with type 2 diabetes mellitus (T2DM), being an important cause of morbidity and mortality among these individuals (Luo et al., 2013; ADA, 2018). DN occurs in family groups; thus genetic factors have been investigated to elucidate susceptibility to development of this disease (Doi et al., 1996).

The angiotensin-converting enzyme (ACE) gene, located on chromosome 17, expressed in a vast variety of tissues, including lungs, vascular endothelium, kidney, heart and testicles, has been the object of studies as an important regulator of blood pressure and kidney electrolyte homeostasis through the renin-angiotensin system (RAS) (Freathy et al., 2006). The RAS is able to contribute significantly to hemodynamic stability through blood pressure regulation, fluid homeostasis, glucose and electrolyte metabolism, and in the modulation of cell growth and proliferation of various tissues (Ahluwalia et al., 2009).

Changes in blood pressure levels linked to chronic diabetic hyperglycemia adversely affect nephrons, which may present sclerosis by a direct effect on cellular hypertrophy, influencing proliferation and rupture of the extracellular matrix (Kennon et al., 1999; Tomino et al., 1999), generating a predisposition to DN by kidney vascular modulation. Various studies indicate that the genes involved in this RAS are highly polymorphic and most DNA variants result in inefficient hemodynamic stability.

It is known that *ACE* I/D polymorphism (rs1799752), caused by insertion (I allele) or deletion (D allele) of 287bp in intron 16, causes instability of RAS and dysregulates micro and macrocirculation and kidney electrolytic homeostasis (Pereira et al., 2003). Around 1995 the first reports appeared of the *ACE* Insertion (I) / Deletion (D) polymorphism, related to three possible genotypes (wild I/I, heterozygous I/D and mutant D/D) (Schmidt et al., 1995, Fujisawa et al., 1995, Mizuiri et al., 1995). The variants of this polymorphism reflect different levels of serum ACE in healthy individuals, therefore suggesting that the *ACE* gene plays a significant role in the pathogenesis of essential hypertension, resulting in increased intraglomerular pressure and decreased kidney function (Pereira et al., 2003).

The severity of DN is related not only to exposure time, but also to the success of disease control, associated risk factors, and comorbidities involved (Higgins et al., 2008). Genomic variations in populations play a fundamental role in the natural selection of the most advantageous genotypes in a given ecological condition; therefore, these characteristics distributed to their descendants become increasingly heterogeneous, making it difficult to associate genetic factors with the development of chronic diseases, such as DN (Arnett et al.,

2018). The aim of our study was to investigate whether ACE I/D polymorphism is associated with DN risk.

MATERIAL AND METHODS

Protocol and Search

The search protocol was deposited in PROSPERO (CRD42018083792) published January 10, 2018. The following search terms were used: "polymorphism" AND "*ACE* gene" AND "diabetic nephropathy".

Information Sources

The research was done in three stages from July 2017 to May 2018. First, a search was made using words and terms that define the article. Secondly, all the key-words and terms were identified in electronic bibliographic databases National Center for Biotechnology Information (NCBI/PubMed) and Cochrane Library. Third, the reference lists of selected studies were also checked for other potentially relevant studies.

Eligibility Criteria

For this study we considered case-controlled-studies published and not published (Grey literature), without language restriction, from 1995 to 2018. Information was carefully extracted from all eligible publications independently by two of the investigators, according to the inclusion criteria: a) studies with human beings; b) adults and ethnicities all of the countries; c) individuals diagnosed with T2DM according to the World Health Organization (WHO); d) all participants in the studies should have been evaluated for kidney function, through the rate of albumin excretion and glomerular filtration rate, following the reference values: microalbuminuria between 20-200 μg / min or 30-300 mg per 24 h; macroalbuminuria ≥ 300 mg / 24 hours with persistent proteinuria greater than 500 mg / 24 h; Glomerular filtration rate (GFR) ≤ 60 mL/min/ 1.73 m2; e) minimum time of 3 years of diagnosis of T2DM for both groups to be analyzed; f) genotype of the *ACE* polymorphism (I/I, I/D and D/D); g) molecular analysis of the polymorphism detected by the Polymerase Chain Reaction (PCR) technique.

After the search, all quotes were linked and loaded in library software Mendeley and duplicates removed. The titles and abstracts were selected by two independent reviewers (L.C.S.) and (E.G.S.) for assessment in relation to the inclusion criteria recovered in their entirety and their details were included in The System for the Unified Management, Assessment and Review of Information (SUMARI) the Joanna Briggs Institute's (JBI) software for the systematic review of literature. Full-text articles were saved and evaluated in detail in relation to the inclusion criteria being submitted to a critical assessment. The results of the research are fully reported and presented in the flow diagram PRISMA. Any disagreement between reviewers was resolved by discussion with a third coauthor.

Statistical analysis

For the application of a more adequate meta-analytical model and obtaining the combined estimate, its variance and confidence intervals, consideration should be given to the classification of statistical tests. The I^2 test was used to estimate the proportion of total

variability in percentages attributed to heterogeneity other than that due to chance. Data were pooled according to level of between-study heterogeneity, using the following strategy: I^2 <25%, fixed effects meta-analysis to estimate the common prevalence (CI-95%), assuming that all or most between-study variability is due to chance; I^2 25-75%, random effects meta-analysis to estimate the average prevalence (CI-95%); I^2 >75%, heterogeneity too great for summary estimate to be calculated. RStúdio® software was used to assemble the results of included studies.

RESULTS

After the initial search, 2310 studies were reviewed based on the title and abstract. Of these, 637 were removed as duplicates, 1557 excluded because they did not meet any inclusion criteria (not an original investigation, not observational design, not in humans, not in adults), 116 were recovered as full- text. After reading the texts, 83 studies were of the cohort type or did not present methodologies with outcomes that were relevant to our study, 33 studies were included in the qualitative synthesis for evaluation of methodological quality (Table 1), after assessment thre studies were excluded because they did not present the score determined by the authors for quality (1 study presented the time of exposure different for control group and for the case group, two other studies presented confounding factors, not allowing to the standardized quality evaluation, validity and reliable methodological system), 30 studies were included for quantitative synthesis (meta-analysis) (Figure 1). In the study of Parchawani et al. (2014), two different communities were evaluated (Ahri community and Rabari community), duplicating the results in A and B for the same reference.

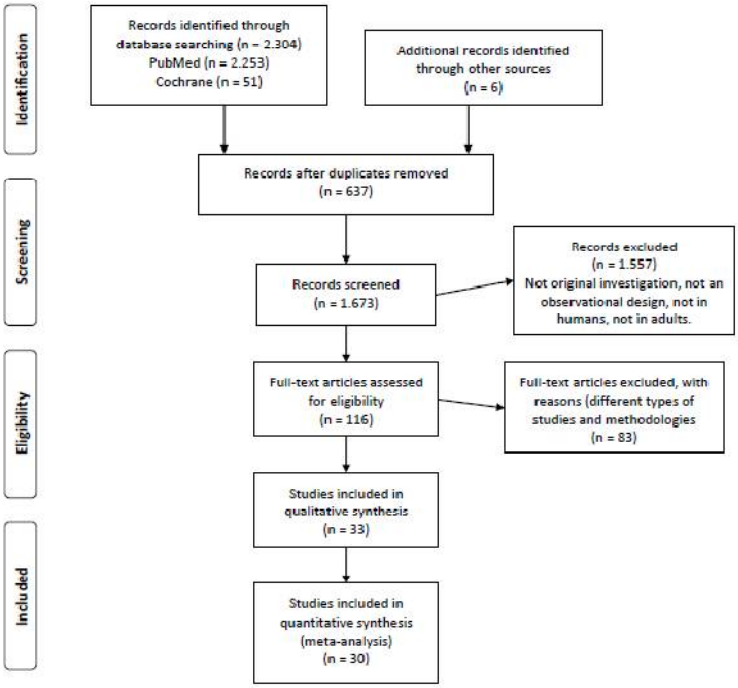

Figure 1. Flowchart of database searching and the record screening process.

Table 1. Assessment of methodological quality of the studies.

CITATION	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1.Fujisawa 1995	Y	Y	Y	Y	Y	N	N	Y	Y	Y
2.Mizuiri 1995	Y	Y	Y	Y	Y	N	N	Y	Y	Y
3.Panagiotopoulos 1995	Y	Y	Y	Y	Y	N	N	Y	Y	Y
4. Doi 1996	Y	Y	Y	Y	N	N	N	U	Y	Y
5.Ringel 1997	Y	Y	Y	Y	Y	N	N	Y	Y	Y
6.Schimidt 1997	Y	Y	Y	Y	Y	N	N	Y	Y	Y
7.Grzeszczak 1998	Y	Y	Y	Y	Y	N	N	Y	Y	Y
8.Hanyu 1998	Y	Y	Y	Y	Y	N	N	Y	Y	Y
9.Tomino 1999	Y	Y	Y	Y	Y	N	N	Y	Y	Y
10.Solini 1999	Y	Y	Y	Y	Y	N	N	Y	Y	Y
11.Taniwaki 2001	Y	Y	Y	Y	Y	N	N	Y	Y	Y
12.Viswanathan 2001	Y	Y	Y	Y	Y	N	N	Y	Y	Y
13.Arzu 2004	Y	Y	Y	Y	Y	N	N	Y	Y	Y
14.Prasad 2006	Y	Y	Y	Y	N	N	N	Y	Y	Y
15.Ng 2006	Y	Y	Y	Y	Y	N	N	Y	Y	Y
16.Nikzamir 2006	Y	Y	Y	Y	Y	N	N	Y	Y	Y
17.Uddin 2007	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
18.Arfa 2008	Y	Y	Y	Y	Y	N	N	Y	Y	Y
19.Ezzidi 2009	Y	Y	Y	Y	Y	N	N	Y	Y	Y
20.Ahluwalia 2009	Y	Y	Y	Y	Y	N	N	Y	Y	Y
21.Palomo-Pinon 2009	Y	Y	Y	Y	Y	N	N	Y	Y	Y
22.El-Bazz 2011	Y	Y	Y	U	U	N	N	Y	Y	U
23.Al-Harbi 2011	Y	Y	Y	Y	Y	N	N	Y	Y	Y
24.Felehgari 2011	Y	Y	Y	N	Y	N	N	Y	N	Y
25.Vashrambhai 2011	Y	Y	Y	Y	Y	N	N	Y	Y	Y
26.Pacheco 2012	Y	Y	Y	Y	Y	N	N	Y	Y	Y
27.El-Baz 2012	Y	Y	Y	Y	Y	N	N	Y	Y	Y
28.Shaikh 2012	Y	Y	Y	Y	Y	N	N	Y	Y	Y
29.Bhaskar 2013	Y	Y	Y	U	Y	N	N	Y	Y	Y
30.Shaker 2014	Y	Y	Y	Y	Y	N	N	Y	Y	Y
31-A.Parchwani 2014	Y	Y	Y	Y	Y	N	N	Y	Y	Y
31-B.Parchwani 2014	Y	Y	Y	Y	Y	N	N	Y	Y	Y
32.Sancakdar 2015	Y	Y	Y	U	Y	N	N	N	Y	Y
33.Fawwaz 2017	Y	Y	Y	Y	Y	N	N	Y	Y	Y
%	**100%**	**100%**	**100%**	**88%**	**90%**	**6%**	**0%**	**94%**	**97%**	**97%**

Y=yes, N=no, U=unclear.

Question 1. Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?
Question 2. Were cases and controls matched appropriately?
Question 3. Wer the same criteria used for the identification of cases and controls?
Question 4. Was exposure measured in a standard, valid and reliable way?
Question 5. Was exposure measured in the same way for cases and controls?
Question 6. Wer confounding factors identified?
Question 7. Wer strategies to deal with confounding factors stated?
Question 8. Wer outcomes assessed in a standard, valid and reliable way for cases and controls
Question 9. Was the exposure period of interest long enough to be meaningful?
Question 10. Was an appropriate statistical analysis used?

In this meta-analysis, a total of 9131 patients with T2DM were genotyped, being 4774 (52.3%) individuals with DN and 4357 (47.7%) individuals without DN. The genotypes for each group, presented I/I (23.6%), I/D (45.8%) and D/D (30.6%) for the case group, and I/I (28.6%), I/D (46.4%) and D/D (25%) for the control group. For the individuals of the case group and control group the genotype I/D had a greater prevalence with 45.8 and 46.4% respectively.

In the demographic characteristics, when comparing the case and control groups in relation to gender, the case group consisted of 4136 individuals (2235 males and 1901 females), the mean age being 58.34 ± 4.18 and a predominance of males (54.04%). The control group

consisted of 3,834 individuals (1813 males and 2021 females), the mean age of the patients was 58.52 ± 3.71 and the women predominated in the group (52.7%). The statistical analysis of the distribution by gender presented significant differences, indicating that there is heterogeneity between the groups studied. When age was observed, the groups did not present significant differences, indicating homogeneity between them.

The Test for Hardy-Weinberg Equilibrium showed that in the case group with 4774 subjects; the frequency of the mutant D allele was 54% and the wild-type I allele was 46%.

The control group with 4,357 individuals had the wild-type I allele in 52% and the mutant D allele with 48%.

The graphs in the meta-analysis are forest-plot. In this context, two graphs were generated and there no association was found between *ACE* I/D polymorphism and DN, but an association of polymorphism D/D with DN was found. The forest-plot for genotype I/D with and without DN showed heterogeneity: I^2 59%; P < 0.01; OR=0.97; CI- 95% = [0.84; 1.12] (Figure 2) and for the genotype D/D with and without DN showed heterogeneity: I^2 69%; P < 0.01; OR=1.54; CI- 95% = [1.27; 1.86] (Figure 3).

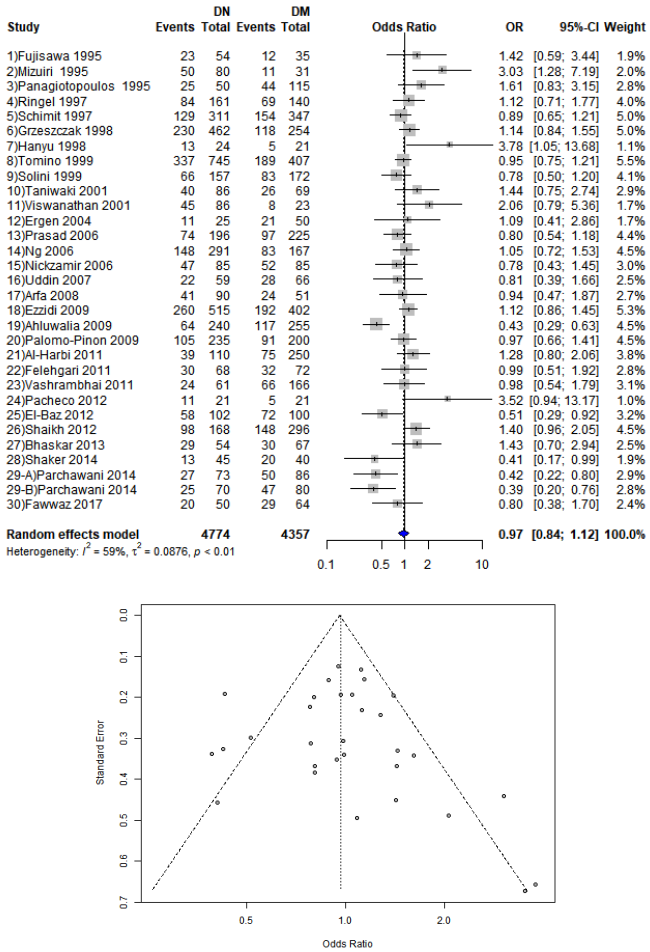

Figure 2. Forest-plot and funnel-plot for the genotype I/D. Odds ratio (OR) and confidence interval of 95% (CI-95%) with lower and upper limits, with the I^2 test of heterogeneity use of the DerSimonian-Laird test.

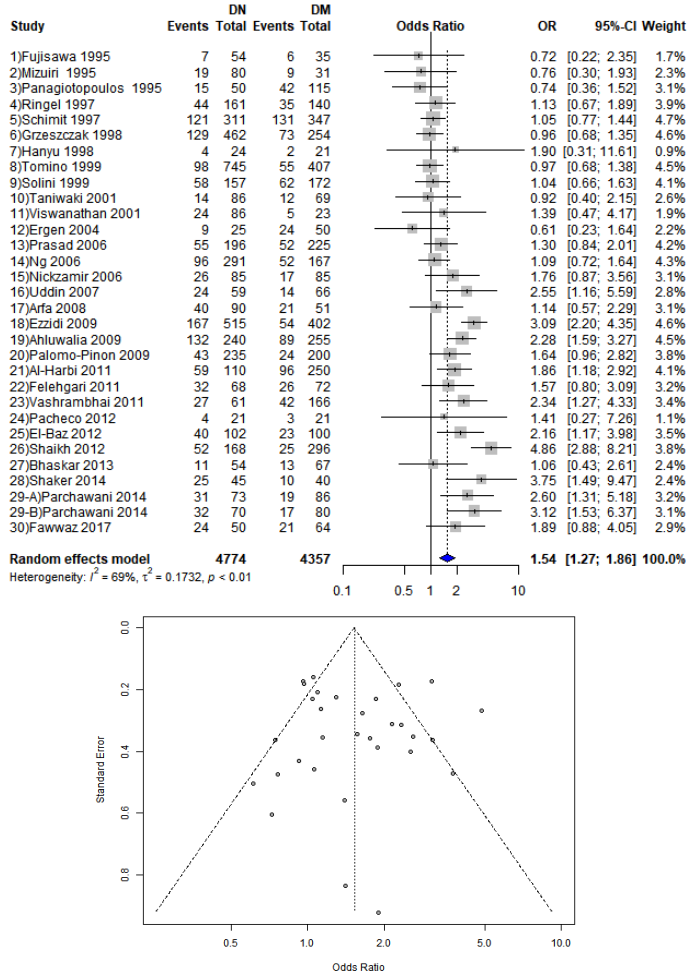

Figure 3. Forest-plot and funnel-plot for the genotype D/D. Odds ratio (OR) and confidence interval of 95% (CI-95%) with lower and upper limits, with the I^2 test of heterogeneity use of the DerSimonian-Laird test.

DISCUSSION

Numerous investigations into the potential role of the *ACE* gene involved in susceptibility to DN were made in the last decades; but the results are still controversial. Previous meta-analyses (Fujisawa et al., 1998; Young et al., 1998; Viana et al., 2012; Luo et al., 2013) have tried to reconcile these findings; however, by attempting to draw conclusions with limited data they were particularly impaired when examining specific subgroups of patients.

Since 1992 (Cambien et al., 1992) the D/D genotype, which denotes homozygous deletion of this 287 bp, has been found to be associated with a significantly higher risk of developing chronic diseases, such as cardiovascular metabolic diseases and, mainly T2DM. This polymorphism was associated with DN, a progression of T2DM, due to its role in the

implication of pathological mechanisms through alteration of endothelial β cells, oxidative stress, and renal microvascular complications (Panagiotoupolos et al., 1995).

Onset and progression to DN have been investigated in various ethnic groups with inconsistent results. Rahimi (2012) suggested that ethnicity is one of the most important factors, which determines the role of ACE polymorphism in the susceptibility to DN. Moreover, different regions of the world showed a contradictory frequency distribution of the mutant D allele in T2DM patients who developed DN complication (Bhaskar et al., 2013). The articles are inconclusive in all populations studied for the genotypic association of *ACE* I/D polymorphism and DN, but in allelic frequency analysis shown a strong association of the D allele with diabetic proteinuria in T2DM linked DN (Doi et al., 1996; Ohno et al., 1996, Yoshida et al., 1996, Viswanathan et al., 2001).

Ezzidi et al (2009) compared the effects of the *ACE* polymorphism (rs1799752) on the risk of DN. For this polymorphism, the D/D genotype was associated with a significant increase in albumin to creatinine ratios levels, and D/D carriers had elevated low-density lipoprotein, total cholesterol, and urea. These authors described that D allele is a DN-predisposing factor (OR = 2.29, 95% CI =1.90–2.77). Moreover, the D allele was associated with increased serum ACE activity in relation to I allele. On the other hand, the hyperglycemia affects the glomerular filtration rate (GFR) in diabetics with the DD genotype and it induces a GFR increase that correlates with ACE plasma levels (Ruggenenti et al., 2008).

The D/D genotype was associated with albuminuria in females indicating a possible gender effect associated with the renal disease (Yoshida et al., 1996). Modulating the estradiol levels and menopause symptoms with genetic factors may influence in susceptibility of the disease (Nikzamir et al., 2006). Therefore, the treatment with estradiol decreases ACE activity (Palomo-Pinon et al., 2009) suggesting that estrogens may play an important role in attenuating the vascular and renal response to angiotensin II. Additionally, that hormone regulates the stimulatory effects of transforming growth factor – β1 (TGF- β1) to the process of apoptosis of mesangial cells, characteristic of renal progression in DN (Gallagher et al., 1999, He et al., 2007; Rogers et al., 2007).

In our study, the case and control group showed a mean age compatible with the age of menopause onset, although the case group presented more males than the control group. In allelic frequency, the D allele is present in most nephropathy patients and is a minority in the normoalbuminuric patients, presenting 54% in the case group and 48% in the control group; it is a significant risk factor for the development of the disease, while allele I is a protective factor (Uddin et al., 2007).

Biomarkers (Jha et al., 2013), including various detectable tubular proteins and enzymes due to the involvement of the tubules and the kidney interstitium, precede glomerular involvement and a drop in GFR, The increasingly common recognition of the kidney disease phenotype does not characterize albuminuric syndrome by isolated reduction of GFR, thus, their choice to diagnose DM for inclusion in normoalbuminuric groups is representative and reliable for the target population (Malta et al., 2010).

Consequently, genotype I/I for the *ACE* polymorphism could be a reduced risk marker, meaning that patients are less likely to develop DN during the course of T2DM (Boright et al., 2005; Freathy et al., 2006; Bhaskar et al., 2013; Shaker et al., 2014; Mizuiri et al., 1995; Verlengia et al., 2014). Our study demonstrated that the D/D genotype evaluated separately is a marker for predicting the susceptibility to DN of T2DM carriers

even after adjustments for potential confounders, such as gender, age, and mainly duration of diabetes.

The lack of glycemic control in DM is presented as a metabolic risk factor for DN, since hyperglycemia contributes to glomerulosclerosis, interstitial fibrosis, and tubular atrophy. In addition, the contribution of the *ACE* I/D polymorphism should be considered, as in other genes involved in important pathological mechanisms, which characterize the complication of DN as a polygenic disease (Potier et al., 2001).

It should be noted that the discrepancies in polymorphism association studies was due to several factors, including variations in the ethnic background of the cases and controls, the possible criteria of inclusion and exclusion of cases and lack of statistical power due to small sample sizes. Thus, undoubtedly, studies of genetic biomarkers contribute to the understanding of the pathophysiology of DM, as well as providing support for strategies for prevention of onset (primary care), acute and chronic complications (secondary care) and rehabilitation or limitation of the disabilities produced by complications (tertiary care).

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahluwalia TS, Ahuja M, Rai TS, Kohli HS, et al. (2009). ACE variants interact with the RAS pathway to confer risk and protection against type 2 diabetic nephropathy. *DNA Cell Biol*. 28 (3): 141-50.

Al-Harbi EM, Farid EM, Gumaa KA, Masuadi EM, et al. (2011). Angiotensin-converting enzyme gene polymorphisms and T2DM in a case-control association study of the Bahraini population. *Mol. Cell Biochem*. 350: 119-125.

American Diabetes Association (ADA) (2018). Introduction: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 41: S1-S2.

Arfa I, Abid A, Nouira S, Elloumi-Zghal H, et al. (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.

Arnett DK and Claas SA (2018). Omics of Blood Pressure and Hypertension. *Circ. Res.* 122(10): 1409-1419.

Arzu Ergen H, Hatemi H, Agachan B, Camlica H, et al. (2004). Angiotensin-I converting enzyme gene polymorphism in Turkish type 2 diabetic patients. *Exp. Mol. Med.* 36(4): 345-350.

Bhaskar LV, Mahin S, Ginila RT and Soundararajan P (2013). Role of the ACE ID and PPARG P12A Polymorphisms in Genetic Susceptibility of Diabetic Nephropathy in a South Indian Population. *Nephrourol. Mon*. 5: 813-817.

Boright AP, Paterson AD, Mirea L, Bull SB, et al. (2005). Genetic variation at the *ACE* gene is associated with persistent microalbuminuria and severe nephropathy in type 1 diabetes: the DCCT/EDIC Genetics Study. *Diabetes*. 54: 1238-1244.

Cambien F, Poirier O, Lecerf L, Evans A, et al. (1992). Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature*. 359(6396): 641-644.

Doi Y, Yoshizumi H, Yoshinari M, Iino K, et al. (1996). Association between a polymorphism in the angiotensin-converting enzyme gene and microvascular complications in Japanese patients with NIDDM. *Diabetologia*. 39: 97-102.

El-Baz R, Settin A, Ismaeel A, Khaleel AA, et al. (2012). MTHFR C677T, A1298C and ACE I/D polymorphisms as risk factors for diabetic nephropathy among type 2 diabetic patients. *J. Renin. Angiotensin Aldosterone Syst.*13: 472-477.

El-Bazz WF, Mohamed KY, Mansour HH, Mohamed RR, et al. (2011). Detection of Angiotensin Converting Enzyme Gene Polymorphism in Egyptian Patients with Diabetic Nephropathy. *AJBAS.* 5(11): 244-251.

Ezzidi I, Mtiraoui N, Kacem M, Chaieb M, et al. (2009). Identification of specific angiotensin-converting enzyme variants and haplotypes that confer risk and protection against type 2 diabetic nephropathy. *Diabetes Metab. Res. Rev.* 25: 717-724.

Fawwaz S, Balbaa M, Fakhoury H, Borjac J, et al. (2017). Association between Angiotensin-converting Enzyme Insertion/Deletion Gene Polymorphism and End-stage Renal Disease in Lebanese Patients with Diabetic Nephropathy. *Saudi J. Kidney Dis. Transpl*. 28: 325-329.

Felehgari V, Rahimi Z, Mozafari H and Vaisi-Raygani A (2011). ACE gene polymorphism and serum ACE activity in Iranians type II diabetic patients with macroalbuminuria. *Mol. Cell Biochem*. 346: 23-30.

Freathy RM, Lonnen KF, Steele AM, Minton JA, et al. (2006). The impact of the angiotensin-converting enzyme insertion/deletion polymorphism on severe hypoglycemia in Type 2 diabetes. *Rev. Diabet. Stud*. 3: 76-81.

Fujisawa T, Ikegami H, Kawaguchi Y, Hamada Y, et al. (1998). Meta-analysis of association of insertion/deletion polymorphism of angiotensin I-converting enzyme gene with diabetic nephropathy and retinopathy. *Diabetologia*. 41: 47-53.

Fujisawa T, Ikegami H, Shen G-Q, Yamato E, et al. (1995). Angiotensin I-Converting Enzyme Gene Polymorphism Is Associated With Myocardial Infarction, but Not With Retinopathy or Nephropathy, in NIDDM. *Diabetes Care*. 18(7): 983-5.

Gallagher PE, Li P, Lenhart JR, Chappell MC, et al. (1999). Estrogen Regulation of Angiotensin-Converting Enzyme mRNA. *Hypertension Journal.* 33(1): 323–8.

Grzeszczak W, Zychma MJ, Lacka B and Zukowska-Szczechowska E (1998). Angiotensin I-converting enzyme gene polymorphisms: relationship to nephropathy in patients with non-insulin dependent diabetes mellitus. *JASN.* (9)9: 1664-1669.

Hanyu O, Hanawa H, Nakagawa O, Tani N, et al. (1998). Polymorphism of the Angiotensin I-Converting Enzyme Gene in Diabetic Nephropathy in Type I1 Diabetic Patients with Proliferative Retinopathy. *Ren. Fail*. 20: 125-133.

He L, Xiong D-H, Liu Y, Zhang F, et al. (2007). Association study of the estrogen signaling pathway genes in relation to age at natural menopause. *J. Genet*. 86: 269-276.

Higgins JPT, White IR and Wood AM (2008). Imputation methods for missing outcome data in meta-analysis of clinical trials. *Clinical Trials.* 5(3): 225-239.

Jha V, Garcia-Garcia G, Iseki K, Li Z, et al. (2013). Chronic kidney disease: Global dimension and perspectives. *Lancet*. 382: 260-272.

Kennon B, Petrie JR, Small M and Connell JMC (1999). Angiotensin-converting enzyme gene and diabetes mellitus. *Diabet. Med*. 16: 448-458.

Luo R, Li X, Wang Y, Li Y, et al. (2013). The Influence of Angiotensin Converting Enzyme and Angiotensinogen Gene Polymorphisms on Hypertrophic Cardiomyopathy. *PLoS One*. 8(10): e77030.

Malta M, Cardoso LO, Bastos FI, Magnanini MMFC, et al. (2010). STROBE initiative: guidelines on reporting observational studies. *Rev. Saúde Pública*. 44: 559-65.

Mizuiri S, Hemmi H, Inoue A, Yoshikawa H, et al. (1995). Angiotensin-Converting Enzyme Polymorphism and Development of Diabetic Nephropathy in Non-Insulin-Dependent Diabetes mellitus. *Nepron.* 70: 444-459.

Ng DP, Placha G, Choo S, Chia KS, et al. (2006). A disease haplotype for advanced nephropathy in type 2 diabetes at the ACE locus. *Diabetes*. 55: 2660-2663.

Nikzamir AR, Golmohamadi T, Nakhjavani M, Zahraei M, et al. (2006). Angiotensin Converting Enzyme Gene Polymorphism in Iranian Patients with Type 2 Diabetes. *Iran Journal Immunol*. 3: 23-29.

Ohno T, Kawazu S and Tomono S (1996). Association analyses of the polymorphisms of angiotensin-converting enzyme and angiotensinogen genes with diabetic nephropathy in Japanese non-insulin-dependent diabetics. *Metabolism.* 45: 218-222.

Pacheco EP, Paz EMC, Jasul GV, Cruz MCA, et al. (2012). Angiotensin I-Converting Enzyme Gene Polymorphism and Diabetic Nephropathy in Filipino Type 2 Diabetes Mellitus Patients. *JAFES.* 27(1): 87-90.

Palomo-Pinon S, Gutierrez-Rodriguez ME, Daz-Flores M, Sanchez-Barrera R, et al. (2009). DD genotype of angiotensin-converting enzyme in type 2 diabetes mellitus with renal disease in Mexican Mestizos. *Nephrology*. 14: 235-239.

Panagiotoupolos S, Smith, TJ, Aldred GP, Baker, EJ, et al. (1995) Angiotensin-Converting Enzyme (ACE) Gene Polymorphism in Type II Diabetic Patients With Increased Albumin Excretion Rate. *J. Diabetes Complications*, 9: 272-276.

Parchwani DN, Kesari MG, Patel DD and Patel DJ (2014). Influence of genetic variability at the ACE locus in intron 16 on Diabetic Nephropathy in T1DM patients. *Indian J. Physiol. Pharmacol*. 58: 327-337.

Pereira AC, Mota GFA, Cunha RS, Herbenhoff FL, et al. (2003). Angiotensinogen 235T allele “dosage” is associated with blood pressure phenotypes. *Hypertension*. 41: 25-30.

Potier M, Elliot SJ, Tack I, Lenz O, et al. (2001). Expression and regulation of estrogen receptors in mesangial cells: influence on matrix metalloproteinase-9. *J. Am. Soc. Nephrol.* 12: 241-251.

Prasad P, Tiwari AK, Kumar KMP, Ammini AC, et al. (2006). Chronic renal insufficiency among Asian Indians with type 2 diabetes: I. Role of RAAS gene polymorphisms. *BMC Med. Genet*. 7(42): 1-9.

Rahimi Z (2012). ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. *J. Nephropathol.* (3): 143-151.

Ringel J, Beige J, Kunz R, Distler A, et al. (1997). Genetic variants of the renin-angiotensin system, diabetic nephropathy and hypertension. *Diabetologia*, 40(2): 193-199.

Rogers JL, Mitchell AR, Maric C, Sandberg K, et al. (2007). Effect of sex hormones on renal estrogen and angiotensin type 1 receptors in female and male rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 292: 794-799.

Ruggenenti P, Bettinaglio P, Pinares F and Remuzzi G (2008). Angiotensin Converting Enzyme Insertion/Deletion Polymorphism and Renoprotection in Diabetic and Nondiabetic Nephropathies. *Clin. J. Am. Soc. Nephrol*. (5): 1511-1525.

Sancakdar E, Ateş K, Kaman D, Deveci K, et al. (2015). Role of ADDUCIN Gly460Trp, ACE I/D and AGT M235T Gene Polymorphisms in Genetic Susceptibility to Diabetic Nephropathy. *Eur. J. Gen. Med*. 12(2): 118-124.

Schmidt S and Ritz E (1997). Angiotensin I converting enzyme gene polymorphism and diabetic nephropathy in type II diabetes. *Nephrol Dial Transplant*, 12: 37-41.

Schmidt S, Schone N and Ritz E (1995). Association of ACE gene polymorphism and diabetic nephropathy? *Kidney Int.* 47: 1176-1181.

Shaikh R, Shahid SM, Nawab SM, Mansoor Q, et al. (2012). Distribution of ACE I/D Polymorphism in the Patients of Diabetes and Nephropathy in Pakistan. *Int. J. Hum. Genet.* 12(3): 133-138.

Shaker OG, Ismail MF, Ashour E, Yousif HM, et al. (2014). ACE gene polymorphism and serum ACE level with Progression of Nephropathy in Type 2 Diabetic Patients. *J. Adv. Chem*. 9: 2023-2032.

Solini A, Giacchetti G, Sfriso A, Fioretto P, et al. (1999). Polymorphisms of angiotensin-converting enzyme and angiotensinogen genes in type 2 diabetic sibships in relation to albumin excretion rate. *Am. J. Kidney Dis.* 34(6): 1002-9.

Taniwaki H, Ishimura E, Matsumoto N, Emoto M, et al. (2001). Relations Between ACE Gene and ecNOS Gene Polymorphisms and Resistive Index in Type 2 Diabetic Patients With Nephropathy. *Diabetes Care.* 24(9): 1653-60.

Tomino Y, Makita Y, Shike T, Gohda T, et al. (1999). Relationship between polymorphism in the angiotensinogen, angiotensin-converting enzyme or angiotensin II receptor and kidney progression in Japanese NIDDM patients. *Nephron*. 82: 139-144.

Uddin M, Azam M, Chowdhury N and Akhteruzzaman S (2007). Angiotensin I-converting enzyme gene polymorphism in type 2 diabetic patients with nephropathy. *J. Med. Sci*. 7: 682-685.

Vashrambhai H, Kalia K and Mannari J (2011). Angiotensin converting enzyme (ACE) gene polymorphism increases the susceptibility of diabetic nephropathy in Western Indian Type 2 diabetic patients. *Int. J. Diabetes Dev. Ctries*. 31(4): 223-228.

Verlengia R, Rebelo AC, Crisp AH, Kunz VC, et al. (2014). Lack of Association Between ACE Indel Polymorphism and Cardiorespiratory Fitness in Physically Active and Sedentary Young Women. *Asian J. Sports Med*. 5: e22768.

Viana LV, Gross JL, Camargo JL, Zelmanovitz T, et al. (2012). Prediction of cardiovascular events, diabetic nephropathy, and mortality by albumin concentration in a spot urine sample in patients with type 2 diabetes. *J. Diabetes Complications*. 26: 407-412.

Viswanathan V, Zhu Y, Bala K, Dunn S, et al. (2001). Association between ACE gene polymorphism and diabetic nephropathy in South Indian patients. *JOP*. 2: 83-7.

Yoshida H, Kuriyama S, Atsumi Y, Tomonari H, et al. (1996). Angiotensin I converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus. *Kidney Int*. 50: 657-664.

Young RP, Chan JC, Critchley JA, Poon E, et al. (1998) Insertion/Deletion Polymorphisms Chinese Type 2 Diabetic Patients. *Diabetes Care* 21(3): 431-438.