Role of angiotensin converting enzyme and angiotensinogen gene polymorphisms in angiotensin converting enzyme inhibitor-mediated antiproteinuric action in type 2 diabetic nephropathy patients

Neerja Aggarwal, Pawan Kumar Kare, Parul Varshney, Om Prakash Kalra, Sri Venkata Madhu, Basu Dev Banerjee, Anil Yadav, Alpana Raizada, Ashok Kumar Tripathi

Data sharing statement: There is no additional data available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Invited manuscript

Correspondence to: Ashok Kumar Tripathi, PhD, Professor, Biochemistry and Immunology Laboratory, Department of Biochemistry, University College of Medical Sciences (University of Delhi) and Guru Teg Bahadur Hospital, Dilshad Garden, Delhi 110095, India. aktripathiucms@gmail.com
Telephone: +91-11-22582972

Received: July 11, 2016
Peer-review started: July 14, 2016
First decision: September 12, 2016
Revised: October 6, 2016
Accepted: November 21, 2016
Article in press: November 23, 2016
Published online: March 15, 2017

Abstract

AIM
To investigate the role of genetic variants of angiotensin converting enzyme (ACE) and angiotensinogen (AGT) genes in the antiproteinuric efficacy of ACE inhibitor therapy in diabetic nephropathy (DN) patients.
INTRODUCTION

Diabetic nephropathy (DN) is a clinical syndrome that occurs approximately in 20%-30% of patients with diabetes mellitus (DM). Nephropathy gradually progresses and makes the patient dependent on renal replacement therapy. DN patients clinically present with persistent micro-albuminuria ($\geq 30$ to $299$ mg/g creatinine) which subsequently progresses to macro-albuminuria ($\geq 300$ mg/g creatinine)\(^{(1)}\). Later severity of the disease is characterized by a fall in estimated glomerular filtration rate (eGFR) as a consequence of renal impairment, ultimately leading to end stage renal disease (ESRD)\(^{(2)}\). Various factors including poor glycemic control, family history of diabetes or hypertension may predispose to the development of DN; however; not all DM patients tend to develop nephropathy\(^{(3)}\).

The renin-angiotensin-aldosterone system (RAAS), which plays an important role in regulating blood pressure, is involved in the pathophysiology of renal complications including DN. Polymorphisms of various genes of RAAS, particularly angiotensin converting enzyme (ACE) and angiotensinogen (AGT) genes, have been strongly implicated in the development and progression of nephropathy\(^{(4,5)}\). ACE is a zinc-dependent di-peptidase enzyme which catalyzes the conversion of inactive angiotensin (angiotensin-I ) to angiotensin-II \(^{(6)}\). The ACE gene is located at the 17q23 locus and known to be associated with the pathogenesis of DN, including progression to overt proteinuria. The ACE gene is highly polymorphic in nature. Of the 160 polymorphisms known, insertion/deletion (I/D) polymorphism is the most studied as it affects ACE enzyme activity in blood. I/D polymorphism involves the presence or absence of a 287 bp Alu repeat in intron 16 of the gene. It has been observed that DD genotype is associated with higher ACE activity and II genotype is associated with the lowest ACE activity\(^{(7)}\).

The AGT gene (rs 699) is located at chromosome 1 and consists of five exons, and it has more than 23 variants\(^{(8)}\). The common polymorphism of the AGT gene is M235T, which encodes threonine instead of methionine at position 235 in exon 2\(^{(9)}\). T allele of the M235T variant is associated with a higher plasma AGT level\(^{(10)}\). A number of drugs that block the RAAS like ACE inhibitors and angiotensin receptor blockers (ARB) are often prescribed to control hypertension; in addition, these drugs are known to control proteinuria either alone or in combination in DN patients\(^{(11)}\). However, the reno-protective response to ACE inhibitor therapy is not uniform in all patients. The reasons behind the uneven antiproteinuric response to these drugs are not completely understood. The polymorphisms of genes of RAAS may be possibly involved in this process.

Despite several studies on association of ACE and AGT gene polymorphisms with ACE inhibitor treatment in type 2 DM (T2DM) patients with nephropathy, no substantial data are available on the role of ACE and AGT gene polymorphisms in antiproteinuric efficacy of ACE inhibitors.
inhibitors in the Indian context. In the present study, we examined the association of ACE and AGT gene polymorphisms with antiproteinuric response to ACE inhibitor therapy in north Indian type 2 diabetic patients with nephropathy.

MATERIALS AND METHODS

Subjects
This study was designed as a single arm prospective longitudinal study to evaluate the antiproteinuric effect of ACE inhibitor therapy based on change in albumin/creatinine ratio (ACR), with the baseline data serving as reference values (control). The required number of cases for 80% power at 5% type I error in detecting a reduction of proteinuria to at least 30% of pretreatment value for a given odds ratio of 1.5 is 221, based on the frequency of mutant ACE gene allele in the Asian population as 40%[12]. In order to accommodate drop out during the course of the study, we recruited 270 patients with T2DM having persistent microalbuminuria (30-300 mg/g creatinine) or overt albuminuria (> 300 mg/g creatinine), of whom 18 could not complete the follow-up. The patients were enrolled from Department of Medicine, Diabetic and Nephrology Clinic at Guru Teg Bahadur Hospital, Delhi, India. Patients having an age between 30 to 65 years and a duration of diabetes ≥ 5 years, with the evidence of diabetic retinopathy and stages 1 to 3 chronic kidney disease (CKD), were recruited. Patients intolerant to ACE inhibitors, pregnant or lactating women, patients taking aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) were excluded from the study. Diagnosis of DM was based upon the 2012 American Diabetes Association (ADA) guidelines. Patients having evidence of 1+ or more proteinuria by urinary dipstick test were included in the study. In addition, patients with dipstick negative proteinuria were screened by urinary dipstick for the presence of microalbumin. Patients with evidence of micro-albuminuria or overt proteinuria on two separate occasions at least 6 wk apart were included in the study and assessed for urinary ACR.

The study was approved by Institutional Ethics Committee-Human Research (IEC-HR) of University College of Medical Sciences and written informed consent was obtained from all patients. All enrolled patients were under satisfactory glycemic control and were under well-controlled blood pressure. The patients were followed after 6 mo of initiation of ACE inhibitor therapy. All were treated initially with ramipril 5 mg/d along with anti-diabetic therapy. The dose was up-titrated to a maximum of 20 mg/d at one or two equally divided doses.

Clinical response assessment
The decrease in urinary ACR (ACR%) was calculated as (baseline value - follow-up value) × 100/baseline value.

Patients were classified as responders when they had a decrease in urinary ACR ≥ 30% or as non-responders when they had a decrease in urinary ACR > 30% at the end of 6 mo follow-up[13,14].

Measurement of biochemical parameters
Blood samples (5 mL) were collected for biochemical analysis and genotype study. Blood was centrifuged at 1000 g for 15 min for serum separation. Serum samples were frozen at -80 °C until assayed. All parameters were determined within a month after sample collection. Morning spot urine samples were collected for urine albumin and urine creatinine tests.

The plasma glucose level was measured by glucose oxidase-peroxidase method and quantified spectrophotometrically at 500 nm. HbA1c was estimated by micro-column based technique and quantified spectrophotometrically at 500 nm. Total cholesterol (TC), serum sodium, potassium and hemoglobin were determined using routine clinical assays in hospital laboratory. Average of three blood pressure readings taken 15 min apart was calculated, and all patients underwent fundus examination for the detection of diabetic retinopathy.

Urine and serum creatinine levels were estimated by alkaline picate Jaffe’s method (kinetic method). Urine albumin was measured by an immuno-turbidometric assay (Nephelometer, Nephstar) after calibration of the instrument by the standard provided. The minimum sensitivity is 10 mg/L. The result is expressed as ACR in terms of mg/g creatinine.

Determination of genotypes
ACE I/D gene polymorphism: The ACE gene (rs4646994) I/D polymorphism was determined by polymerase chain reaction (PCR) using a flanking primer pair that recognizes the insertion-specific sequence. The 25 μL PCR reaction mixture contained 100 ng of genomic DNA and amplification buffer containing 20 mmol/L Tris (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 200 μmol/L of dNTPs, 10 pmol of each primer, and 1.0 U of Taq DNA polymerase (RBC, India). The DNA was amplified by cycling at 94 °C for 2 min, at 60 °C for 45 s, and at 72 °C for 2 min (Eppendorf PCR machine, Germany). After 30 cycles, the reaction was extended for an additional 8 min at 72 °C. The oligonucleotide sequences of the primers were: 5′-CTGAGAGCCACTCCCCATCTTGTCT-3′ and 5′-GATGTTGCCATCACATTGCTGAT-3′. The PCR products were separated by 1.5% agarose gel electrophoresis, and a 490 bp band with insertion (I allele) and a 190 bp band with deletion (D allele) were visualized with ethidium bromide staining in the UVP Bio-documentation System.

AGT M235T gene polymorphism: The AGT gene (rs699) M235T polymorphism was determined by PCR-restriction fragment length polymorphism (PCR-RFLP) assay. The 25 μL PCR reaction mixture contained 100 ng of genomic DNA and amplification buffer containing 20 mmol/L Tris (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 200 μmol/L of dNTPs, 10 pmol of each primer, and 1.0 U of Taq DNA polymerase (RBC, India). The DNA was amplified by cycling at 94 °C for 1 min, at 68 °C for 45 s, and at 72 °C
Table 1: Demographic and clinical characteristics of patients

| Parameter                      | Type 2 diabetes mellitus with nephropathy |
|-------------------------------|------------------------------------------|
| Number of patients (n)        | 270                                      |
| Gender (male/female)          | 128/142                                  |
| Age (yr)                      | 52.23 ± 6.011                            |
| Duration of diabetes (yr)     | 8.31 ± 3.091                             |
| Family history of diabetes    | 105/165                                  |
| Family history of hypertension| 65/207                                   |
| Medications                   |                                          |
| Insulin (yes/no)              | 115/155                                  |
| Metformin (yes/no)            | 153/117                                  |
| Glimipideride (yes/no)        | 129/141                                  |

For 2 min (Eppendorf PCR machine, Germany). After 30 cycles, the reaction was extended for an additional 10 min at 72 °C. The oligonucleotide sequences of the primers were: 5’-CCGTTTTGTCCAGGGCCTGCTCTCTCT-3’ and 5’-CAGGGTGCTGTCACACTGGACCCC-3’. The PCR product was digested with restriction enzyme Thn111 I (Fermentas) to identify the M/T polymorphism at 37 °C for 16 h. Digested DNA fragment products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The presence of an uncut 165 bp band indicated homozygous MM genotype, 141 bp and 24 bp bands indicated TT homozygous genotype, and 165 bp, 141 bp and 24 bands indicated MT heterozygous genotype.

**Statistical analysis**

The statistical methods of this study were reviewed by the Department of Biostatistics, UCMS and GTB Hospital, Delhi, India. Data of all the parameters were collected at enrollment and at 6 mo after ramipril treatment. Analyses of obtained data were performed using SPSS, version 20.0. P-values < 0.05 were considered significant. \( \chi^2 \) test was applied to compare genotype data of ACE and AGT genes in all groups. For biochemical parameters, paired student’s t-test was applied to compare the baseline values with the values obtained at 6 mo. ACR values follow the skewed distribution, hence we applied non-parametric method (Wilcoxon-signed rank test) to compare the baseline ACR values with the values obtained at 6 mo.

**RESULTS**

Demographic and biochemical data at baseline and at 6 mo after ACE inhibitor therapy

The demographic and biochemical data are listed in Tables 1 and 2. The age of the patients ranged from 30 to 60 years. The duration of diabetes ranged from 5 years to 20 years and mean duration of diabetes was 8.31 years. Approximately 39% of enrolled patients had a family history of diabetes and 23% had a family history of hypertension. Biochemical data before treatment and after 6 mo of treatment with ramipril are listed in Table 2. There was no significant change in blood urea, serum sodium, serum potassium, fasting plasma glucose, post prandial plasma glucose, systolic and diastolic blood pressure, hemoglobin or HbA1c level after follow-up. Also, the differences in serum creatinine and eGFR levels after treatment were not statistically significant.

**Antiproteinuric effect of ACE inhibitor therapy**

The antiproteinuric effect of ACE inhibitor therapy was evaluated by urinary ACR values. Patients with a decrease of more than 30% in ACR values were considered as responders to ACE inhibitor treatment. ACR values of enrolled patients at baseline varied widely and ranged from 30 to 14573 mg/g creatinine. An overall significant decrease in ACR values was observed after ACE inhibitor treatment as compared to baseline values (Table 3). Taken together, 48% of enrolled patients were found as responders to ACE inhibitor therapy. Subsequently, based on the ACR, patients were grouped as micro-albuminuric (ACR < 30 and ACR ≤ 300 mg/g creatinine) and macro-albuminuric (ACR > 300 mg/g creatinine). A significant decrease in ACR was observed in both the micro- and macro-albuminuric DN groups. In the micro-albuminuric DN group (n = 170), the percentage of responders was 45% whereas in the macro-albuminuric group (n = 82), the percentage was 55% at 6 mo follow-up.

**Distribution of genotypes of ACE and AGT genes**

For ACE I/D polymorphism was studied by sequence specific PCR method and AGT M235T polymorphism was studied by PCR-RFLP method. Genotype distribution and allele frequency for ACE and AGT genes are listed in Table 4. Distribution of all genotypes was in Hardy-Weinberg equilibrium for all the subgroups of ACE and AGT genes. For ACE gene, the genotype frequency of II, ID, and DD was found to be 31%, 53% and 16%, respectively. For AGT gene, the genotype frequency of MM, MT, and TT was found to be 25%, 53% and 22%, respectively.

**ACE and AGT polymorphisms and response to ACE inhibitor therapy**

Table 5 shows the genotype distribution of DN patients based on the response to ACE inhibitor therapy. No significant change in the genotype distribution was observed among responders and non-responders with regard to ACE and AGT genes. When the patients were grouped as micro- and macro-albuminuric based on their ACR values (Table 6), no inter-genotype differences were observed in subgroups. However, macro-albuminuric patients carrying ACE I/D genotypes were responding in a better way to therapy compared with micro-albuminuric patients. Seventy-two percent of macro-albuminuric patients having TT genotype responded to therapy, although the difference was not statistically significant.

**DISCUSSION**

In the present study, we examined the antiproteinuric
Table 2  Biochemical parameters before and after treatment with angiotensin converting enzyme inhibitor

| Parameter                             | Baseline 1 | 6 mo 1,2 | P-value |
|---------------------------------------|------------|----------|---------|
| No. of patients                       | n = 252    | n = 252  |         |
| Blood urea (mmol/L)                   | 2.22 ± 0.86| 2.01 ± 0.77| 0.661   |
| Serum creatinine (µmol/L)             | 95.47-30   | 99.28-28 | 0.068   |
| Serum sodium (mmol/L)                 | 139.47 ± 4.11| 135.14 ± 3.88| 0.512   |
| eGFR (MDRD) mL/min per 1.73 m²        | 73.65 ± 24.71| 68.90 ± 24.44| 0.081   |
| Fasting plasma glucose (mmol/L)       | 7.63 ± 0.60| 6.693 ± 0.81| 0.08    |
| Post-prandial plasma glucose (mmol/L) | 10.53 ± 1.62| 8.52 ± 1.3 | 0.076   |
| HbA1c (%)                             | 6.52 ± 1.71| 6.1 ± 1.14 | 0.06    |
| Hemoglobin (g/L)                      | 123.8 ± 23 | 111.2 ± 31| 0.65    |
| Systolic blood pressure (mmHg)        | 132.30 ± 10.03| 130.12 ± 10.46| 0.71   |
| Diastolic blood pressure (mmHg)       | 86.10 ± 10.03| 84.07 ± 8.32| 0.68    |

1Data are presented as mean ± SD; 2P < 0.05. HbA1c: Hemoglobin A1c; eGFR: Estimated glomerular filtration rate.

Table 3  Responders and non-responders before and after treatment with angiotensin converting enzyme inhibitor therapy

| Patients              | Urinary ACR at baseline 1 | Urinary ACR at 6 mo 1 | P-value | R 1 (%) | NR 1 (%) |
|-----------------------|---------------------------|-----------------------|---------|---------|----------|
| Overall (n = 252)     | 185.97 (55.66-222.20)     | 118.64 (96.24-146.26) | < 0.001 | 121     | 131      |
| Micro-albumin (n = 170)| 78.79 (71.30-87.07)       | 53.67 (44.46-64.79)   | < 0.001 | 76      | 94       |
| Macro-albumin (n = 82)| 1068.7 (879.62-1298.28)  | 596.45 (451.60-787.68)| < 0.001 | 45      | 37       |

1Median (IQR); 2A decline of > 30% in ACR value at 6 mo is considered as R. R: Responders; NR: Non-responders; ACR: Albumin/creatinine ratio.

Table 4  Genotype distributions and allele frequency for angiotensin converting enzyme and angiotensinogen gene polymorphisms

| Gene        | n = 252 | Genotype/Allele | Percentage (%) |
|-------------|---------|-----------------|----------------|
| ACE (I/D)   |         | Genotypic       | 31             |
|             |         | frequency       | 53             |
|             |         | DD              | 16             |
|             |         | Allele          | 57             |
|             |         | D               | 43             |
| AGT (M235T) |         | Genotypic       | 25             |
|             |         | frequency       | 53             |
|             |         | MT              | 33             |
|             |         | TT              | 22             |
|             |         | Allele          | 51             |
|             |         | T               | 49             |

ACE: Angiotensin converting enzyme; AGT: Angiotensinogen.

The effect of ACE inhibitor (ramipril) in DN patients by following urinary ACR. ACE inhibitors are commonly used for inhibition of the RAAS and are known to have renoprotective efficacy in both diabetic and non-diabetic kidney diseases and antiproteinuric efficacy of ACE inhibitors are more pronounced than any other antihypertensive drugs. However, there are variable responses regarding antiproteinuric efficacy of RAAS blockers among patients and a 20%-80% reduction was observed. In the present study, overall we observed a 36% reduction in ACR values and about 48% of patients responded to therapy. Our finding is in accordance with previous studies showing overall decrease in albumin excretion after treatment with ACE inhibitors. According to the NKF KDOQI guidelines, ACE inhibitors reduced protein excretion by approximately 35% to 40%, which is greater than other antihypertensive agents when the effect of blood pressure has been taken into account. Hence, in the present study patients with an ACR change ≥ 30% were considered as responders to ACE inhibitor therapy. When subdividing our study subjects as micro- and macro-albuminuric, it was observed that 55% of patients with macro-albuminuria responded in a better way to ACE inhibitor therapy. Earlier antiproteinuric effect of ACE inhibitor has been shown to be more pronounced in macro-albuminuric patients. The mechanism leading to the antiproteinuric effect of ACE inhibitors has not been elucidated fully. However, it is thought that ACE inhibitors cause efferent arteriolar vasodilation of glomerulus and thereby decrease the intraglomerular hypertension, leading to anti-proteinuric effect. Recently it has been shown that ACE inhibitors ameliorate the glomerular membrane size-selective dysfunction, thus resulting in anti-proteinuric effect.

In order to find out the reason behind differential responses to ACE inhibitor therapy in DN patients, we studied the polymorphisms of ACE and AGT genes as these polymorphisms are strongly associated with the progression of DN. The genotype distribution of ACE gene observed in our study subjects is in line with most of the previous studies on the Indian population. In the present study, the percentage of responders did not differ significantly with regard to ACE I/D genotypes, indicating that the antiproteinuric effect of ACE inhibitors is independent of ACE genotype. Similarly, the finding that the anti-proteinuric effect of ACE inhibitors is independent of ACE genotypes has been reported by...
several authors. So et al. have reported that ACE II genotype with a cumulative genetic risk score of < 1 in normoalbuminuric T2DM patients, is coupled with better response to ACE inhibitors, although no significant difference was found in renoprotective effect of ACE inhibitor therapy based on ACE I/D genotypes after 3 years of follow-up. The antiproteinuric effect of RAAS inhibitors in patients with macro-albuminuria is also found to be independent of ACE I/D genotypes. However, there are a number of controversies about the association of ACE I/D genotypes with therapeutic efficacy of ACE inhibitors. In Korean and Caucasian patients, DD genotype has been shown to be more responsive to ACE inhibitor therapy. In conclusion, ACE inhibitor treatment in DN patients appears to cause a significant reduction in urinary protein excretion and macro-albuminuric patients exhibit better response. The antiproteinuric effect of ACE inhibitor therapy in patients is independent of ACE I/D or AGT M235T genotypes. Long term follow-up of larger populations with ACE inhibitor therapy may validate the present findings.

### COMMENTS

**Background**

Angiotensin converting enzyme (ACE) inhibitors are the standard therapy for patients with hypertension, proteinuria and kidney diseases. The use of ACE inhibitors delays the progression of diabetic and non-diabetic kidney diseases. Various polymorphisms of the renin-angiotensin-aldosterone system (RAAS) have been implicated in the pathology of diabetic nephropathy. Of these,
polymorphism of the ACE gene is the most important. The current study was designed to evaluate the therapeutic efficacy of ACE inhibitor in terms of proteinuria and the role of ACE and AGT gene polymorphisms in ACE inhibitor-mediated antiproteinuric response in diabetic nephropathy patients.

Research Frontiers
Patients on ACE inhibitor therapy have improved proteinuria. In this study, the authors observed that ACE and AGT gene polymorphisms do not have any role in reducing albuminuria in patients with diabetic nephropathy.

Innovations and Breakthroughs
The literature suggests a mixed role of ACE gene polymorphisms in renoprotective action in diabetic patients. However, the present study suggests no role of ACE I/D and AGT M235T gene polymorphisms in modulating the renoprotective efficacy of ACE inhibitors in terms of reducing albuminuria in diabetic nephropathy patients.

Applications
The authors’ study provides additional evidence supporting the therapeutic role of ACE inhibitors in reducing albuminuria in patients with diabetic nephropathy. However, the present study suggests that patients on ACE inhibitor therapy have improved proteinuria. In this study, the authors observed that ACE and AGT gene polymorphisms do not have any role in reducing proteinuria and the role of ACE and AGT gene polymorphisms in ACE inhibitor-mediated antiproteinuric response in diabetic nephropathy patients.

Terminology
Diabetic nephropathy: It is the damage to kidneys due to diabetes; Polymorphism: The presence of genetic variation within a population.

Peer Review
This is a good paper.

REFERENCES
1 Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH, Steffels MW. Nephropathy in diabetes. *Diabetes Care* 2004; 27 Suppl 1: S79-S83 [PMID: 14693934 DOI: 10.2337/ diacare.27.2007.579]
2 Dabla PK. Renal function in diabetic nephropathy. *J Diabetes 2010; 15: 48-56* [PMID: 21537427 DOI: 10.4239/wjd.v12.i48]
3 Kalra OP, Datta SK, Kumar S. Genetic basis of diabetic nephropathy. *Med Updat 2010; 20: 695-701*
4 Hadajd J, Tarnow L, Forsblom C, Kazeem G, Marre M, Groop LC, Parving HH, Cannabin F, Tregouet DA, Gut IG, Théva A, Gauguer D, Farrall M, Cox R, Matsuda F, Lathrop M, Hager-Viennet N. 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: 1284-1291* [PMID: 17376814 DOI: 10.1681/ASN.2006101102]
5 Ilki V, Ilic M, Soldatov I, Popovic S, Magi C. Association of renin-angiotensin system genes polymorphism with progression of diabetic nephropathy in patients with type 1 diabetes mellitus. *Vijnovacini Pregl 2014; 71: 627-633* [PMID: 25100106 DOI: 10.2296/VSP1407027]
6 Gesualdo L, Grandiziano G, Masica L, Pertosa G, Schena FP. Acute renal failure in critically ill patients. *Intensive Care Med 1999; 25: 1188-1190* [PMID: 10551984 DOI: 10.1007/s001340051037]
7 Rahimi Z. ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. *J Nephropathol 2012; 1: 143-151* [PMID: 24475405 DOI: 10.5812/nephropathol.8109]
8 Say VH, Ling KH, Duraisamy G, Isac S, Rosli R. Angiotensinogen M235T gene variants and its association with essential hypertension and plasma renin activity in Malaysian subjects: a case control study. *BMC Cardiovasc Disord 2005; 5: 7* [PMID: 15811183 DOI: 10.1186/1471-2261-5-7]
9 Gaillard I, Clauser E, Corvol P. Structure of human angiotensinogen gene. *DNA 1989; 8: 87-99* [PMID: 2924688 DOI: 10.1089/ dna.1989.8.87]
10 Jeunemaître X, Soubrier F, Kotelevtsiev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM. Molecular basis of human hypertension: role of angiotensinogen. *Cell 1992; 71: 169-180* [PMID: 1394429 DOI: 10.1086/0092-8674(92)90275-H]
11 Tobihi JE, Bevice P, Di Gennaro F, Madalena L, Cao G, Angerosa M. Understanding the mechanisms of proteinuria: therapeutic implications. *Int J Nephrol 2012; 5: 546039* [PMID: 22844592 DOI: 10.1155/2012/546039]
12 Hypertension and the ACE Gene-GB Healthwatch. Available from: URL: http://www.gbhealthwatch.com/GND-Hypertension-ACE.php
13 Bakris GL. Slowing nephropathy progression: focus on proteinuria reduction. *Clin J Am Soc Nephrol 2008; 3 Suppl 1: S3-S10* [PMID: 18179794 DOI: 10.2215/CJN.03250907]
14 National Kidney Foundation. KDOQI Clinical Practice Guidelines on Hypertension and Antihypertensive Agents in Chronic Kidney Disease, 2004. Available from: URL: http://www2.kidney.org/professionals/kdoqi/guidelines_bp/guide_11.htm
15 Narita I, Goto S, Saito N, Song J, Omori K, Kondo D, Sakatsume M, Geyjo F. Renoprotective efficacy of renin-angiotensin inhibitors in IgA nephropathy is influenced by ACE A2350G polymorphism. *J Med Genet 2003; 40: e130* [PMID: 14664698 DOI: 10.1136/jmg.40.12. e130]
16 Han SY, Kwon YJ, Jo SK, Shin JH, Cha DR, Cho WY, Pyo HY, Kim HK. ACE gene polymorphism and renal responsiveness to ACE inhibitors in IgA nephropathy patients. *Korean J Intern Med 2000; 15: 13-18* [PMID: 10714086 DOI: 10.1007/ Klopp/1.5.13]
17 Ha SK. ACE insertion/deletion polymorphism and diabetic nephropathy: clinical implications of genetic information. *J Diabetes Res 2014; 2014: 846068* [PMID: 25587546 DOI: 10.1155/2014/846068]
18 Li P, Chen Y, Liu J, Hong J, Deng Y, Yang F, Jin X, Gao J, Li J, Fang H, Liu G, Shi L, Du J, Li Y, Yan M, Wen Y, Wang Y. Efficacy and safety of safety ofangshen formula on patients with type 2 diabetic kidney disease: a multicenter double-blinded randomized placebo-controlled trial. *PLoS One 2015; 10: e0126027* [PMID: 25938778 DOI: 10.1371/journal. pone.0126027]
19 Remuzzi G, Maia M, Ruggenenti P. Prevention and treatment of diabetic renal disease in type 2 diabetes: the BENEDICT study. *J Am Soc Nephrol 2006; 17: 890-897* [PMID: 16565256 DOI: 10.1681/ASN.2005121324]
20 Van de vaal RM, Gansvoort RT, Harst PV, Boomsma F, Plokker HWT, Veldhuisen DJV, De Jong PE, van Gilst WH, Voors AA. Predictors of Angiotensin-Converting Enzyme Inhibitor-Induced Reduction of Urinary Albumin Excretion in Non-diabetic Patients. *Hypertension 2006; 48: 870-876* [DOI: 10.1161/01. HYP.0000204402.26923.d1]
21 Anantharaman R, Bhansali A, Bhadada SK, Kohli HS, Dutta P, Walia R, Jayaprakash P, Upreti V. Anti-albuminuric efficacy of a combination of angiotensin converting enzyme inhibitor & amp; angiotensin receptor blocker in type 1 DM with nephropathy. *Indian J Med Res 2010; 132: 42-47* [PMID: 20693588]
22 Vejakama P, Thakkinistian A, Lerrtattanadow D, Insafithi A, Ngarmukos C, Attia J. Renoprotective effects of renin-angiotensin system blockade in type 2 diabetic patients: a systematic review and network meta-analysis. *Diabetologia 2012; 55: 566-578* [PMID: 22189484 DOI: 10.1007/s00125-011-2398-8]
23 Woo KT, Lau YK, Wong KS, Chang GS. ACEI/ATRA therapy decreases proteinuria by improving glomerular permeability in IgA nephritis. *Kidney Int 2000; 58: 2485-2491* [PMID: 11155082 DOI: 10.1046/j.1523-1755.2000.00432.x]
24 Gagliardini E, Corna D, Zoea C, Sangali F, Carrara F, Rossi M, Conti S, Rottoli D, Longaretti I, Remuzzi A, Remuzzi G, Benigni A. Unlike each drug alone, lisinopril if combined with avosentan promotes reduction of renal lesions in experimental diabetes. *Am J Physiol Renal Physiol 2009; 297: F1448-F1456* [PMID: 19675181 DOI: 10.1152/ajprenal.00340.2009]
25 Choudhury I, Jothimalar R, Patra AK. Angiotensin Converting Enzyme Gene Polymorphism and its Association with Hypertension in South Indian Population. *Indian J Clin Biochem 2012; 27: 265-269* [PMID: 26405385 DOI: 10.1007/s12291-012-0217-8]
Aggarwal N et al. RAAS and ACE in diabetic nephropathy

26 Kumari S, Sharma N, Thakur S, Mondal PR, Saraswathy KN. Beneficial role of D allele in controlling ACE levels: a study among Brahmins of north India. J Genet 2016; 95: 291-295 [PMID: 27350671 DOI: 10.1007/s12041-016-0649-7]

27 Jayapalan J, Muniandy S, Pheng CS. Angiotensin Converting Enzyme Insertion/Deletion Gene Polymorphism: An Observational Study among Diabetic Hypertensive Subjects in Malaysia. Trop J Pharm Res 2010; 9: 431-439 [DOI: 10.4314/tjphr.v9i6.1052]

28 Jayapalan J, Muniandy S, and Chan SP. Null association between ACE gene I/D polymorphism and diabetic nephropathy among multiethnic Malaysian subjects. Indian J Hum Genet 2010; 16: 2 [DOI: 10.4103/0970-9966.69351]

29 So WY, Ma RCW, Oktai R, Tong PCY, Ng MCY, Ho CS, Lam CWK, Chow CC, Chan WB, Kong APS, Chan JCN. Angiotensin-converting enzyme (ACE) gene insertion/deletion polymorphism (ACE I/D) modifies the response to ACE inhibitor therapy in type 2 diabetic patients. Kidney Int 2006; 69: 1438-43 [DOI: 10.1038/sj.ki.5000997]

30 Ha SK, Lee SY, Park, HS, Shin JH, Kim SJ, Kim DH, Kim KR, Lee HY, Han DS. ACE DD genotype is more susceptible than ACE II and ID genotypes to the antiproteinuric effect of ACE inhibitors in patients with proteinuric non-insulin-dependent diabetes mellitus. Nephrol Dial Transplant 2000; 15: 1617-1623 [DOI: 10.1093/ndt/15.10.1617]

31 Scharplatz M, Puhan MA, Steurer J, Perna A, Bachmann LM. Does the Angiotensin-converting enzyme (ACE) gene insertion/deletion polymorphism modify the response to ACE inhibitor therapy? A systematic review. Curr Control Trials Cardiovasc Med 2005; 6: 16 [PMID: 16242049 DOI: 10.1186/1468-6708-6-16]

32 Nakayama Y, Nonoguchi H, Kohda Y, Inoue H, Memetimin H, Izumi N, Tomita K. Different mechanisms for the progression of CKD with ACE Gene Polymorphisms. Nephron Clin Pract 2009; 111: c240-c246 [PMID: 19293592 DOI: 10.1159/000029150]

33 Penno G, Chaturvedi N, Talmud PJ, Cotonero P, Manto A, Nunnipieri M, Luong LA, Fuller JH. Effect of angiotensin-converting enzyme (ACE) gene polymorphism on progression of renal disease and the influence of ACE inhibition in IDDM patients: findings from the EUCLID Randomized Controlled Trial. EURODIAB Controlled Trial of Lisinopril in IDDM. Diabetes 1998; 47: 1507-1511 [PMID: 9726242 DOI: 10.2337/diabetes.47.9.1507]

34 Jacobsen P, Rossing K, Rossing P, Tarnow L, Mallet C, Poirier O, Cambien F, Parving HH. Angiotensin converting enzyme gene polymorphism and ACE inhibition in diabetic nephropathy. Kidney Int 1998; 53: 1002-1006 [PMID: 9551410 DOI: 10.1111/j.1523-1755.1998.00847.x]

35 Miraoui N, Ezzidi I, Turki A, Chaiab M, Mahjoub T, Almawi WY. Renin-angiotensin-aldosterone system genotypes and haplotypes affect the susceptibility to nephropathy in type 2 diabetes patients. J Renin Angiotensin Aldosterone Syst 2011; 12: 572-580 [PMID: 21421655 DOI: 10.1177/1470320310396542]

36 Freire MB, Ji L, Onuma T, Orban T, Warram JH, Krolewski AS. Gender-specific association of M235T polymorphism in angiotensinogen gene and diabetic nephropathy in NIDDM. Hypertension 1998; 31: 896-899 [PMID: 9535411 DOI: 10.1161/01.HYP.31.4.896]

37 Erg ulu Z, Cetinkalp S, Erdogan M, Kosova B, Karadeniz M, Kutukculer A, Gunduz C, Tetik A, Topcuoglu N, Ozgen AG, Tuzun M. 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 Complications 2006; 22: 186-190 [PMID: 18413162 DOI: 10.1016/j.jdiacomp.2006.12.004]

38 Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Ricard S, Poirier O, Parving HH. Angiotensin gene polymorphisms in IDDM patients with diabetic nephropathy. Diabetes 1996; 45: 367-369 [PMID: 8593440 DOI: 10.2337/diabetes.45.3.367]

39 Fradin S, Goulet-Salmon B, Chantepie M, Grandhomme F, Morello R, Jauzac P, Reznik Y. Relationship between polymorphisms in the renin-angiotensin system and nephropathy in type 2 diabetic patients. Diabetes Metab 2002; 28: 27-32 [PMID: 11938025]

40 Hadjadj S, Bellamou S, Bouhanick B, Galfroy Y, Guilloteau G, Chatellier G, Alhenc-Gelas F, Marre M. Prognostic value of angiotensin-I converting enzyme I/D polymorphism for nephropathy in type 1 diabetes mellitus: a prospective study. J Am Soc Nephrol 2001; 12: 541-549 [PMID: 11181802]

41 Narita I, Goto S, Saito N, Song J, Ornori K, Kondo D, Sakatsume M, Gejyo F. Angiotensinogen gene variation and renoprotective efficacy of renin-angiotensin system blockade in IgA nephropathy. Kidney Int 2003; 64: 1050-1058 [PMID: 12911556 DOI: 10.1046/j.1523-1755.2003.00187.x]

P- Reviewer: Kumaresan R, Shukla R, Wada J S- Editor: Qiu S L- Editor: Wang TQ E- Editor: Lu YJ
