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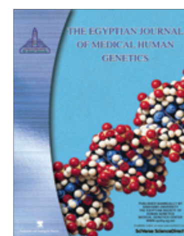

ORIGINAL ARTICLE

Null genetic risk of *ACE* gene polymorphisms with nephropathy in type 1 diabetes among Egyptian population

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Abstract Reported to date, strong evidence exists in multiple studies for genetic predisposing in the development of diabetic nephropathy, and no studies addressed this issue among Egyptian population. The results of angiotensin converting enzyme gene (ACE) in the susceptibility to nephropathy in type 1 diabetes with nephropathy are conflicting. We aim to identify the associations of two *ACE* gene polymorphisms (*Pst*I, A > G substitution and a 287-bp insertion/deletion) with nephropathy in type 1 diabetes in Egyptian children/adolescents. Our case-control study contained 140 diabetic individuals; 80 diabetic with nephropathy as cases, and 60 diabetic subjects without nephropathy as control group. Amplified DNA from peripheral leucocytes/buccal mucosa was genotyped for using polymerase chain reaction and enzymatic assay. We found no significant differences in the distribution of *ACE* insertion/deletion and *Pst*I genotypes or allele frequencies were observed between the examined groups. Frequencies of *Pst*I–indel haplotypes were similar in all of our study groups. In both cases and control subjects, ACE activity and microalbuminuria were highest among D/D homozygotes and lowest in I/I homozygotes, while a dissimilar result was seen

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in *Pst*I polymorphism. Our findings in Egyptian population strongly conclude that there is no association between the *ACE* gene I/D and *Pst*I polymorphisms with nephropathy in type 1 diabetes.
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1. Introduction

Diabetes mellitus (DM), a multifactorial chronic disease, has rapidly increased to epidemic proportions over the past few decades. The number of patients with DM worldwide was estimated at 173 million in 2002 and is predicted to increase to 350 million cases by 2030 (www.who.int/whr/2006/en) [1]. The disease is associated with severe complications including nephropathy, neuropathy, retinopathy and accelerated cardiovascular disease. Nephropathy is a frequent complication of long-term diabetes. Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in developed countries (www.who.int/whr/2006/en) [1]. It is the major determinant of premature morbidity and mortality in insulin-dependent diabetes mellitus (IDDM) [2]. About 30% of IDDM (known as type 1 diabetes 'T1D') patients is susceptible to diabetic nephropathy [3], characterized by hypertrophy of glomeruli, hyperperfusion, thickening of basement membranes and glomerular hyperfiltration [4]. In addition, there is microalbuminuria and subsequently progressive glomerulosclerosis, and tubulointerstitial fibrosis causing reduction in glomerular filtration rate (GFR) may occurs later [5,6]. The majority of patients with T1D will either develop nephropathy within the first 15 years after diagnosis or will remain relatively protected thereafter [3].

Differential disease risk in diabetic nephropathy may be partly attributable to genetic susceptibility. Evidence for a genetic component to diabetic nephropathy comes from family studies displaying familial aggregation of diabetic nephropathy both in type 1 and in type 2 diabetes mellitus [7–9], as well as differences in the prevalence of diabetic nephropathy between ethnic groups [8,9].

Some variants have been used to associate the genotypes with diabetic nephropathy [10]. These include the following genes: angiotensin converting enzyme (*ACE*, MIM 106180), apolipoprotein E (*APOE*, MIM 107741), erythropoietin (*EPO*, MIM 133170), nitric oxide synthase (*NOS3*, MIM 163729), heparan sulfate proteoglycan (*HSPG2*, MIM 142461), vascular endothelial growth factor A (*VEGFA*, MIM 192240), ferm domain-containing 3 (*FRMD3*, MIM 607619), and cysteinyl-tRNA synthetase (*CARS*, MIM 123859).

ACE gene, located on chromosome 17q23, comprises 26 exons; exons 1–12 encode for the amino domain, exons 13–26 encode for the carboxyl domain. The first polymorphism found in this gene was a 287-bp insertion/deletion (indel) located in intron 16, and the D allele was associated with higher serum ACE activity [11], together with enhanced conversion of angiotensin I to angiotensin II [12], inactivation of bradykinin [13] and more rapid progression of renal disease [14]. Five other polymorphisms (1Dde, 2*Rsa*I, 2*Rsa*II, 4*Alu*I, and 4*Alu*II) in the *ACE* gene were identified by Doria et al. [15].

Some reports supported the *ACE* indel polymorphism with the development of diabetic nephropathy [16,17], while some studies did not confirm this finding [18,19]. Representation of cases from Egypt was abandoned; none from the Egyptian subjects with nephropathy of type 1 diabetes investigated the association of genetic variations of *ACE* gene polymorphisms.

Here, the current study presents a genetic risk of the *ACE* gene polymorphisms with nephropathy in type 1 diabetes among Egyptian children/adolescents.

2. Subjects and methods

2.1. Eligibility criteria

We searched for studies comparing genetic variants in type 1 diabetes (T1D) with nephropathy, relative to T1D patients without nephropathy. We limited our analyses to studies investigating established and advanced diabetic nephropathy. A standard questionnaire was used to obtain the medical history and demographic data to patients attending the Pediatrics Hospital in Ain Shams University, Cairo.

2.2. Subjects

All individuals recruited ($n = 140$; male 74, female 66) with T1D were born of Egyptian parents and lived in Cairo Governorate. A physical examination contained height, weight and blood pressure measurements (Table 1). In addition retinopathy status was assessed by reviewing medical records.

Individuals with nephropathy ($n = 80$; microalbuminuria cases = 70 plus overt nephropathy cases = 10) had diabetes for at least 10 years before the onset of persistent proteinuria (>0.5 g protein/24 h), hypertension (blood pressure > 140/90 mmHg and/or treatment with anti-hypertensive medication) and diabetic retinopathy.

Microalbuminuria could be considered if the albumin/creatinine ratio ranged from 30 to 299 μg/mg. Overt nephropathy was defined if the albumine/creatinine ratio was >300 μg/mg. Normoalbuminuria was classified if values <30 μg/mg [20].

Individuals recruited as control subjects ($n = 60$) had T1D for a minimum duration of 15 years were not taking anti-hypertensive medication and did not demonstrate any evidence of renal disease. The presence of non-diabetic renal disease was exclusion criteria for this collection.

Blood samples were drawn for biochemical determinations and for isolation of DNA. The study protocol was approved by the ethics committee of the same institute, and a parental informed consent was obtained prior to documenting individuals in the study.

2.3. Methods

2.3.1. HbA1c measurement

Mean glycosylated hemoglobin (HbA1c) as a reflection of long-term glycemic control was determined using high perfor-

Table 1 Clinical characteristics in type 1 diabetic individuals with nephropathy (cases) and without nephropathy (controls).

Characteristics	Cases ($n = 80$)	Controls ($n = 60$)	p-Value
Age (years)	14.4 ± 2.3	13.7 ± 2.4	0.1
Disease duration (years)	10.8 ± 2.4	8.1 ± 2.0	< 0.05
HbA1c (%)	9.30 ± 2.5	7.1 ± 2.0	< 0.001
BMI (kg m^{-2})	27.9 ± 6.9	32.5 ± 5.0	> 0.05
Systolic blood pressure (mmHg)	147.0 ± 12.5	127.0 ± 14.9	< 0.001
Diastolic blood pressure (mmHg)	83.0 ± 11.5	75.0 ± 12.4	< 0.001
Random blood glucose (mg/dl)	170.6 ± 65.8	144.5 ± 56.2	< 0.05
ACE (IU/l)	71.0 ± 25.6	13.7 ± 2.4	< 0.0001
Microalbuminuria (μg/mg)	98.0 ± 66.0	19.4 ± 7.2	< 0.0001

mance liquid chromatography (HPLC) using the VARIANT II Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA).

2.3.2. Determination of the albumin/creatinine ratio

The albumin excretion rate was determined in three 24-h urine collections performed at least 1 month apart. The urine samples were stored at 4 °C and assayed within 1 week. Urinary albumin was determined by enzyme-linked immunosorbent assay (ELISA). The urinary creatinine was determined using automated a Kodak Ektachem 700 Analyzer (Rochester, NY, USA).

2.3.3. Serum ACE activity measurement

Serum ACE activity was measured by a kinetic assay on the basis of converting angiotensin I into angiotensin II using a colorimetric ACE kit (Bühlmann Laboratories AG, Baselstr., Switzerland). We used ACE high sensitive assay (order code: KK-ACF) with a detection limit of 1 U/l. A 250 μl of the substrate was added to 25 μl of each calibrator, control and serum patient sample and the reaction mixture was incubated at 37 °C for 5 min and the color was measured at 340 nm twice in a time interval of exactly 10 min. One unit of ACE activity is defined as the amount of enzyme required to release 1 μmol of hippuric acid expressed as nmol/ml/min. Serum sample from individuals of 20–70 years were collected. The serum range (mean ± SD) has been determined to be 12–68 ACE unit.

2.3.4. DNA isolation

Genomic DNA was extracted from peripheral blood (200 μl) using the QIAamp DNA Blood kit (Qiagen, Hilden, Germany). In some cases, DNA was prepared *in situ* by gently scraping the buccal mucosa for 30 s using a cytobrush [21]. The cells obtained were treated directly with diluted NaOH solution, heated, and neutralized with Tris-Cl, pH 8.0. A 2.5-μl volume of buccal cells typically sufficed for amplification by polymerase chain reaction (PCR).

2.3.5. ACE gene genotypes

We selected two polymorphic regions; intron 7 (amino domain) and intron 16 (carboxy domain) loci. The *Pst*I polymorphism was analyzed using the flanking primers 5′-CTC GGC TTG GGA CTT CTA-3′, and the anti-sense 5′-AGA GCT GGT CCA TCG TGA-3′ [22]. Genotyping for the indel polymorphism was performed using a PCR fragment amplified by the sense 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and anti-sense oligo 5′GAT GTG GCC ATC ACA TTC GTC AGA T-3′ [23].

For PCR amplification, DNA was added to a 25-μl reaction mixture containing 0.1 μM of each primer, 200 mM of each dNTP, 67 mM Tris–HCl, 16 mM $(NH_4)_2SO_4$, 0.01% Tween-20, 1 mM $MgCl_2$, and 0.45 U *Taq* DNA polymerase. The samples were then subjected to 30 rounds on PCR Engine Dyad (Bio-Rad Laboratories, USA) with annealing temperature at 58 °C for either indel or *Pst*I polymorphism for 1 min, followed by final extension of 7 min at 72 °C. The PCR amplicon of intron 7 was digested with *Pst*I restriction enzymes (New England Biolabs, Beverly, MA, USA) at 37 °C for 2 h. The product was separated on a 3% MetaPhor agarose gel (BMA, Rockland, ME, USA), stained by ethidium bromide and photographed using a gel-documentation system (G-Box, SynGene, Frederick, MD, USA). The (−) allele was detected as a single band (800 bp), and the (+) allele was detected as two fragments (600 bp and 200 bp). Fragments of 450 bp and 190 bp indicate the presence of the insertion/deletion heterozygous status, respectively.

2.3.6. ACE haplotypes

The two polymorphisms were also analyzed jointly, and the haplotype frequencies were compared between the study groups. Frequencies of haplotypes in the groups were calculated according to pairwise distribution of I/D and *Pst*I genotypes.

2.4. Data analysis

Statistical analysis was performed using the SPSS 16.0 (SPSS, Chicago, IL, USA). The data are presented as mean ± standard deviation. Nonparametric measures were used to calculate chi-square values. A probability < 0.05 was considered statistically significant. Odds ratios with 95% confidence intervals were calculated.

3. Results

Eighty nephropathy with type1 diabetes (T1D) cases and 60 T1D controls without nephropathy were recruited in this study. The diabetic nephropathy cases had age ranges from 7 to 18 years (mean, 14.4 ± 2.3 years), and their duration of diabetes were 5–14 years (mean, 10.8 ± 2.4 years). The diabetic controls had age ranges from 6 to 18 years (mean, 13.7 ± 2.4 years), and their duration of diabetes were 5–10 years (mean, 8.1 ± 2.0 years). There was no significant difference between cases and

Table 2 Genotypes distribution and allele frequencies of *ACE* gene polymorphisms in nephropathy with type 1 diabetic individuals.

Variable	Cases (%) $n = 80$	Controls (%) $n = 60$	χ^2 (p-value)
PstI polymorphism			
'+/+'	50 (62.5)	44 (73.3)	0.3 (0.59)
'+/−'	24 (30.0)	10 (16.7)	2.0 (0.18)
'−/−'	6 (7.5)	6 (10.0)	0.14 (0.71)
χ^2 (p-value)	18.4 ($p < 0.0001$)	18.6 ($p < 0.0001$)	
PstI allele frequency			
'+'	124/160 (0.77)	98/120 (0.82)	> 0.05
'−'	36/160 (0.23)	22/120 (0.18)	> 0.05
Indel polymorphism			
'D/D'	38 (47.5)	30 (50.0)	0.03 (0.87)
'I/D'	30 (37.5)	18 (30.0)	0.33 (0.56)
'I/I'	12 (15.0)	12 (20.0)	0.29 (0.59)
χ^2 (p-value)	6.65 ($p = 0.036$)	2.6 ($p = 0.27$)	
Indel allele frequency			
'D'	106/160 (0.66)	78 (0.65)	> 0.05
'I'	54/160 (0.34)	42 (0.35)	> 0.05

Table 3 Frequencies of *Pst*I and indel ACE haplotypes in the study groups.

Population	Combined genotypes									
	n	+ +/DD	+ −/DD	− −/DD	+ +/ID	+ −/ID	− −/ID	+ +/II	+ −/II	− −II
Control	60	20	4	6	12	6	0	12	0	0
Cases	80	20	12	6	18	12	0	12	0	0
	Haplotypes (frequency)									
	n	+ D	− D	+ I	− I					
Control	96	50 (0.52)	16 (0.17)	30 (0.31)	0 (0.0)					
Cases	140	74 (0.53)	24 (0.17)	42 (0.30)	0 (0.0)					

controls groups observed in age at diagnosis and body mass index (BMI) (Table 1). Clearly, the diabetic nephropathy had higher systolic/diastolic blood pressure (147/83 mmHg) compared to controls (127/75 mmHg) with a high significant difference ($p < 0.001$). Significant differences were found higher in duration of diabetes and in HbA1c for cases compared to controls ($p < 0.001$). Moreover, serum ACE activity and microalbuminuria were significantly higher in diabetic nephropathy cases compared to diabetic controls (< 0.0001 each).

In the present study, the associations between *ACE* gene polymorphisms in intron 7 (*Pst*I) and in intron 16 (indel) and diabetic nephropathy were examined separately (Table 2). The *Pst*I '+/+' genotype was a more frequent genotype (63.3%) than '+/−' (30%) and '−/−' (6.7%). The differences of the genotype distributions in cases were independently significant ($\chi^2 = 18.4$, 2 *df*, $p < 0.0001$). The difference was due to an excess of *Pst*I '+/+' homozygotes. The differences between the *Pst*I genotypes in cases and controls were not significant ($p > 0.05$ each).

For the indel polymorphism among the diabetic nephropathy, the D/D genotype in the *ACE* gene was a more frequent genotype (47.5%) than I/D (37.5%) and I/I (15%), with a significant difference ($\chi^2 = 6.65$, 2 *df*, $p = 0.036$), while the genotype distributions in control group were not significantly different ($\chi^2 = 2.6$, 2 *df*, $p = 0.27$) (Table 2). All indel possible genotypes were not significant compared to these genotypes in diabetic control group ($p > 0.05$ each).

Although the frequencies of the '+' allele or the 'D' allele were the most frequent in cases or controls independently; all alleles in this study were not defined as a risk factor in diabetic nephropathy cases compared to diabetic controls (> 0.05 each) (Table 2).

Table 3 showed the possible combined genotypes in the two ACE polymorphisms in the study. Combined haplotypes showed that the frequencies of the different *Pst*I–indel haplotypes were similar in all of our study groups (Table 3).

In both cases and control subjects, ACE activity and microalbuminuria were highest among D/D homozygotes and lowest in I/I homozygotes, with I/D heterozygotes having intermediate values (Table 4). A dissimilar relationship was shown for *Pst*I genotypes, in which the ACE activities were nearly comparable (Table 4). There were no differences in the ACE activity between cases and control subjects.

4. Discussion

The present study provides the first data on the genetic risk of *ACE* gene polymorphisms in type 1 diabetes with nephropathy in Egyptian population. Our study focused on the *Pst*I (in intron 7) and indel (in intron 16) loci, which we found, linked the risk of diabetic nephropathy in Egyptian children/adolescents. To our knowledge, only three studies in literature dealt with the effect of

Table 4 Serum ACE levels in groups with different *ACE* genotypes.

ACE genotypes ($n = 80$)	Total number of cases	Serum ACE activity (IU/l)	Microalbuminuria (μg/mg)
PstI polymorphism			
'+/+'	50	84.9 ± 50.0*	139.0 ± 66.2
'+/−'	24	82.7 ± 26.5*	115.5 ± 47.2
'−/−'	6	73.1 ± 19.8	64.8 ± 17.8
Indel polymorphism			
D/D	38	89.8 ± 19.2*	140.0 ± 47.0*
I/D	30	57.5 ± 12.4*	59.9 ± 23.3
I/I	12	55.4 ± 29.2	55.0 ± 19.4

Values are mean ± SD.
* $p < 0.001$ versus I/I genotype by Student's *t*-test.

*Pst*I polymorphism in diabetic nephropathy among Polish and Jewish ethnic population [24–26].

The '+/+' genotype showed a more likely higher frequency rather than other genotypes in diabetic individuals with nephropathy (cases) and without nephropathy (controls). We found no association between the *Pst*I alleles or genotypes in cases in the studied group. This is in agreement with Grzeszczak et al. in this issue [24].

A similar results were coming up with the 'D' allele in the ACE indel polymorphism. The latter finding is in agreement of other studies [18,19,26,27] that found no associations with the D allele and diabetic nephropathy. Unexpected results in Malays have been reported that there is a likely higher I/I genotype frequency in this ethnic population [28].

Interestingly, we also found an independent association between indel genotypes and diabetic nephropathy among the Egyptians. Although the '+/+' and the 'D/D' genotypes were generally the most frequent type of *ACE* polymorphisms, the probabilities of these genotypes as potential predictors for diabetic nephropathy is highly unlikely. In addition, the D/D, I/D, I/I genotypes were previously demonstrated to display highest, intermediate and lowest levels of serum ACE levels, respectively [11]. However, the DD genotype has always been held accountable for exerting its deleterious effects on various disease pathogenesis including diabetic nephropathy.

In this study, all potential clinical risk factors including diabetes duration, blood pressure, HbA1c, ACE activity, and microalbuminuria that might have predictive effects for diabetic nephropathy, while gender, age of subjects, and BMI had no effect on the development of diabetic nephropathy.

5. Conclusion

We conclude that in this study the role of the *ACE PstI* (in intron 7) and insertion/deletion (in intron 16) in the susceptibility to diabetic nephropathy was not demonstrated. Potential clinical risk factors of diabetes duration, blood pressure, HbA1c, ACE activity, and microalbuminuria did not alter the null influences of *ACE PstI* and indel polymorphisms on disease patterns in our population. Although statistically sufficient sample sizes were used in the present study, this sample size might be considered too small to permit any conclusion about the two *Pst*I–indel haplotypes associated in the risk of diabetic nephropathy.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

[1] World Health Organization (WHO). www.who.int/whr/2006/en.
[2] Hostetter TH. Diabetic nephropathy. In: Brenner BM, Rector FC, editors. The kidney. Philadelphia: WB Saunders; 1991. p. 1695–727.
[3] Krolewski AS, Warram JH, Rand LI, Kahn CR. Epidemiologic approach to the etiology of type I diabetes mellitus and its complications. N Engl J Med 1987;317:1390–8.
[4] Ziyadeh FN. Renal tubular basement membrane and collagen type IV in diabetes mellitus. Kidney Int 1993;43:114–20.
[5] Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med 1993;329:1456–62.
[6] Remuzzi A, Perico N, Amuchastegui CS, Malanchini B, Mazerska M, Battaglia C, et al. Short- and longterm effect of angiotensin II receptor blockade in rats with experimental diabetes. J Am Soc Nephrol 1993;4:40–9.
[7] Seaquist ER, Goetz FC, Rich S, BarbosaFamilial clustering of diabetic kidney disease J. Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. N Engl J Med 1989;320:1161–5.
[8] Pettitt DJ, Saad MF, Bennett PH, Nelson RG, Knowler WC. Familial predisposition to renal disease in two generations of Pima Indians with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1990;33:438–43.
[9] Quinn M, Angelico MC, Warram JH, Krolewski AS. Familial factors determine the development of diabetic nephropathy in patients with IDDM. Diabetologia 1996;39:940–5.
[10] Mooyaart AL, Valk EJ, van Es LA, Bruijn JA, de Heer E, Freedman BI, Dekkers OM, Baelde HJ. Genetic associations in diabetic nephropathy: a meta-analysis. Diabetologia 2011;54(3):544–53.
[11] Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angioten-

sin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86:1343–6.

[12] Ueda S, Elliot HL, Morton JJ, Connell JMC. Enhanced pressor response to angiotensin I in normotensive men with the deletion genotype DD for angiotensin-converting enzyme. Hypertension 1995;25:1266–9.

[13] Brown NJ, Blais Jr C, Gandhi SK, Adam A. ACE insertion/deletion genotype affects bradykinin metabolism. J Cardiovasc Pharmacol 1998;32:373–7.

[14] Rudberg S, Rasmussen LM, Bangstad HJ, Osterby R. Influence of insertion/deletion polymorphism in the ACE-I gene on the progression of diabetic glomerulopathy in type 1 diabetic patients with microalbuminuria. Diabetes Care 2000;23:544–8.

[15] Doria A, Warram JH, Rich SS, Krolewski AS. Angiotensin I-converting enzyme (ACE): estimation of DNA haplotypes in unrelated individuals using denaturing gradient gel blots. Hum Genet. 1994;94(2):117–23.

[16] Doria A, Warram JH, Krolewski AS. Genetic predisposition to diabetic nephropathy. Evidence for a role of the angiotensin I-converting enzyme gene. Diabetes 1994;43(5):690–5.

[17] 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(3):384–8.

[18] Schmidt S, Ritz E. Angiotensin-1 converting enzyme gene polymorphism and diabetic nephropathy in type 2 diabetes. Nephrol Dial Transplant 1997;12:37–41.

[19] Tarnow L, Gluud C, Parving HH. Diabetic nephropathy and the insertion/deletion angiotensin-converting enzyme gene. Nephrol Dial Transplant 1998;13:1125–30.

[20] Tabaei B, Al-Kassab A, Lag L, Zawacki C, Herman W. Does microalbuminuria predict diabetic nephropathy? Diabetes Care 2001;24(9):1560–6.

[21] Elhawary NA, Shawky RM, Elsayed N. High-precision DNA microsatellite genotyping in Duchenne muscular dystrophy families using ion-pair reversed-phase high performance liquid chromatography. Clin Biochem 2006;39(7):758–61.

[22] Yoshida H, Mitarai T, Kawamura T, Kitajima T, Miyazaki Y, Nagasawa R, et al. Role of the deletion of polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. J Clin Invest 1995;96(5):2162–9.

[23] Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res 1992;20:1433.

[24] 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.

[25] Grzeszczak W, Zychma M, Zukowska-Szczechowska E, Lacka B. Is PstI polymorphism of the angiotensin I converting enzyme gene associated with nephropathy development in non-insulin-dependent diabetes mellitus (preliminary study). Pol Arch Med Wewn. 1997;98:19–25.

[26] 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(10):2553–8.

[27] 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(2):78–86.

[28] Jayapalan JJ, Muniandy S, Chan SP. Angiotensin-1 converting enzyme I/D gene polymorphism: scenario in Malaysia. Southeast Asian J Trop Med Public Health 2008;39(5):917–21.