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Full Length Research Paper

Lack of association of insertion/deletion polymorphism in angiotensin converting enzyme gene with nephropathy in type 2 diabetic patients in Punjabi population of Pakistan

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The pathogenesis of diabetic nephropathy is not clearly understood. Beside haemodynamic alterations, genetic factors may also contribute to diabetic nephropathy leading to renal failure. Previous studies suggest that renin-angiotensin system may play critical role in progression and inhibition of diabetic nephropathy. Angiotensin converting enzyme gene insertion/deletion polymorphism is correlated to serum angiotensin converting enzyme activity that may be associated with diabetic nephropathy. We investigated the association of diabetic nephropathy with angiotensin converting enzyme gene insertion/deletion polymorphism in type 2 diabetes mellitus patients, in a case control study among 195 unrelated patients with type 2 diabetes mellitus and 65 age and sex matched non diabetic controls. Our study revealed that the distribution of DD, ID and II genotypes did not significantly differ between diabetic patients with diabetic nephropathy and without diabetic nephropathy (DD, 18.1%; ID, 44.6 %; II, 37.3%; vs. DD, 29.7%; ID, 38.6%; II, 30.7% respectively). We also compared different clinical and biochemical characteristics of the study population. In the present preliminary study the insertion/deletion polymorphism within angiotensin converting enzyme gene is not likely to be associated with nephropathy in type 2 diabetic patients of Punjabi population of Pakistan.

Key words: Angiotensin converting enzymes, insertion/deletion polymorphism, albuminuria and type 2 diabetes mellitus.

INTRODUCTION

Diabetic nephropathy is a leading cause of diabetic related morbidity and mortality. It is the leading cause of end-stage renal disease (ESRD) worldwide, and it is estimated that approximately 20% of type 2 diabetic patients reach ESRD during their lifetime (Movva et al., 2007). It is also the leading cause of ESRD requiring dialysis or transplantation in developing countries in Asia (Ng et al., 2005). Kidney disease in diabetic patients is clinically characterized by increasing rates of urinary albumin excretion, starting from normoalbuminuria, which progresses to microalbuminuria, macroalbuminuria/overt nephropathy, and eventually to ESRD. Microalbuminuria is the earliest clinically detectable stage of diabetic kidney disease at which appropriate interventions can retard, or

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Abbreviations: ACE; Angiotensin converting enzyme, **ARB;** angiotensin receptor blockers, **BMI;** body mass index, **CI;** confidence interval, **ESRD;** end-stage renal disease, **EDTA;** ethylene diamine tetra-acetic acid, **I/D;** Insertion /deletion, **OR;** odds ratio, **PCR;** polymerase chain reaction, **RAS;** ranin angiotensin system, **W:H;** waist to hip ratio.

reverse, the progress of the disease (Unnikrishnan et al., 2007).

Available data suggest that multiple factors such as haemodynamic alterations, metabolic abnormalities, various growth factors and genetic factors contribute to the pathogenesis of diabetic nephropathy (Tarnow, 1996). However, not all diabetic patients develop renal complications. Clustering of diabetic nephropathy in families and the large variation in its prevalence among different diabetic populations suggest the involvement of genetic factors (Fradin et al., 2002). The genetic basis of renal complication in diabetes is not clearly understood and many candidate genes have been shown to be associated with diabetic nephropathy. Genes encoding components of the renin-angiotensin system (RAS) are suggested as logical susceptibility determinants as angiotensin-II, the final product of the RAS increases intraglomerular capillary pressure causing glomerus-clerosis (Tripathi et al., 2006). Considerable interest is focused on angiotensin converting enzyme (ACE) gene encoding a key enzyme in the RAS that catalyses the conversion of angiotensin-I to angiotensin-II in liver and inactivates bradykinin in many tissues (Feng et al., 2002).

The patho-physiological effects of angiotensin-II are predominantly mediated through the angiotensin-II Type 1 receptor. A number of genetic polymorphisms with potential or established regulatory roles in the RAS have recently been identified. Although the insertion/deletion (I/D) polymorphism is in the intronic region of the ACE gene, this polymorphism is of a functional significance as the ACE levels have been shown to be genetically controlled (Jacobsen, 2005). Up to 50% of the inter-individual variations in plasma ACE levels can be attributed to an I/D polymorphism of the ACE gene with the highest ACE levels found in the DD homozygotes and the lowest found in II homozygotes while I/D subjects having inter-mediate levels (Cambien et al., 1992). Besides good glycemic control, measures such as the attenuation of the RAS using ACE inhibitors/angiotensin receptor blockers (ARB) are important in the clinical management of this complication (Lewis et al., 1993). However, the efficacy of ACE inhibitors/ARB in treating diabetic nephropathy can be influenced by genetic variation at the ACE gene locus (Jacobsen et al., 1998). ACE gene polymorphism is co-related to serum and tissue ACE activity; thus, ACE gene polymorphism may be associated with diabetic nephropathy in type 2 diabetes mellitus (Penno et al., 1998).

The human ACE gene is located in chromosome 17q23 with 25 introns and 26 exons and spans 21 kilo base pair (kbp), exons ranging in size from 88 bp to 481 bp, encoding for 4020 bp cDNA. Exons 1 to 12 encode for the amino domain, exons 13 to 26 encode for the carboxyl domain (Grzeszczak et al., 1998). The gene has been described with I/D polymorphism. The polymorphism consists of the presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence within intron 16, therefore, three genotypes are defined (DD, II, homozygotes and ID heterozygotes), and the D allele is associated with higher serum ACE activity (Koyama et al., 2008). According to previous studies, there are differences in the frequencies of I/D polymorphism in different ethnic groups. Conflicting findings in various populations have been obtained with regard to the role of ACE I/D polymorphism in type 2 diabetic patients regarding causation of albuminuria leading to nephropathy. Ethnic factors might contribute to variability between reports evaluating the role of ACE I/D polymorphism. Although, several studies on ACE gene polymorphism in type 2 diabetes mellitus and its association with diabetic nephropathy have been performed in Caucasian and Japanese populations, no substantial data is available about ACE gene polymorphism in patients of type 2 diabetes in Pakistan. Therefore, this study is designed to determine whether ACE gene polymorphism is associated with diabetic nephropathy in patients of type 2 diabetes mellitus in local population.

MATERIALS AND METHODS

Subjects

Patients with type 2 diabetes mellitus having duration of disease for more than 10 years of both sexes between the age groups of 40 to 65 years were selected from Diabetes Management Center, which is one of the super specialty centers in Services Institute of Medical Sciences, Lahore, Pakistan, having very well equipped computerized data base system of the diabetic patients.

Depending on the Urinary albumin excretion, the patients were divided into three groups including diabetes without nephropathy (normal albumen excretion), diabetes with incipient nephropathy (microalbuminuria) and diabetes with overt nephropathy (macroalbuminuria).

Phenotyping

For each of the patients, the information was collected in the Performa for other criteria too. That include age, gender, duration of diabetes mellitus, waist to hip (W:H) ratio, body mass index (BMI), systolic and diastolic blood pressure, fasting and random blood sugar levels and HbA1c pencentage. A total number of 195 patients were included in the study. All patients with hematuria, pyuria, positive urine culture and end stage renal disease were excluded in the study.

A total of 65 normal healthy age, sex and ethnically matched controls were recruited from general population. A written consent was obtained from the patients and the controls and it was documented in the detailed consent Performa. The study was approved by the ethical committee of Sheikh Zayed Federal Post Graduate Medical Institute, Lahore, Pakistan.

Sample collection and storage

After twelve hours overnight fast, blood samples were drawn by venipuncture and collected into lavender top vacutainers [containing ethylene diamine tetra-acetic acid (EDTA)] for determination of

Table 1. Clinical and biochemical characteristics of the study population.

Parameter	Group 1	Group 2	Group 3	Group 4
N	65	101	71	23
Age (years)	53.25±8.51	54.56±7.8	54.37±7.12	51.57±9.14
Sex (male, female)	25:40	38:63	24:47	11:12
Duration of Diabetes (years)		14.18±4.20	13.48±3.87	14.09±4.56
Waist to hip ratio	0.939±0.065[a]	0.984±0.072	0.998±0.065	0.984±0.063
BMI Body Mass Index (Kg/m^2)	24.42±4.12[b]	27.12±3.97	26.90±4.36	25.48±2.76
Systolic blood pressure (mmHg)	118.81±7.84[c]	123.29±18.53	127.25±19.85	127.17±21.36
Diastolic blood pressure (mmHg)	78.88±7.85	77.65±10.63	79.54±9.87	80.87±10.83
Fasting blood glucose (mg/dl)	81.45±10.02[d]	158.16±68.01 [e]	185.59±87.99	187.91±77.14
Random blood glucose (mg/dl)	109.91±14.98[f]	258.92±95.07	285.49±110.22	293.04±89.25
HbA1c (%)	----	8.29±1.08 [g]	9.73±1.30[h]	11.39±2.11[i]

Group 1, normal controls; group 2, diabetes without nephropathy; group 3, diabetes with microalbuminuria and group 4, diabetes with macroalbuminuria. Data are expressed as mean ± S.D. $P^a < 0.05$ as compared to group 2, 3, 4; $P^b < 0.05$ as compared to group 2, 3; $P^c < 0.05$ as compared to group 3, 4; $P^d < 0.001$ as compared to group 2, 3, 4; $P^e < 0.05$ as compared to group 3; $P^f < 0.001$ as compared to group 2, 3, 4; $P^g < 0.001$ as compared to group 3, 4; $P^h < 0.001$ as compared to group 2, 4; $P^i < 0.001$ as compared to group 2, 3.

HbA1C, plasma glucose and DNA extraction. Second blood sample was collected after two hours post prandial for estimation of plasma glucose. A fasting urine sample was collected for urine routine examination and culture sensitivity.

Determination of genotype

For the determination of ACE I/D polymorphism, genomic DNA was extracted by standard inorganic method from peripheral blood leukocytes already collected on EDTA (pH = 8) (Lahiri et al., 1992). The ACE I/D gene polymorphism was determined by polymerase chain reaction (PCR) using primers flanking the polymorphic region of intron 16 with the following primer sequences: 5'–CTGGAGACCACTCCCATCCTTTC and 5'–GATGTGGCCATC-ACATTCGTCAG. PCR was carried out in 25 µl volumes under standard conditions (3 mmol/l $MgCl_2$, 500 µmol/l for each dNTP, 10 mmol/l Tris/HCl, 50 mmol/l KCl, 0.2 µmol/l primers, 1 U Taq DNA polymerase per sample) in a thermocycler with 30 s denaturing time (94°C) 30 s annealing time (56°C) and 30 s extension time (72°C) for a total of 35 cycles followed by final extension for 5 min (72°C). Electrophoresis of amplified products in 2% agarose gel containing ethidium bromide allowed the detection of human ACE gene I/D polymorphism, characterized by the presence (insertion) or absence (deletion) of a 287 bp Alu repeat sequence in intron 16. The homozygous individuals for insertion allele (II genotype) were identified by the presence of single 490 bp product, the homozygous individuals for deletion allele (DD genotype) were identified by the presence of a single 190 bp product and the heterozygous individuals with insertion, deletion (ID genotype) were identified by the presence of both 190 and 490 bp product (Feng et al., 2002). Mistyping between DD and ID genotypes was overcome by a second, allele specific PCR, which was identified by the presence or absence of I allele.

Estimation of microalbuminuria

Microalbumin concentration was measured in a fasting urine sample using albumin:creatinine ratio (microgram per milligram of creatinine) by an immunoturbidometric assay (Hitachi 902 autoanalzyer, Roche diagnostics) after calibration of the instrument.

Statistical analysis

Data was expressed as Mean ± standard deviation (S.D). Student t-test or one way analysis of variance [ANOVA (Tukey's honestly significant difference comparison)] was used to compare continuous variables and x^2 –test was used to compare proportions among groups. A p-value less than 0.05 was considered statistically significant. Comparison of categorical data that is, different ACE genotype among controls and patients (with or without nephropathy) was done by Fisher's exact test and likelihood ratio. All statistical analysis was done using window based SPSS statistical package (version 15; SPSS, Chicago, IL).

RESULTS

The study group of this report comprises 65 ages and sex matched normal controls and 195 patients with type 2 diabetes mellitus for more than 10 years. All the subjects were divided into four groups: group 1, normal controls; group 2, diabetes without albuminuria; group 3, diabetes with microalbuminuria; group 4, diabetes with macroalbuminuria. All the four groups received a base line examination of clinical and biochemical characteristics which are summarized in Table 1. At base line the four groups were similar with regard to age. We did not find any significant difference of gender between the first three groups but percentage of males were found higher in group 4 as compared to group 3.

Diabetic patients had significantly higher value of W:H, fasting and random plasma sugar levels as compared to controls. BMI was significantly higher in diabetic patients both having normo and microalbuminuria but not with patients having macroalbuminuria as compared to controls. Diabetic patients having micro and macro-albuminuria have higher values of systolic blood pressure as compare to controls. HbA1c had significantly higher value in patients having overt nephropathy as compared to normo/microalbuminuria. We did not find any

Table 2. Clinical and biochemical characteristics of the type 2 Diabetic patients according to ACE genotype.

Parameter	ACE genotype		
	ID	DD	II
N	81	47	67
Age (years)	54.85±7.72	54.15±6.57	53.23±8.55
Sex (male, female)	31:50	19:28	23:44
Duration of Diabetes (years)	14.30±4.55	13.34±3.81	13.85±3.74
Waist to hip ratio	0.987±0.066	0.989±0.067	0.993±0.073
BMI (Kg/m^2)	27.17±3.78	25.96±3.73	27.07±4.42
Systolic blood pressure (mmHg)	124.72±18.06	126.81±23.04	124.63±18.28
Diastolic blood pressure (mmHg)	78.23±9.96	79.32±11.11	78.88±10.55
Fasting plasma glucose (mg/dl)	163.10±74.13	185.11±83.72	172.57±77.57
Random plasma glucose (mg/dl)	259.63±94.97	291.43±102.15	275.13±105.71
HbA1c (%)	8.89±1.69	9.57±1.58	9.24±1.70
Urinary Albumin (µg/mg of creatinine)	209.87±539.5	312.21±779.95	131.32±368.02
Nephropathy (%)	39/81 (48%)	30/47 (63%)	32/67 (47%)

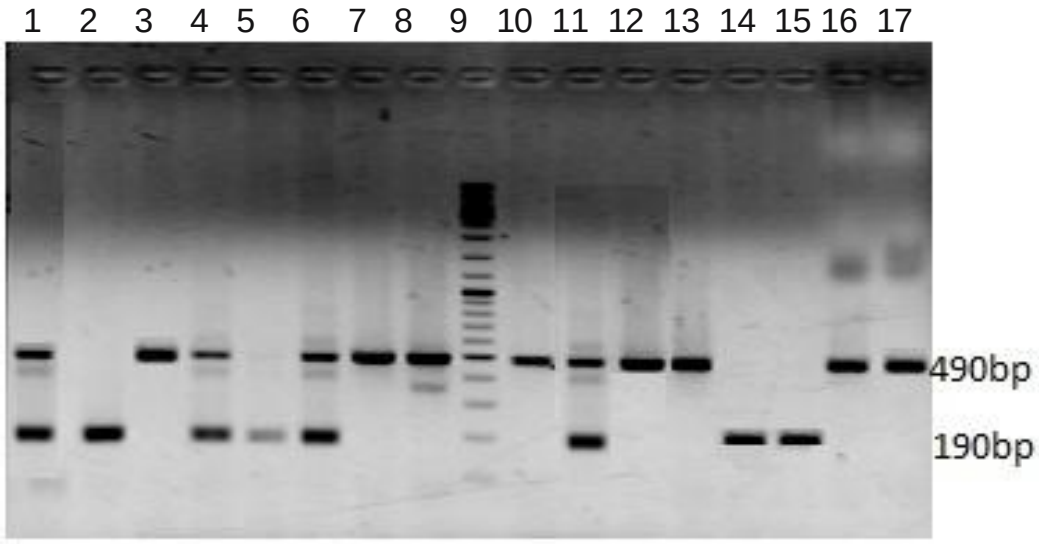

Figure 1. Ethidium bromide stained 2% agarose gel of 16 representative samples demonstrating PCR amplified DNA fragments showing homozygous DD, homozygous ID genotypes. Lane 2, 5, 14 & 15: Homozygous DD sample. Lane 3, 7, 8, 10, 12, 13, 16, & 17: Homozygous II samples. Lane 1, 4, 6 & 11: Heterozygous ID sample. Lane 9: DNA marker (# SM0403, Fermentas).

difference in duration of diabetes mellitus in all the diabetic patients.

After the clinical characterization of the four groups we further characterized type 2 diabetic patients according to ACE genotype by PCR using the previously described flanking primers as shown in Table 2 and Figure 1. There were no significant differences according to ACE genotype status with respect to age, duration of diabetes, W:H ratio, BMI, systolic and diastolic blood pressures, fasting and random plasma sugar and HbA1c. The percentages of patients having nephropathy (47 vs 63%, P is 0.12) were higher in patients with ACE DD genotype than in patients with ACE II genotype.

We further analyzed the distribution of ACE genotype and allele frequencies in type 2 diabetic patients without nephropathy with diabetic patients having nephropathy. Table 3 shows comparison of ACE genotype distribution and allele frequencies. The frequency of ACE D allele was 40.43% in patients without nephropathy compared to 49% in nephropathy patients. The difference in allele frequency was not statistically significant in both of these groups (p = 0.103). However, the ACE DD genotype was more frequent in patients having nephro-pathy (29.7%) compared to patients without nephropathy (18.1%) but the difference did not reach statistically significant levels (p = 0.066) when compared with other groups (ID + II).

Table 3. Distribution of ACE genotypes and allele frequencies in patients with type 2 diabetes with and without Nephropathy.

Genotypes	Type 2 diabetes mellitus without nephropathy	Type 2 diabetes mellitus with nephropathy
N	94	101
II	35 (37.3%)	32 (30.7%)
ID	42 (44.6%)	39 (38.6%)
DD	17 (18.1%)	30 (29.7%)
Alleles		
I	112 (59.6%)	103 (51%)
D	76 (40.4%)	99 (49%)

There was no significant difference in proportion of DD genotype with II or ID (p = 0.126, 0.100) respectively, when patients with or without nephropathy were compared.

DISCUSSION

The data presented in this study is the first from Pakistani population of Punjab regarding the role of genetic variation of ACE gene in causation of nephropathy in type 2 diabetes mellitus patients. The findings clearly establish that there is no association of nephropathy with genotypic variation in ACE I/D polymorphism (OR = 0.51, P = 0.1).

Originally, it was shown that an I/D polymorphism of the ACE gene was associated with the risk of myocardial infarction in patients with type 2 diabetes (Ruiz et al., 1994). The role of polymorphism in diabetic nephropathy is more ambiguous. There is an initial observation that type 1 diabetes patients with an insertion in the ACE gene has less frequent albuminuria and diabetic nephropathy (Marrc et al., 1994). This proposition was later on tested by a number of investigations (Parving et al., 1996; Tarnow et al., 1995). In type 2 diabetes no definite evidence have been found of a relationship between I/D polymorphism and presence or absence of nephropathy at least in our study of Pakistani population, although in Japanese and North Indian populations a significant association with D allele have been noted (Triapthi et al., 2006; Doi et al., 1996; Ohno et al., 1996).

In contrast to our study a Meta analysis conducted by Kunz and coworkers showed that the risk of nephro-pathy was increased in the presence of DD or ID genotypes in Asian patients with diabetes mellitus (Kunz et al., 1998). Similar findings were reported by Jeffers and coworkers showing an association between ACE DD genotype and diabetic nephropathy (Jeffers et al., 1997).

On the other hand lack of association was documented between ACE allele and genotype frequency in diabetic patients with and without nephropathy (Arfa et al., 2008). Our study did not show a positive association between the DD genotypes of ACE gene polymorphism in diabetic nephropathy and type 2 diabetic patients without nephropathy. Relative risk for DD homozygous subjects was 1.33 (95% CI = 1.013 - 1.748, p = 0.066) as compared with II genotype.

We also investigated the frequency between the D allele (DD + ID) and II genotype in two groups. The D allele frequency in diabetic nephropathy group was found slightly higher than that of the type 2 diabetic patients without nephropathy (OR = 0.781, 95% CI: 0.432-1.413, P = 0.452) that indicated the D allele was not associated with diabetic nephropathy.

Our study did not show any positive relationship between the D allele and development of diabetic nephropathy in type 2 diabetic patients (95% CI: 0.97-1.51, RR = 1.21, p = 0.10). Our finding is not in accordance with data reported by Ohno (OR = 2.6) and Yoshida (OR = 4.60), (Ohno et al., 1995; Yoshida et al., 1996).

In conclusion, data of this investigation concluded that DD genotype and D allele in type 2 diabetic patients with and without nephropathy were insignificant. There is need to investigate along with ACE, other genetic variants including polymorphism of angiotensin-II type 1 receptor gene (AT1 A1166C) and angiotensinogen gene (M235T) polymorphism for association with diabetic nephropathy. However, this work is still in progress in our laboratory.

REFERENCES

Arfa I, Abid A, Nouira S, Elloumi-Zghal H, Maloucbe D, Mannai I Zorgati MM, Ben Alaya N, Rebai A, Zouari B, Ben Ammar S, Ben Rayana MC, Hmida S, Blousa-Chabchoub S, Abdelhak S (2008). Lack of association between the angiotensin-converting enzyme gene (I/D) polymorphism and diabetic nephropathy in Tunisian type 2 diabetic patients. J. Renin-Angio-Aldo. S. 9: 32-36.

Cambien F, Poirier O, Lecerf I, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S (1992). Deletion polymorphism in the gene for angiotensin converting enzyme is a potent risk factor for my ocardial infarction. Nature, 359: 641-644.

Doi Y, Yoshizumi H, Yoshinari M, Lino K, Yamamoto M, Ichikawa K, Iwase M, Fujishima M (1996). Association between a polymorphism in the angiotensin converting enzyme gene and microvascular complications in Japanes patients with NIDDM. Diabetologia. 39: 97-102.

Feng Y, Niu T, Xu X, Chen C, Li Q, Qian R, Wang G, Xu X (2002). Insertion/Deletion polymorphism of the ACE gene is associated with

type 2 diabetes. Diabetes, 51: 1986-1988.
Fradin S, Salmon BG, Chantepie M, Grandhomme F, Morello R, Jauzac PH, Reznik Y (2002). Relationship between polymorphisms in the rennin angiotensin system and nephropathy in type 2 diabetic patients. Diabetes Metab. 28: 27-32.
Grzeszczak W, Zychma MJ, Lacka B, Szczechowsaa EWAZ (1998). Angiotensin converting enzyme gene polymorphism: Relationship to nephropathy in patients with non-insulin dependent diabetes mellitus. J. Am. Soc. Nephrol. 9: 1644-1649.
Jacobsen PK (2005). Preventing end stage renal disease in diabetic patients- genetic aspect (part I). J. Renin-Angio-Aldo. S. 6: 1-14.
Jeffers BW, Estacio RO, Raynolds MV, Schrier RW (1997). ACE gene polymorphism in NIDD and its relationship with diabetic nephropathy. Kidney Int. 52: 473-477.
Koyama RG, Casto RMRPS, Mello MT, Tufik S, Pedrazzole M (April 2008). Simple detection of large InDeLS by DHPLC: The Ace gene as a model. J. Biomed. Biotechnol. [5 Pages]. Available from: URL:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2358980/?tool=pubmed.
Kunz R, Bork JP, Fritche L, Ringel J, Sharma AM (1998). Association between the angiotensin-converting enzyme insertion/deletion polymorphism and diabetic nephropathy: a methodological appraisal and systematic review. J. Am. Soc. Nephrol. 9: 1653-1663.
Lahiri DK, Bye S, Nurnberger Jr. JI, Hodes ME, Crisp M (1992). A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. J. Biochem. Bioph. Method, 25: 193-205.
Marrc M, Bernadet P, Gallois Y, Svagner F, Guyene TT, Hallab M, Cambien F, Passa P, Alhenc-Gelas F (1994). Relationship between angiotensin-I converting enzyme gene polymorphism, plasma levels and diabetic retinal and renal complications. Diabetes, 43: 384-388.
Movva S, Alluri RV, Komandur S, Vattam K, Eppa K, Mukkavali KK, Mubigonda S, Saharia S, Shastry JC, Hasan Q (2007). Relationship of angiotensin converting enzymes gene polymorphism with nephropathy associated type 2 diabetes mellitus in Asian Indians. J. Diabetes Complicat. 21: 237-241.
Ng DPK, Tai BC, Koh D, Tan KW, Chia KS (2005). Angiotensin-I converting enzyme insertion/deletion polymorphism and its association with diabetic nephropathy: a meta-analysis of studies reported between 1994 and 2004 comprising 14727 subjects. Diabetologia, 48: 1008-1016.
Ohno T, Kawazn S, Tomono S (1995). Association analyses of the polymorphism of ACE and angitensinogen genes with diabetic nephropathy in Japanese non-insulin-dependent diabetics. Metabolism, 45: 218-222.
Parving HH, Tarnow L, Rossing P (1996). Genetics of diabetic nephropathy. J. Am. Soc. Nephrol. 7: 2509-2517.
Penno G, Chaturvedi N, Talmud PJ, Cotroneo P, Manto A, Nannipieri M, Philippa J, Luong LA, Fuller JH (1998). 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, 47: 1507-1511.
Ruiz J, Blanche H, Cohen N, Velho G, Cambien F, Cohen D, Passa P, Froguel P (1994). Insertion/deletion polymorphism of angiotensin-converting enzyme gene is strongly associated with coronary heart disease in non-insulin-dependent diabetes mellitus. Proc. Natl. Acad. Sci. USA. 91: 3662-3665.
Tarnow L, Cambien F, Rossing P, Nielson FS, Hansen BV, Lecerf L, Poirier O, Danilov S, Parving HH (1995). Lack of relationship between an insertion/deletion polymorphism in the angioternsn I converting enzyme gene and diabetic nephropathy in IDDM patients. Diabetes, 44: 489-494.
Tarnow L (1996). Genetic patterns in diabetic nephropathy. Nephrol. Dial. Transplant, 11: 410-412.
Tripathi G, Dharmani P, Khan F, Sharma RK, Pandirikkal V, Agrawal S (2006). High prevalence of ACE DD genotype among north Indian end stage renal disease patients. BMC Nephrol. [Seven screens]. Available from: URL:http://www.biomedcentral.com/1471-2369/7/15.
Unnikrishnan R, Rema M, Pardeepa R, Deepa M, Shanthirani CS, Deepa R, Mohan V (2007). Prevalence and risk factors of diabetic nephropathy in an urban South Indian Population. Diabetes Care, 30: 2019-2024.
Yoshida H, Kuriyama S, Asumi Y, Tomonari H, Mitarai T, Hamaguchi A, Kubo H, Kawaguchi Y, Kon V, Matsuoka K, Ichikawa I, Sakai O (1996). Angiotensin converting enzyme genepolymorphism in NIDDM. Kidney Int. 50: 657-664.