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# Association between ACE Gene Polymorphism and Diabetic Nephropathy in South Indian Patients

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## ABSTRACT

**Objective** To study the association of ACE gene polymorphism and diabetic nephropathy in South Indian subjects.

**Setting** Outpatient clinic of a specialized hospital.

**Patients** The study included 109 South Indian type 2 diabetic patients (72 males and 37 females; age 56.7±9.0 years, mean±SD). The patients were subdivided into two groups: nephropathic (n=86) and normoalbuminuric patients (n=23).

**Interventions** Genomic DNA was isolated from the peripheral blood leukocytes. To determine the ACE genotype, genomic DNA was amplified by PCR initially using a flanking primer pair and, subsequently when necessary, with a primer pair that recognizes the insertion specific sequence for confirmation of the specificity of the amplification reactions.

**Main outcome measures** ACE genotype distribution in the two study groups.

**Results** In the nephropathic patients, ID and DD genotypes were present in 52.3% and 27.9% of the patients, respectively as compared to 34.8% and 21.7% respectively in those with normoalbuminuria. The D allele was present in 80.2% of the nephropathic patients and 56.5% of the normoalbuminuric patients ($\chi^2$=4.28, P=0.039; odds ratio 3.12). Therefore, the higher percentage of II genotype in the normoalbuminuric group was 43.5% as compared to the 19.8% in nephropathic patients.

**Conclusions** This study showed a positive association between the D allele (ID and DD genotype) of the ACE polymorphism and diabetic proteinuria in South Indian type 2 diabetic patients. Our findings are in keeping with several earlier studies showing a strong association of the D allele of the ACE gene with diabetic nephropathy.

## INTRODUCTION

South Asian type 2 diabetic patients have been shown to have a higher prevalence of nephropathy when compared to Europeans [1, 2].

ACE polymorphism appears to have a significant impact on the progression of diabetic nephropathy [3]. Several Japanese studies have found the D allele to be an independent risk factor for diabetic nephropathy [4]. It is important to look for the gene association in the Asian Indian population, in view of the high prevalence of diabetic nephropathy and to see whether the association differs from other populations. To our knowledge, there have been no studies on ACE

gene polymorphism in the native Indian population.

## METHODS

### Patients

The study was carried out in 109 South Indian type 2 diabetic patients (72 males and 37 females; age 56.7±9.0 years, mean±SD). Inclusion criteria were: patient age greater than or equal to 30 years, duration of type 2 diabetes greater than or equal to 5 years. Patients not on oral hypoglycemic agents and/or with congestive cardiac failure were excluded from the study.

Eighty-six of these 109 patients were consecutive cases with nephropathy referred as outpatients at the hospital during a 7-month period. These patients were selected on the basis of:

- presence of persistent proteinuria greater than or equal to 500 mg/dL;
- presence of hypertension;
- presence of diabetic retinopathy;
- normal urine microscopy.

In addition, 23 patients with normoalbuminuria, normal urine microscopy and absence of both hypertension and retinopathy were studied. Normoalbuminuria was defined on the basis of an albumin excretion rate less than or equal to 30 μg/mg creatinine in early morning urine samples using the immunoturbidimetry method for albumin. These patients were selected from the outpatients treated in the hospital in the same time period.

Data on the duration of the diabetes, blood pressure, prevalence of hypertension, glycosylated hemoglobin (HbA1) and body mass index (BMI, $Kg/m^2$) were recorded for each patient.

### DNA Isolation and Determination of ACE Genotype

Genomic DNA was isolated from peripheral blood leukocytes according to published protocols for extracting DNA from human nucleated cells [5]. To determine the ACE genotype, genomic DNA was amplified by PCR [6] initially using a flanking primer pair and subsequently when necessary, with a primer pair that recognizes the insertion specific sequences for confirmation of the specificity of the amplification reactions.

The flanking primer pair used was 5'CTGGAGACCACTCCCATCCTTTCT3' and 5'GATGTGGCCATCACATTCGTCACGAT3'. Amplification with this primer pair results in 490 bp and 190 bp amplification products corresponding to the I and D alleles, respectively. PCR amplification used 25 μL reactions (0.5 μg genomic DNA, 200 pmol of each primer, 0.5 mM each of deoxy-ATP, GTP, CTP, thymidine 5-triphosphate (TTP), 3 mM $MgCl_2$, 1 unit of Taq DNA polymerase (Perkin-Elmer, Norwalk, CT, USA), 0.001% gelatin and 10 mM Tris-HCl, pH 8.3) with 10 min. denaturation at 94 $^oC$, followed by 30 cycles of one min. at 94 $^oC$, one min. at 58 $^oC$ (annealing) and two min. at 72 $^oC$ (extension) in a thermal cycler. PCR products were detected on a 2% agarose-gel containing ethidium bromide.

Mistyping of ID heterozygotes as D homozygotes may occur due to the preferential amplification of the D allele and inefficiency in the amplification of the I allele [7]. To increase the specificity of DD genotyping, PCR amplifications were also performed with an insertion specific primer pair (5'TGGGACCACAGCGCCCGCCACTAC3' and 5'TCGCCAGCCCTCCCATGCCCATAA3') in all samples that were found to be DD after amplification with the flanking primers. Briefly, insertion-specific amplification was performed using 25 μL reactions (0.5 μg genomic DNA, 200 pmol of each primer, 0.5 mM each of deoxy-ATP, GTP, CTP, TTP, 3 mM $MgCl_2$, 0.5 units of Taq DNA polymerase, 0.001% gelatin and 10 mM Tris-HCl, pH 8.3) with one min. denaturation at 94 $^oC$, followed by 30 cycles of 30 sec. at 94 $^oC$, 45 sec at 67 $^oC$ (annealing), and two min. at 72 $^oC$ (extension). Under these conditions, only the I allele produced a 335 bp amplicon. The 335 bp

**Table 1.** Clinical details of 109 South Indian type 2 diabetic patients.

| | Nephropathic patients (No. 86) | Normoalbuminuric patients (No. 23) | Statistics | P value |
|---|---|---|---|---|
| **Sex: males / females** | 57 (66.3%) / 29 (33.7%) | 15 (65.2%) / 8 (34.8%) | $\chi^2$=0.00 | 1.000 |
| **Age (years)** | 56.7±8.9 | 56.7±9.3 | *t*=0.00 | 1.000 |
| **BMI (Kg/m$^2$)** | 25.9±4.2 | 25.7±3.5 | *t*=0.21 | 0.834 |
| **Duration of diabetes (years)** | 13.4±6.9 | 13.2±5.1 | *t*=0.13 | 0.897 |
| **Duration of hypertension (years)** | 6.1±3.5 | 5.7±3.8 | *t*=0.48 | 0.634 |
| **Systolic blood pressure (mmHg)** | 146.6±18.6 | 132.0±13.6 | *t*=3.52 | <0.001 |
| **Diastolic blood pressure (mmHg)** | 85.1±8.5 | 80.9±10.0 | *t*=2.03 | 0.045 |
| **HbA1 (%)** | 9.8±1.1 | 9.9±1.0 | *t*=-0.40 | 0.689 |

fragment was identified on 2% agarose-gel containing ethidium bromide. The reaction yields no products in samples of DD genotype.

## ETHICS

The study was approved by the ethical committee of the Diabetes Research Centre of Madras. Informed oral consent was obtained from each patient.

## STATISTICS

Data are reported as mean ± standard deviation (SD). Statistical comparisons between group means were done by the unpaired Student *t*-test, while proportions were compared by means of the Yates' corrected chi-squared test ($\chi^2$). The odds ratio (OR), together with the 95% confidence interval (CI), comparing the allelic distributions in the two study groups were also calculated. Two-tailed P values less than 0.05 were considered significant. The SPSS/PC+ 4.01 package was used to perform statistical analyses.

## RESULTS

As shown in Table 1, the two study groups were well-matched for gender, age, body mass index (BMI), duration of both diabetes and hypertension, and HbA1 values. Nephropathic patients had significantly higher systolic and diastolic blood pressure values.

Table 2 shows the ACE genotype distribution in nephropathic and normoalbuminuric patients. Among the total patients, 27 (24.8%) patients had the II genotype, while the ID genotype was present in 53 patients (48.6%) and the DD genotype in 29 patients (26.6%). In nephropathic patients, the ID and DD genotypes were present in 52.3% and 27.9% of patients, respectively, as compared to 34.8% and 21.7% in normoalbuminuric patients. Total D allele was present with a significantly higher prevalence ($\chi^2$=4.28, P=0.039) in nephropathic

**Table 2.** Distribution of ACE genotype in 109 South Indian type 2 diabetic patients.

| ACE genotype | Nephropathic patients | | Normoalbuminuric patients | |
|---|---|---|---|---|
| | No. of cases (% vs. total D) | % vs. overall cases | No. of cases (% vs. total D) | % vs. overall cases |
| **ID** | 45 (65.2%[b]) | 52.3% | 8 (61.5%[b]) | 34.8% |
| **DD** | 24 (34.8%[b]) | 27.9% | 5 (38.5%[b]) | 21.7% |
| **Total D alleles (ID+DD)** | 69 | 80.2%[a] | 13 | 56.5%[a] |
| **II** | 17 | 19.8%[a] | 10 | 43.5%[a] |
| **Overall No. of cases** | **86** | | **23** | |

[a] $\chi^2$ = 4.28; P=0.039. Total D and II allele distributions (nephropathy vs. normoalbuminuria)

[b] $\chi^2$ = 0.00; P=1.000. ID and DD distributions within total D alleles (nephropathy vs. normoalbuminuria)

patients (80.2%) than in normoalbuminuric patients (56.5%); therefore, the percentage of II genotype was higher in normoalbuminuric patients (43.5%) as compared to the nephropathic patients (19.8%). According to these prevalence values, the odds ratio (OR) related to the association of the D allele with nephropathy was 3.12 (95% CI: 1.17-8.32). No significant differences between nephropathic and normoalbuminuric patients were observed as far as the distribution of the ID and DD alleles within the total D class were concerned ($\chi^2$=0.00, P=1.000). Taking into account nephropathic patients only, the severity of proteinuria, as well as the blood levels of urea and creatinine, were not significantly related to the different genotypes (considering II, ID, and DD alleles, respectively: proteinuria: 1,940±1,401, 2,017±1,632, and 1,965±1,603 mg/dL; urea 43±31, 38±14, and 38±17 mg/dL; creatinine 1.3±1.0, 1.1±0.6, and 1.1±0.6 mg/dL).

## DISCUSSION

This study demonstrated a positive association between the D allele (ID and DD genotype) of the ACE polymorphism and diabetic nephropathy in South Indian type 2 diabetic patients. Several Japanese studies had also found the D allele to be an independent risk factor for diabetic nephropathy in type 2 diabetic patients [4, 6, 8]. The odds ratio noted in our study for the association of D allele with nephropathy (OR=3.12) was comparable to the report of Ohno *et al.* [8] (OR=2.6) and Yoshida *et al*. [6] (OR=4.6) in similar analyses. Jeffers studied 509 type 2 Caucasian diabetic patients and found the DD genotype to be an independent risk factor for diabetic nephropathy with an OR equal to 2.8 [9].

A meta analysis showed that patients who were homozygous for the deletion allele (DD genotype) had a rapid decline in renal function and the D allele also appeared to be significantly associated with diabetic nephropathy [10]. We did not observe any association between the D allele and severity of nephropathy. This is probably related to the small numbers in each allelic group. Kennon *et al.* also, in their review of the literature, found a significant association between the DD genotype and diabetic and non-diabetic renal disease [3]. The French and Belgian GENEDIAB study demonstrated that the D allele was associated with both an increased incidence and severity of diabetic nephropathy in a large group of type 1 diabetic patients [11].

We have not studied the distribution of ACE gene polymorphism in the general population. In the diabetic nephropathy group, there was an elevated association with the D allele. This was in keeping with the observations in several populations. Follow-up studies of the patients are being carried out to study the relationship of the genotypes with the severity and the rate of decline of kidney function.

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**Key words** Diabetes Mellitus, Non-Insulin-Dependent; Diabetic Nephropathies; India; Peptidyl-Dipeptidase A; Polymorphism (Genetics)

**Abbreviations** TTP: thymidine 5-triphosphate

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