

Lack of association between the angiotensin-converting enzyme gene (I/D) polymorphism and diabetic nephropathy in Tunisian type 2 diabetic patients

Imen Arfa,* Abdelmajid Abid,[†] Sonia Nouira,* Houda Elloumi-Zgbal,* Dhafer Malouche,[†] Imen Mannai,^{*†} Mohamed Majdi Zorgati,* Nissaf Ben Alaya,[^] Ahmed Rebai,[#] Béchir Zouari,[‡] Slim Ben Ammar,* Mohamed Chibeb Ben Rayana,⁺ Slama Hmida,[∞] Samira Blousa-Chabchoub,⁺ Sonia Abdelhak*

Key words: angiotensin-converting enzyme, diabetic nephropathy, insertion-deletion polymorphism, type 2 diabetes

* Molecular Investigation of Genetic Orphan Diseases Research Unit, Institut Pasteur de Tunis, Tunis, Tunisia.

[†] Engineering school of statistic and information analysis (LEGI-EPT-ESSAIT), University of 7th November at Carthage Tunis, Tunisia.

⁺ National Institute of Nutrition, Tunis, Tunisia.

[^] Laboratory of Epidemiology, Institut Pasteur de Tunis, Tunis, Tunisia.

[#] Center of Biotechnology Sfax, Tunisia.

[‡] Department of Epidemiology and Statistics, School University of Medicine, Tunis, Tunisia.

[∞] National Center of Blood Transfusion Tunis, Tunisia.

Journal of the Renin-Angiotensin-Aldosterone System

(Including other Peptidergic Systems)

March 2008
Volume 9
Number 1

Abstract

Objective. The aim of the present study was to investigate whether the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism is associated with diabetic nephropathy and type 2 diabetes in the Tunisian population.

Design. A case-control study was conducted among 141 unrelated type 2 diabetic patients with (90 patients) or without nephropathy (51 patients) and 103 non-diabetic controls with normal fasting blood glucose. Genotyping was performed using a nested polymerase chain reaction amplification in order to identify correctly heterozygous individuals.

Results. The distribution of DD, ID and II genotypes did not significantly differ between type 2 diabetic patients with or without nephropathy (DD: 44%; ID: 46%; II: 10% *vs.* DD: 41%; ID: 47 %; II: 12%, respectively). There was also no significant statistical difference between the genotype distribution and allele frequencies of the (I/D) polymorphism in all type 2 diabetic subjects compared to non-diabetic controls with normal fasting blood glucose (DD: 43%; ID: 46%; II: 11% *vs.* DD: 37%; ID: 48%; II: 15%, respectively). **Conclusions.** In the present preliminary study, the (I/D) polymorphism within the ACE gene is likely not associated with diabetic nephropathy nor with type 2 diabetes in the Tunisian studied population.

Introduction

Type 2 diabetes is a common metabolic disorder that has an evident genetic component as shown by the strong familial aggregation and high concordance in twins.^{1,2} Poor glycaemic control, disease duration and blood pressure (BP) leads to the development of diabetic microvascular complications. Diabetic nephropathy is a serious microvascular complication of diabetes mellitus, accounting for 22% of patients on dialysis in Tunisia (Kheder 2005, personal communication). However, not all diabetic patients develop renal complications.³ Clustering of diabetic nephropathy in families^{4,5} and the large variation in its prevalence among different diabetic populations suggest the involvement of genetic factors. The genetic basis of renal

complication in diabetes is not clearly understood but many candidate genes have been shown to be associated with diabetic nephropathy.^{6,8} Genes encoding components of the renin-angiotensin system (RAS) are suggested as logical susceptibility determinants as angiotensin (Ang) II the final product of the RAS increases intraglomerular capillary pressure causing glomerulosclerosis.^{9,10} Considerable interest was focused on angiotensin-converting enzyme (ACE) gene encoding a key enzyme in the RAS that catalyses the conversion of Ang I to Ang II in liver and inactivates bradykinin in many tissues.^{11,12} Studying the ACE gene is supported by clinical and experimental studies showing that treatment with ACE-inhibitors prevents and reduces progression of diabetic nephropathy¹³⁻¹⁵ and suppresses hepatic glucose production in type 2 diabetic patients.¹⁶ ACE gene spans 21 Kb on chromosome 17 and is characterised by the presence (insertion [I]) or absence (deletion [D]) of a 287 bp Alu repeat sequence within intron 16.¹⁷ Although the (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. Patients homozygous for the II have the lowest ACE levels whilst patients with DD genotype have the highest levels.^{17,18} Conflicting findings in various populations have been obtained with regard to the role of ACE (I/D) polymorphism in diabetic nephropathy¹⁹⁻²¹ and in type 2 diabetes.²²⁻²⁴ Ethnic factors might contribute to variability between reports evaluating the role of ACE (I/D) polymorphism. To our knowledge, there is no available data testing the involvement of this polymorphism in North Africans. In the present study we investigated whether the ACE (I/D) polymorphism is associated with diabetic nephropathy and type 2 diabetes in the Tunisian population.

Material and methods

Study population

For this study we explored 141 unrelated type 2 diabetic patients (WHO criteria) attending the National Institute of Nutrition (Tunis, Tunisia), a referral diabetes Medical Center in Tunisia (85 women and 56 men, aged 61±9.9 years, known

Correspondence to:
Dr Sonia Abdelhak
Molecular Investigation
of Genetic Orphan
Diseases Research Unit,
Institut Pasteur de Tunis,
Tunis, Tunisia.
Tel: +216 71 78 96 08
Fax: +216 71 79 18 33
E-mail: sonia.abdelhak
@pasteur.rns.tn

Accepted for publication
8th November 2007

JRAAS 2008;9:32-36

type 2 diabetes from 17.1±6.8 years). All patients gave their fully informed consent and were screened for renal complication due to diabetes. Diabetic nephropathy was defined by persistent microalbuminuria (incipient nephropathy: albumin excretion rate [AER] of 30–300 mg/24 hour) or macroalbuminuria (established nephropathy: AER > 300 mg/24 hour) on at least two consecutive occasions over the previous six months. All subjects with renal disease other than diabetic nephropathy were excluded by appropriate clinical investigations. Patients with AER < 30 mg/24 hour were normoalbuminuric and had no diabetic nephropathy. To avoid misclassification of diabetic individuals as having no nephropathy because of diabetes duration, we excluded patients with diabetes duration < 10 years from our study. Fifty-one diabetic patients without nephropathy who fulfilled this criterion were compared to 90 diabetic patients with nephropathy. To assess the relationship between ACE (I/D) polymorphism and type 2 diabetes we recruited 103 non-diabetic controls (63 women and 40 men, aged 38.6±8 years) with normal fasting blood glucose. Clinical and biochemical measurements of patients with type 2 diabetes were performed after at least eight hours of fasting. Weight and height were measured to calculate the body mass index (BMI), systolic BP (SBP) and diastolic BP (DBP) was measured in a sitting position after at least five minutes of rest. Plasma levels of glucose, total cholesterol and triglycerides were analysed by enzymatic methods using Beckman reagents on a Synchron C x 9 Beckman analyser. Haemoglobin A_{1C} (HbA_{1C}) was analysed by HPLC using Biorad analyser. The AER was measured in an overnight urine collection using an immunoturbidimetric assay.

Determination of the ACE genotype

Determination of the ACE genotypes was performed according to Marre *et al.*,²⁵ with minor modifications. After informed consent, 10 ml of whole blood were taken from each patient (into ethylenediamine tetra-acetic acid [EDTA]), lymphocytes were isolated and genomic DNA was extracted using a phenol/chloroform standard method. The (I/D) polymorphism in intron 16 of ACE gene was determined by nested polymerase chain reaction (PCR) using flanking primer pairs GIIS (5'-CTCAAGCAGCCCCCTCAC-3') and GAS (-5'GATGTGGCCATCACATTTCGTC-3'). A specific primer pair FYM (5'-ATCAGGAGTCAGGAGATCGA-3') corresponding to the insertion sequence was added. PCR amplification was performed in a single tube using 50 µl reactions (200 ng genomic DNA, 10 µM each primer, 250 µM each deoxyribonucleotides triphosphates (dNTP), 1.5 mM magnesium chloride (MgCl₂), 1U *thermus aquaticus* (Taq) DNA polymerase, amplification buffer contained 20mM Tris-hydrochloric acid (TRIS-HCL) (ph 8.8) and 50 mM KCL) with five minutes of initial denaturation at 96°C followed by 20 cycles of amplification using GIIS and GAS primers pairs (one minute at 94°C, one minute at 62°C, one minute at 72°C). To avoid preferential amplification of the D allele, the primer FYM recognising the insertion specific sequence and GIIS were added and PCR reaction continued for 15 cycles in the same conditions, followed by a final extension at 72°C for seven minutes. The amplified products were detected on 2% agarose gel containing ethidium bromide. Amplification with GIIS and GAS produced a fragment of 274 bp for the D allele and a fragment of 561 bp for the I allele. Further extension between GIIS and FYM generated a 376 bp fragment for the insertion.

Table 1

Characteristics and clinical data of Tunisian type 2 diabetic patients with or without nephropathy.

Variables	Type 2 diabetic patients (n=141)		p-value
	Without nephropathy (n=51)	With nephropathy (n=90)	
Sex (M/F)	17/34	39/51	0.24
Age (years)	61.6±10.1	60.6±9.7	0.59
Diabetes duration (years)	17.1±5.2	17.1±7.6	0.9
BMI (Kg/m ²)	27.26±5.5	28.47±4.77	0.2
SBP (mmHg)	139.5±14.4	147.9±16.6	< 0.01
DBP (mmHg)	81.8±4.7	83.2±5.7	0.1
Cholesterol (mmol/L)	4.91±0.78	5.43±0.88	< 0.01
TG (mmol/L)	1.34 (0.43–3.14)	1.73 (0.48–5.62)	< 0.01
Fasting plasma glucose (mmol/L)	11.91±2.48	12.21±3.44	0.6
2 hour plasma glucose (mmol/L)	15.44±3.24	16.26±4.16	0.2
HbA _{1C} (%)	8.97±1.25	9.45±1.92	0.1

Key: Data are given as means±SD or medians (min-max); BMI = body mass index; DBP = diastolic blood pressure; HbA_{1C} = glycated haemoglobin; SBP = systolic blood pressure; TG = triglyceride.

Table 2
Genotype distribution and allele frequencies of the (I/D) polymorphism in Tunisian patients with type 2 diabetes with or without nephropathy.

Variables	No nephropathy (n=51)	Incipient nephropathy (n=36)	Established nephropathy (n=54)
Genotypes			
DD	41 (28–56)	42 (25–59)	46 (33–60)
ID	47 (33–61)	44 (28–62)	46 (33–60)
II	12 (4–24)	14 (5–29)	7 (2–18)
Alleles			
D	0.65	0.64	0.69
I	0.35	0.36	0.31

Key: Data are % (exact binomial 95% CI), $X^2=1.16$, p -value=0.88. I = insertion; D = deletion.

Table 3
Genotype distribution and allele frequencies of the (I/D) polymorphism in Tunisian patients with type 2 diabetes with or without nephropathy.

Variables	Type 2 diabetic patients (n=141)	Normal controls (n=103)
Genotypes		
DD	43 (35–52)	37 (28–47)
ID	46 (38–55)	48 (39–59)
II	11 (6–17)	15 (8–23)
Alleles		
D	0.66	0.61
I	0.34	0.39

Key: Data are % (exact binomial 95% CI), $X^2 = 1.42$; p -value=0.49; I = insertion; D = deletion.

ACE (I/D) polymorphism

The genotype distribution and allele frequencies of type 2 diabetic subjects with or without renal complications after at least 10 years diabetes duration are shown in table 2. The genotype distribution in control samples was in very good agreement with a Hardy-Weinberg equilibrium ($X^2=0.049$, $p=0.82$).

The distribution of the ACE gene I/D polymorphism in subjects with diabetic nephropathy (n=90) (44% DD [n=40], 46% ID [n=41], 10% II [n=9]) did not significantly differ ($X^2=0.19$, p -value=0.9) from those without diabetic nephropathy (n=51) (41% DD [n=21], 47% ID [n=24], 12% II [n=6]). Table 3 shows that there is no significant statistical difference ($X^2=1.42$, p -value=0.49) between the genotype distribution and allele frequencies of all type 2 diabetic subjects (n=141) compared to non-diabetic controls with normal fasting blood glucose (n=103) (43% DD [n=61], 46% ID [n=65], 11% II [n=15] vs. 37% DD [n=38], 48% ID [n=50], 15% II [n=15], respectively).

Discussion

In this study we examined the role of the ACE gene (I/D) polymorphism in diabetic nephropathy and in type 2 diabetes. Despite reports showing that the D allele of the ACE gene is associated with higher plasma enzyme levels²⁶ and elevated fasting blood glucose levels,²⁷ our results showed that the deletion polymorphism of the ACE gene is likely not to be associated with susceptibility to diabetic nephropathy nor with type 2 diabetes in the studied Tunisian population. The frequency of the D allele in Tunisian normal controls was 0.61, a slightly higher frequency was reported by a recent Tunisian study²⁸ in a sample of 100 healthy subjects (0.67) but there is no statistical difference ($X^2=0.93$, $p=0.33$). Similar frequencies were found among Caucasians,^{21,29} but lower values were reported in Asians.^{20,23} Association studies of ACE I/D polymorphism and diabetic nephropathy in various populations have yielded

Statistical analysis

For quantitative traits, data are reported as means±SD or median (min-max) and comparison between means was analysed by Student's *t*-test. Comparison of genotype and allele distribution between groups was performed by a X^2 test in contingency tables. A p -value < 0.05 was considered to be indicative of statistical significance.

Results

Characteristics of the studied patients

Clinical characteristics of albuminuric and normoalbuminuric patients with type 2 diabetes are summarised in table 1. The two groups were compared with regard to sex, age, diabetes duration, metabolic control (glycaemic levels and HbA_{1C}), BMI, BP and blood levels of cholesterol and triglyceride. Subjects with or without diabetic nephropathy were comparable regarding age and established risk factors of diabetes duration and metabolic control (glycaemic levels, HbA_{1C}). There were also no statistical significant differences between these two groups with respect to sex, BMI and DBP, whereas, patients who had diabetic nephropathy had significantly higher SBP and both cholesterol and triglyceride levels than those without diabetic nephropathy.

