S. M. Ghogomu, PhD, B. Fru Asa, MSc, N. Epole Ngolle, MSc, Molecular and Cell Biology Laboratory, Biotechnology Unit, Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, Cameroon, and R.N. Mouliom, MD, Diabetes and hypertension clinic, Regional Hospital, Buea, Cameroon

Request for reprints to: S. M. Ghogomu, Molecular and Cell Biology Laboratory, Biotechnology Unit, Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, Cameroon

POSITIVE ASSOCIATION OF ACE I/D GENE VARIANTS WITH GENETIC PREDISPOSITION TO DIABETES IN THE BANTU ETHNIC GROUP OF CAMEROON

S. M. GHOGOMU, B. FRU ASA, N. EPOLE NGOLLE and R.N. MOULIOM

ABSTRACT

Background: Pre-disposition to diabetes is possibly associated with gene polymorphisms of the renin–angiotensin system (RAS). Angiotensin-converting enzyme (ACE) gene is one of the genes of the RAS system whose polymorphisms have been suggested to be risk factors for type 2 diabetes melitus (T2DM). The involvement of the ACE gene polymorphism in diabetes show inconsistent results across ethnic groups as revealed by earlier studies.

Objective: To investigate the association between insertion/deletion (I/D) polymorphism of the 287 bp DNA fragment in intron 16 of the ACE gene and genetic pre-disposition to T2DM.

Design: Case–control study

Setting: Bantu ethnic group of South West Cameroon

Subject: Diabetic subjects (n= 50) and non-diabetic subjects (n=50) of both sexes aged between 31 and 73 years.

Results: The distribution of three geno-types (II, ID, and DD) was significantly different between the diabetic and non-diabetic groups ($\chi 2 = 10.3$, $p = 0.001$). Also, frequency of D allele was higher in the diabetic patients than in the non-diabetic subjects ($p= 0.03$).

Conclusion: Our data suggest that the D allele of ACE gene polymorphism is associated with the genetic pre-disposition to develop T2DM.

INTRODUCTION

The prevalence of type 2 diabetes melitus in Cameroon ranges from 1.0% in rural areas to 6.1% in urban areas (1). The susceptibility of T2DM is strongly hereditary with concordance in twins and family aggregations. The risk of an offspring having diabetes doubles if the individual has a history of diabetes and furthermore, the individual has 80% chances of acquiring diabetes (2). Moreover, the fact that susceptibility varies with ethnic group further strengthens the involvement of a genetic factor in predisposition to diabetes (3, 4).

Many studies have showed that the genetic polymorphisms of some genes of the RAS system are associated with T2DM (5) and the angiotensin converting enzyme gene polymorphism is one of them. There is evidence that ACE is associated with T2DM. The renin-angiotensin system (RAS) leads to the production of angiotensin II, a vasoconstrictor, that affects glucose homeostasis and involved in the pathogenesis of DM through inhibition of insulin

signal transduction, reduction of glucose intake, resistance to insulin and destruction of the beta cells of the pancreas by oxidative stress (6). Further more, ACE inhibitors improve glucose utilisation and suppresses hepatic glucose production in type 2 diabetes (7) and there is a correlation between serum ACE activity and ACE polymorphism (8).

This ACE gene consists of a 26 exons and spans 21 kp on chromosome 7. There is a 287 bp DNA fragment insertion (insertion, I)/deletion (deletion, D) polymorphism in intron 16 of ACE gene (9).

The relationship between I/D polymorphism of the ACE gene and T2DM has been inconsistent among different ethnic groups. For example, studies reported a strong association of ACE gene polymorphisms with T2DM in Northern India [10]; Bhavani established positive association of ACE polymorphism with T2DM in south India (11). The ACE I/D polymorphism is significantly associated with T2DM in Arab ethnicity (12, 13), Malaysian subject (14), Indonesia (15), Bahrain (13). In other

reports, the D allele is associated with T2DM in Egyptian patients (9) and the Asian population (16). Contrary to these reports, Prasad and Ajay Kumar reported no relation between ACE gene and T2DM among North Indian (17), Chinese (18, 19), Lebanese (20) and the Tunisian population (21). It is therefore evident that the pre-disposition of to T2DM through ACE polymorphism is dependent on the ethnic group. Such studies have never been carried out in Cameroon. Cameroon is made of Ten Regions divided into three ethnic groups: the Bantu (Centre, South and Littoral, South West and East Regions), Semi-Bantu (Northwest and West Regions) and Fulbe (Adamawa, North and Far North Regions) (22) with rare inter-marriages between ethnic groups either due to traditional, cultural or religious beliefs and dialect barriers. Hence, the principal objective of this work is to investigate the possible relationship that exists between ACE gene polymorphism and risk of developing essential diabetes in the Bantu ethnic group in Buea, South West Region of Cameroon.

MATERIALS AND METHODS

Study population: The study group comprised of 50 diabetic controls and 50 non diabetic subjects of between 31 and 73 years inclusive. All diabetic subjects were resident in Buea, belonging to the Bantu ethnic group without any known ancestors of other ethnic origin and recruited from the diabetic and hypertension clinic at the Regional hospital Buea, South West Region. Diabetic subjects were those with a fasting blood sugar (FBS) of above 110 (mg/dl) or who were already placed on anti-diabetic medication. These patients were required to be free of secondary hypertension [systolic blood pressure (SBP) of at most 140mmHg and a diastolic blood pressure (DBP) of at most 90 mmHg] and must have been diagnosed with diabetes before the age of 70 years. These subjects must have had a family history of diabetes in their parents and/or siblings. Non-diabetic controls were recruited randomly from the Buea Municipality and with a fasting blood sugar (FBS) of 90-110 (mg/dl). These individuals had never been treated with anti diabetic medications, had no family history of diabetes. All of the subjects were unrelated and were native Cameroonians of the Bantu ethnic group of the South West Region. All subjects gave their consent and ethical clearance for this work was obtained from the Faculty of Health Science ethical committee of the University of Buea. Administrative authorization was obtained from the South West Region Delegation of Health.

Collection of anthropometric and biochemical data: A structured questionnaire was used for data collection on anthropometric variables (height, weight, and sex),

duration of diabetes, family history and complications of hypertension. Body mass index (BMI) was calculated as weight, divided by height squared (kg/m^2). Resting blood pressure was measured in the right upper arm of subjects using a cardiocheck PA analyser. FBS was measured using the OneTouch UltraSoft lipid profile strip and analyzer. Serum lipid levels were measured using lipid profile test strips that provided a quantitative measurement of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) in blood. Table 1 shows the mean clinical and anthropometric profile of normal and diabetic subjects

DNA extraction: Whole blood from participants was collected by venepuncture in EDTA-free microtainer tubes by a trained nurse as described by Kiechle *et al* (23). DNA was extracted from blood using the GenElute Blood Genomic DNA kit (Sigma) as described by the manufacturer.

Geno-typing: Geno-typing was carried out with genomic DNA isolated from human leukocytes by a commercial Genomic DNA extraction kit for Blood (Sigma). Angiotensin converting enzyme (ACE) insertion/deletion (I/D) variant [24] was assayed by PCR amplification of targets in a 25 μL reaction mixture comprised of 0.5 μg genomic DNA, 25 μM each primer, 10μL of deionised water and 12.5 μL of PCR master mix containing 2.5 mM each of dNTP (dATP, dCTP, dGTP and dTTP), 2.0 mM $MgCl_2$, 1X Taq buffer and 0.05 U/μL Taq DNA polymerase. Primer sequences used for the amplification of the ACE gene were: 5'-CTGGAGACCACTCCCATCCTTTCT-3' (Forward) and 5'-GATGTGGCCATCACATT CGTCAGAT-3' (Reverse). The predenaturation step was carried out for 3 min at 94°C. This was followed by a denaturation step at 94°C for 45 sec, annealing at 58°C for 45 sec, and polymerisation at 72°C for 45 sec with a final extension at 72°C for 7 min. Forty PCR amplification cycles were carried out in Applied Biosystems thermal cycler. PCR products were visualised on 2% agarose gels containing ethidium bromide.

Statistical analyses: All the statistical analyses were carried out using SPSS (Chicago, IL) software version 14.0 for Microsoft Windows. Continuous variables were compared between the groups using two-tailed student's t-test. Allelic frequencies were calculated by gene-counting method and the genotype distribution with Hardy-Weinberg expectations by a chi-squared test. The statistical significance of the traits and values between geno-types (D/D *vs* I/D or I/D *vs* I/I) were compared using one-way ANOVA. A level of $p < 0.05$ was considered statistically significant.

RESULTS

No statistically significant differences were detected between the studied groups regarding age, sex, TC, HDL-C, SBP and DBP. However, there was a statistically significant difference as regards FBG, disease duration, BMI, LDL-C and TG (p<0.05) (Table 1).

Table 1
*Mean clinical and anthropometric profile of non-diabetic and diabetic subjects*

Parameter	Normal subjects	Diabetic subjects	p values
Age (years)	52 ± 8.7	54.6 ± 10.6	0.212
DM Duration (years)	0.0	7.4 ± 2.1	NA
Sex ratio (male: female)	19:31	17:33	NA
BMI (Kg/m2). RV: 18.5-24.99	29.63 ± 6.91	47.06 ± 10.19	0.0001*
SBP (mm Hg). RV: < 140	132 ± 20	142 ± 24	0.054
DBP (mm Hg). RV: < 90	85 ± 11	90 ± 20	0.129
FBS(mg/dl). RV: 70-110	67 ± 12	148 ± 47	0.0001*
HDL-C (mg/dl). RV: 30-85	59±35	70 ± 24	0.098
LDL-C (mg/dl) RV: < 140	75 ± 37	111 ± 55	0.001*
TG (mg/dl). RV: < 160	91 ± 45	117 ± 45	0.004*
TC (mg/dl). RV< 200	169 ± 44	175 ± 42	0.486

Mean age, diabetes duration, body mass index (BMI), blood glucose level, Systolic blood pressure (SBP) and diastolic blood pressure (DBP), fasting blood sugar (FBS) level, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), Triglyceride (TG), total cholesterol (TC) levels, are summarised as mean ± SD (standard deviation); RV: Reference value; NA: Non applicable, asterisk indicate significance in P-value.

Geno-type distribution and allele frequency: Three geno-types were recognised: II with one band at 490 bp, DD with one band at 190 bp and ID a hetero-zygote type with 2 bands at 490 and 190 bp (Figure 1).

Figure 1
Detection of ACE I/D polymorphism on DNA samples after PCR analysis. Lane M: DNA marker (Lambda Hind III); Lanes 1: Negative control; Lanes 2,4, 5: I/D hetero-zygous (490bp and190bp); Lane 3: D/D homo-zygous (190bp), Lanes 6: I/I homo-zygous (490bp). Arrows indicate the different allele forms
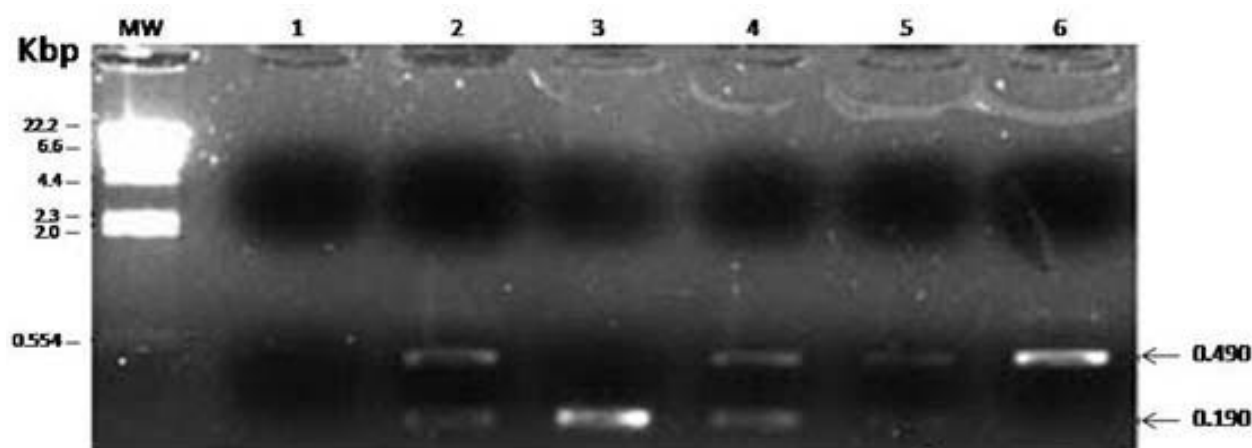
Data for the distribution of ACE insertion/deletion (ACE I/D) geno-types and alleles in the studied groups is illustrated in Table 2.

Table 2
ACE geno-type and allele frequencies in control and diabetic group

Group	n	Geno-types (%)			Allele	
		II	ID	DD	I	D
Control	50	1 (2)	34 (68)	15 (30)	36	64
Diabetic	50	2 (4)	19 (38)	29 (58)	23	77
			p = 0.001; χ^2= 10.		p = 0.003	

The frequency of the DD geno-type of ACE gene was significantly higher ($p<0.05$) in diabetic patients than the controls (58% and 30%respectively) while it was inversely true for the ID geno-type (38% and 68% respectively $p>0.05$).

No significant differences were observed in the II geno-type in diabetic patients and controls (2 vs. 4%). The frequency of ACE D allele was insignificantly higher ($p>0.05$) in the diabetic patients (77%) than controls (64%). Also, I allele frequency was insignificantly lower ($p>0.05$) in the diabetic patients (23%) than controls (36%). ACE geno-type distributions in three groups were in line with Hardy-Weinberg equilibrium (all $P > 0.05$, data not shown).

Comparing the studied parameters in diabetic patients with the risky geno-types DD and DI to those with the II genotype revealed that these risky geno-types were insignificantly associated with serum lipid levels, BMI and blood pressure but significantly associated with higher FBS, ($p <0.05$), these data are summarised in Table 3.

Table 3
Variation of Anthropometric and biochemical variables with ACE geno-type

Geno-type	LDL-C (mg/dl) RV: < 140	HDL-C (mg/dl) RV: 30-85	TG (mg/dl) RV: < 160	TC (mg/dl) RV< 200	Glucose (mg/dl) RV: 70-110	BMI (Kg/m^2) RV: 18.5-24.99	SBP (mm Hg) RV: < 140	DBP (mm Hg) RV: < 90
II (53)	80±32	60±28	92±46	164±41	71±8	29±6	129±11	87±05
ID (44)	107±46	68 ± 30	103±44	170±43	137±29*	30±30	140±09	89±08
DD (3)	110±14	69±15	115±23	176±21	147±21*	32±17	143±05	91±04

Note: Compared to II geno-type: *$P < 0.05$; RV: Reference value

DISCUSSION

Although there is evidence for a significant association between the I/D polymorphism of the ACE gene and the risk of T2DM, the contribution of ACE gene variants to diabetic development still remains controversial. Analysis for the association of the ACE I/D polymorphism with T2DM has been published in different ethnic groups (3, 10, 11, 13, 14, 15). However, some researchers reported no obvious association between a specific ACE geno-type and diabetes in other ethnic groups (17, 18, 19, 21). These reports suggest that the involvement of ACE I/D polymorphism in the T2DM varies among different ethnic/racial groups (25).

In this present study, we found an association between the ACE I/D polymorphism and T2DM. The frequencies of both ID and DD ACE geno-types as well as the minor D allele frequency were significantly higher in the T2DM patients as compared to controls of the studied population. These results indicate that ACE gene deletion polymorphism is associated with T2DM in the Bantu ethnic group of the SWR of Cameroon. These results are consistent with other reports where the DD geno-type and the D allele are associated with T2DM in Egyptian patients (9), Indian (26), Iranian (27) and Tunisian (28) populations. However the reason why ACE gene polymorphism is associated to Cameroonian patients of this ethnic group with type 2 diabetes is unknown and therefore will require further investigations.

In addition, this study also found that in the different geno-types, TG and LDL-C and TC, levels, SBP and DBP in D allele carriers were higher than that of I allele carriers although the differences were not statistically significant. These results indicate that our patients may not have developed the metabolic syndrome but run the risk of developing it. The mechanism by which ACE gene polymorphism affects the lipid levels is unclear and is worth further exploring. On the other hand, levels of FBG were significantly higher in D allele carriers than those of I thereby suggesting that the ACE gene I/D polymorphism is a risk factor for T2DM in the Bantu

ethnic group of the SWR of Cameroon and the D allele may predispose subjects to T2DM.

Identifying the risk factors of T2DM and geno-typing these SNPs among semi-Bantus ethnic group in Buea can help identify at risk individuals in the Bantu ethnic group, hence create awareness by providing better understanding of the disease complications, management, early prevention and thus a change in lifestyle of these subjects. Furthermore these results may initiate the formulation of governmental policies to prevent and control this disease in this population.

In conclusion, the ACE gene I/D polymorphism is a risk factor for T2DM in the Bantu ethnic group of the SWR of Cameroon and the D allele predisposes these subjects to T2DM.

ACKNOWLEDGEMENTS

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