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Implication of insertion/deletion polymorphism of angiotensin converting enzyme gene in the occurrence of type 2 diabetes in the Gabonese Population

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Abstract

Diabetes is a metabolic disease most often associated with complications when the biological parameters are uncontrolled, so management of diabetes remains a challenge in Africa. The aim of this study was to find a relationship between the I/D polymorphism of the gene encoding for angiotensin converting enzyme (ACE) and type 2 diabetes in Gabonese subjects. This study was carried out in the laboratory of the Research Unit of the University of Health Sciences. The study population consisted of 225 subjects. This panel was composed of 88 controls (normoglycemic and non-hypertensive and not having a family history of diabetes) and 137 type 2 diabetic individuals. The genotypic analysis of the *ACE* gene of the different subjects was carried out by the technique of Polymerase Chain Reaction (PCR). Categorical and continuous variables were compared between diabetic patients and controls using the Chi-square test (χ^2) for categorical and the ANOVA test for continuous. The distribution of DD, ID, and II genotypes in controls and diabetics was 63.6%, 33%; 3.4% respectively and 65.7%; 31.4%; 2.9%. In addition, the allelic distribution showed that the alleles I and D in the controls and the diabetic subjects had proportions of 19.9%; 80.1% vs. 18.6%; 81.4% respectively. The genotypic and allelic differences between the two groups were not significant ($p \geq 0.05$). Therefore the allele D would not be the factor involving the I/D polymorphism in the occurrence of type 2 diabetes in the Gabonese population.

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Introduction

Type 2 diabetes (T2D) is a real public health problem, not only because it represents 90% of diabetes but also because of its prevalence, strong growth and pathologies associated with it, mainly cardiovascular diseases are responsible of high morbidity and mortality (Scheen and Paquot, 2012). The number of people with type 2 diabetes is growing rapidly around the world. This increase is associated with the ageing population, economic development, increasing urbanization, a less healthy diet and decreased physical activity (Fédération Internationale du Diabète, 2015).

The disease is a metabolic disorder characterized by hyperglycemia and results from the combination of several susceptibility genes whose expression depends on environmental factors, chief among them, excessive consumption of saturated fats and sugars, and sedentary lifestyle (D'Ivernois and Gagnayre, 2011). Moreover, it appears increasingly that genes of susceptibility or predisposition are especially numerous in the occurrence of type 2 diabetes (Scheen and Paquot, 2012). Thus, a genetic approach should lead to better strategies for the prevention of diabetes (Graham, 2003; Farmer and Avard, 2008). In this context, previous work has investigated the relationship between type 2 diabetes and the insertion/Deletion polymorphism of the gene encoding for the angiotensin converting enzyme (ACE). However, these studies have shown contradictory results (Zhou *et al*, 2013). The *ACE* gene spans 21kb (26 exons). It is located on the *17Q23* human chromosome and encodes 1306 amino acids including the signal peptide. Indeed, this gene shows an insertion or deletion of a 287 bp repeat Alu sequence in intron 16. This polymorphism forms three possible genotypes: genotypes II then ID and DD (Parchwani *et al*, 2005) and explain the variability of plasma concentration of ACE between 30% and 40%. The alleles I and D are codominant, the homozygotes DD and II have the highest and lowest levels of ACE, respectively, whereas the heterozygotes ID have an intermediate level (Rigat *et al*, 1990).

The insertion or deletion polymorphism of the gene encoding angiotensin converting enzyme is involved in changes in concentrations of angiotensin II (Marre *et al*, 1994).

The ACE metalloproteinase Zinc (Zn) is the key enzyme of the renin angiotensin aldosterone system (RAAS), it cleaves the C-terminus of the dipeptide (His-Leu) from angiotensin I to produce angiotensin II (Ag II). The latter is pro-inflammatory and pro-oxidative (Jones and Vinh, 2008; Chmaisse *et al*, 2009; Garg *et al*, 2012; Sung-Kyu, 2014). It is localized in the endothelium of vessels and circulating in biological fluids such as plasma, cerebrospinal and seminal fluid (Sasaki and Inoguchi**,** 2012).

However, In Sub-Saharan Africa countries and Gabon in particular, no studies have been conducted according to this genetic approach. The aim of this study was to investigate the relationship between *ACE* gene I/D polymorphism and type 2 diabetes to highlight the involvement of this gene in the onset of type 2 diabetes in the Gabonese population.

Materials and methods

Ethical clearance

The case-control study was conducted from July to November 2016 at the Department of Chemistry and Biochemistry at the University of Health Sciences (USS). The sampling was done in agreement with patients after informed consent. All the information collected has been treated in strict respect of the anonymity and confidentiality of the persons. And the study has obtained approval from the National Ethics Committee.

Area and study population

The study population consisted of 225 subjects, including 137 type 2 diabetics according to pathology classification criteria (WHO) and 88 controls. The recruitment of diabetics was carried out at the endocrinology service in the Hospital University Center of Libreville (CHUL) while the controls were selected on the one hand at the National Center for Blood Transfusion in Libreville (CNTS) and on the other hand at the at the University of Health Sciences. The controls were non-diabetic subjects confirmed by

a blood glucose test, non-hypertensive following a blood pressure measurement, and not having a family history of diabetes. Anthropometric parameters such as blood pressure (mmHg), weight (kg), height (m), age (years), sexes were noted, and the body mass index was calculated for all participants. The information collected was stored in a database using the Excel software (Microsoft Office 2013).

Sampling and DNA extraction

The blood was collected in 5 ml EDTA (Ethylene diamine tetraacetyl) tubes and stored at + 4°C before use. The genomic DNA was extracted from the whole blood leukocytes using the kit "DNeasy Blood & tissue" of the Qiagen group (Courtaboeuf, France) according to the manufacturer's protocol briefly summarized as follows: 250 µL of blood obtained by sampling in an EDTA tube was transferred into a 1.5 mL sterile tube to which 25µL of proteinase K was added and then 250µL of AL buffer (pH = 7.2), a hypotonic lysis solution for cells. After incubation in a water bath, 250µL of ethanol (96% - 100%) was added to precipitate the nucleic acid molecules. The homogenate obtained was transferred to an anion exchange chromatography column. The column was centrifuged at 8000 (rpm) for 1 minute. It was washed with 500µL of buffer AW1 (pH = 7) and with 500µL of buffer AW2 (pH = 7) and then centrifuged successively for 8000 rpm for 1 minute and 14000 rpm for 3 minutes to remove the impurities. Elution was carried out with 40µL of AE buffer (pH = 9). The eluate composed of nucleic acids was stored at -20°C.

Genic amplification

The sequence of 287 bp in intron 16 of the ACE gene was amplified by PCR. The reaction mixture was prepared as follows: 2µl of PCR buffer (10×), 2µl of $MgCl_2$ (50 mM), 2µl of dNTP (10 mM), 0.24µl of each primer (25pmol/µl), 12.22µl of sterile water, and 0.3µl of T*aq* DNA polymerase (5U/mL) (Invitrogen-Life Technology) supplemented with 4µl of genomic DNA (200 µg/mL). The sens and antisens primers had the sequence ACE1 (sens) 5'-CTGGAGACCACTCCCATCCTTTCT-3' and ACE2 (antisens) 5'-GATGTGGCCATCACATTCGTCAGA-3'respectively.

The amplification was performed using a thermal cycler (Esco Healthcare) programmed at different temperatures consisting of a pre-denaturation (hot start) at 94°C. for 5 min, followed by 30 cycles of 94°C for 45 sec, 64°C for 45 seconds, 72°C for 1 min and 30 sec, a final elongation cycle at 72°C for 5 min and a refrigeration phase.

The I/D polymorphism was demonstrated by detecting the presence of the allele I (insertion) or absence of the D allele (deletion) of a sequence of 287 bp in intron 16 of ACE gene by 1.5% agarose gel electrophoresis, with a dye and a developer, the EZ-vision. The gel was observed under a UV lamp at λ = 260 nm. The 100 bp size marker (invitrogen) was used. The generator (Consort EV243) was set at 110 V, 95 mA for a migration time of up to 1h and 30 min.

Statistics analysis

The collected data was entered in the Excel spreadsheet (Microsoft Office 2013) and analyzed on the Epi Info 2006 software. The continuous variables were expressed as mean ± Standard deviation and the categorical variables were expressed by percentage. All these data have been described in number (n) and percentage (%). A comparison of the distribution of the two variables between the two populations was made using the Pearson Chi² (χ^2) homogeneity test for categorical, the Anova test for continuous and the 95% confidence interval (CI) of the results was calculated. The control of Hardy Weinberg's equilibrium in the distribution of the polymorphism in question among the control and case populations was carried out by a chi-square conformity test. The results were considered significant when the probability p (*p*- value) was equal to or less than 0.05 ($p \leq 0.05$).

Socio-demographic characteristics analysis

The characteristics of the study population are summarized in (Table I).

The two groups were in Hardy-Weinberg equilibrium. Diabetic subjects ($\chi^2 = 0.195$) and controls ($\chi^2 = 0.105$), $P \geq 0.05$.

The subjects were aged from 20 to 84 years and had a mean age of 44.9 ± 13.9 years. The mean age of diabetics was higher than that of controls 49.6 ± 11.7 years and 37.5 ± 13.8 years, respectively. The prevalence of diabetes increases with age, this result is similar to the study done in 2009 having found the same results (Baroudi *et al*, 2009). The mean body mass index (BMI) was higher in diabetic subjects than in normoglycemic subjects. Diabetics had a BMI of 26.67 ± 6.09 kg/m2 and the controls had a BMI of 22.9 ± 4.7%.kg/m2. This shows that diabetic subjects were predominantly overweight. This observation is in agreement with two studies carried out previously (Feng *et al,* 2002; Chmaisse *et al*, 2009). These authors reported that BMI in diabetic subjects was 27.2 ± 4.4 kg/m2; 31.5 ± 6.4 kg/m2 respectively, whereas in the controls BMI was 24.8 ± 2.8 kg/m2 and 24.9 ± 4.3 kg/m2 (Feng *et al*, 2002; Chmaisse *et al*, 2009).Overweight is therefore an aggravating risk factor for patients with type 2 diabetes.

Results and discussion

Analysis of the results showed a prevalence of DD genotype with 146 cases (64.9%) in the general population followed by ID genotype in 72 cases (32.0%) while the genotype II was minority (3.1%).

Table 1. Socio-demographic characteristics of the study population.

Characteristics	Controls	Diabetics subjects	Population	P value
Population n (%)	88 (39.1)	137 (60.9)	225 (100)	
Age (mean± SD) years	37.5 ± 13.8	49.6 ± 11.8	44.9 ± 13.9	0.0001
BMI (Kg/m²)	22.9 ± 4.7	26.7 ± 4.7	25.6.± 5.9	0.0001
Men (%) [CI 95%]	47 (53.4) [42.5-64.1]	80 (58.4) [49.7-6.7]	127 (56.4)	
Women (%) [CI 95%]	41 (46.6) [35.9-57.5]	57 (41.6) [33.3-50.3]	98 (43.6)	

n: number; %:percentage; SD: standard deviation, CI: confidence interval, p: statistical significance. BMI: body mass index.

In diabetics, 65.7% of the DD genotype was found against 31.4% of the ID genotype and 2.9% of II. Similarly, in the controls the genotype DD was found in 63.6% of individuals compared to 33.0% ID and 3.4% of II. The difference in distribution of these genotypes was not significant ($p \geq 0.05$) (Table 2).

Table 2. Proportions of genotypes among cases and controls.

Genotypes	DD	ID	II	*p*-value
Control n (%)	56 (63.6)	29 (33.0)	3 (3.4)	
Diabetic subjets n (%)	90 (65.7)	43 (31.4)	4 (2.9)	0,943

Analysis of the genotypic profiles showed that the distribution of genotypes in the two groups was in Hardy Weinberg equilibrium, the Chi² value obtained in diabetics and controls being less than the theoretical value of 0.105 <3.84 and 0.195 <3.84.

The frequency of DD genotypes was similar in both groups, as were the proportions of homozygotes II ($p = 0.9226$). The same observation was made in a Tunisian study (Arfa *et al*, 2008) and in an Arab population of Dubai (Alsafar *et al*, 2015).

These authors showed that in the two groups, the difference in proportions of the three genotypes was not significant (Arfa *et al*, 2008 ; Alsafar *et al*, 2015).

On the other hand, this observation disagreed with the results obtained in an Arab Tunisian population in 2009 (Baroudi *et al*, 2009) which showed a significant difference in the proportions of the three genotypes.

This would imply allele D to the occurrence of type 2 diabetes in the Tunisian Arab population (Baroudi *et al*, 2009). This divergence can be explained by the environmental factors that can lead to genetic changes or a difference in the genetic heritage between Caucasians and blacks.

Furthermore, the distribution of *ACE* alleles showed a large predominance of allele D (80.9%) over the I allele (19.1%) in the study population.

This order of distribution has been retained in both groups, 80.1% (D) and 19.9% (I) in controls and 81.4% (D) and 18.6% (I) in diabetic with p value not significant P≥ 0.05 (Table 3).

Table 3. Distribution of alleles I and D in the two groups.

Genotypes	D	I	*p*-value
Controls n (%)	141 (80.1)	35 (19.9)	0,923
Diabetic Subjects n (%)	223 (81.4)	51 (18.6)	

Type 2 diabetes is a multifactorial disease. It includes the influences due to the environment, diets and lifestyles. All of these factors were not controlled during the investigations. In addition, other genes might be involved letting suggest the influence of haplotypes in susceptibility to type 2 diabetes (Horikoshi and *al*, 2007; Yasushi, 2008; Van Vliet-Ostaptchouk *et al*, 2008).

The non-control of these factors could explain the lack of research relationship between the I/D polymorphism of the ECA gene and TD2. The distribution of alleles in both groups also showed a non-significant difference between the allelic frequencies of I and D in the two groups, this result was confirmed in a study conducted in a Lebanese population (Arfa *et al*, 2008; Chmaisse *et al*, 2009).

Conclusion

The study showed that there was no significant difference in the distribution of genotypes DD, ID and II and allelic frequencies of I and D *ACE* between control and diabetic subjects.

This suggests that the D allele would not be involved in the occurrence of type 2 diabetes in the Gabonese population. However, other studies will be needed such as assaying plasma ACE activity in diabetic patients, as well as analysis of other genes (HHEX, SLC30A8) that may be related to the onset of the disease in order to highlight a genetic relationship linked to the occurrence of the pathology which can lead to better management of the patients and especially a new clinical approach for an effective diagnosis of diabetics.

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