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Study of Association of CAPN 10 Gene's Polymorphism SNP19 with Type 2 Diabetes in Ethnic Group of Atacora District, Republic of Benin

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Abstract Non-insulin-dependent diabetes (DNID) is a multifactorial disease resulting from the interaction of genetic and environmental factors. The purpose of this study was to evaluate the link between the polymorphism SNP19 of the calpaine 10 gene (CAPN 10) and the occurrence of type 2 diabetes in diabetic patients monitored at the Departmental Hospital Centre (CHD) of Atacora, in Benin. A total of 200 patients with diabetes were included in the study, 121 women and 79 men. The determination of the glycemic parameter after a follow-up period of at least 1 year allowed these patients to be classified into two groups: the normal blood glucose group and those with a blood glucose level greater than 1 g/L. All subjects were analyzed for SNP 19 polymorphism in the CAPN10 gene using the PCR method. Lipid measurements were also performed on each patient by enzymatic method. The results of our study show that there is a difference in genetic susceptibility to type 2 diabetes and that allele 1 of the CAPN10 gene is a risk factor in the Ditamari population. BMI and education were correlated with glycemic status.

Keywords: *Type 2 diabetes, SNP19 of the CAPN10 gene, ethnic group, Atacora, Benin*

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1. Introduction

Type 2 diabetes is a multifactorial metabolic disease characterized by hyperglycemia resulting from dysfunction of insulin secretion by β cells and/or lack of response from target cells. It is a highly genetic disease that interacts with environmental factors [1].

Studies have shown that the inheritability of type 2 diabetes (DT2) is high. Indeed, the concordance rate of the DT2 is very high in monozygous twins (if one develops the disease, the other at 100% risk of also being affected). Similarly, the risk of diabetes is high among offspring of parents with the disease (50% of patients have a family history of diabetes). Finally, having a non-Caucasian and/or migrant origin increases the risk of DT2 [2].

Numerous studies have identified susceptibility genes in DT2, genes that may increase the risk of developing DT2 [3].

Among the genes predisposing to type 2 diabetes is the CAPN10 gene, which according to several studies carried out in different regions is the first gene associated with type 2 diabetes. It consists of 13 exons and 15 introns, and is carried by the short arm of chromosome 2 [4].

Many studies show high prevalence of T2 in some isolated ethnic groups (living in the same environment) [5,6,7]. The STEPS survey, conducted in 2008 in Benin, found that of 3822 subjects surveyed 101 had hyperglycemia of the diabetic type, a prevalence of 2.6%. From a geographical point of view, the Atacora department comes in second place (6%) after that of the Alibori. On the other hand, the Otamari ethnic group (in the Atacora department) taken alone has a prevalence of 6.8% [8]. These figures concern us in several respects. The objective of the present work was to study the link between the polymorphism SNP19 of the calpaine gene 10 and the occurrence of type 2 diabetes in diabetic patients monitored at the Centre Hospitalier Dpartmental (CHD) of Atacora, in Natitingou with associated factors.

2. Materials and Methods

2.1. Patients and Ethics

This study included a sample of 200 diabetic participants who were exhaustively recruited on the basis of the patient admission register in the Atacora CHD diabetology center in Natitingou. The informed oral consent of each patient was sought and obtained prior to winding up in the study and confidentiality was ensured. The study protocol was validated by the ethics committee of the University of Abomey-Calavi. It relates to the socio-demographic characteristics of patients, the collection of anthropometric data, lifestyle and personal and family history. Blood samples were also taken from these patients.

This was a cross-sectional prospective descriptive and analytical study that took place at Atacora CHD in Natitingou from April 2018 to August 2018.

With z =1.96 for a 5% risk

$$n = \frac{z^2 \mathrm{XpXq}}{j^2}$$

p: according to the 2008 STEPS survey of Ncds, the prevalence of diabetes is 2.6% (Houinato *et al.*, 2008) in Benin

$$n = \frac{1.96^2 \mathrm{X0.026X0.974}}{0.03^2} = 108.09.$$

2.2. Methods

From 5 ml blood sample on sterile EDTA anticoagulant tube, the genomic DNA of diabetic patients was extracted from the leukocytes using the Bac Nucleon kit of Amersham's company following the manufacturer's recommendations. All patients were tested for SNP19 polymorphism in the calpaine 10 (CAPN10) gene using the PCR method. The genotyping of the polymorphism UCSNP19 of the CAPN10 gene consists of an insertion/deletion characterized by a two- or three-fold repetition of a sequence of 32 bp [4]. Demonstration of the UCSNP19 mutation of the calpaine 10 gene was performed using F: 5'-GTT TGG TTC TCT TCA GCG TGG AG - 3', and R: 5'-CAT GAA CCC TGG CAG GGT CTA AG-3. The genomic DNA of 50 ng was amplified in a final volume of 25 µL, containing 1 µmol/L of each primer, 1 mM of each dNTP, 1.5 mol/L of Mgcl2, the 5X PCR buffer, and 0.25U of the Taq DNA polymerase (Promega). Amplification conditions were 95°C initial denaturation for 5 minutes, 30 cycles of PCR including 94°C denaturation for 1 minute, 55°C hybridization for 1 minute, elongation at 72°C for 1 minute and final elongation at 72°C for 10 minutes. The amplification products were visualized with UV light after 2% agarose gel electrophoresis using a molecular weight marker of 100bp for the identification of amplification fragments.

2.3. Statistical Analyses

The data were analyzed by R software version i386 3.5.2.

A descriptive analysis was conducted for the entire study population based on the glycemic status of the subjects (diabetic versus normoglycemia). Pearson Chi-2 statistical tests (or the exact Fisher test according to the distribution of the variable) for qualitative variables and Student Test-t for quantitative variables were performed. The relative risk associated with the genotype was estimated by calculating the odds ratio (OR) and its 95% confidence interval. Finally, a logistic regression analysis was performed to identify the factors associated with the glycemic status of the subjects.

3. Results

This study examined the impact of SNP 19 polymorphism in the CAPN10 gene in diabetic subjects followed at CHD-Atacora and the factors associated with treatment response or not. All patients with diabetes who met our inclusion criteria were considered. Thus, 200 patients with diabetes participated in this study. Blood samples were taken and several biochemical and genetic parameters were performed. Based on the blood glucose results obtained from the samples, two groups of diabetic subjects were formed: the group of patients with normoglycemia and those with hyperglycemia.

The characteristics of patients with DT2 and subjects with normal blood sugar levels are given in Table 1. From this table, it appears that it is at the level of BMI that we observe a statistically significant difference between patients with DT2 and subjects with normoglycemia after follow-up (27.95 5.65 versus 24.83-5.34; p = 0.02). For the other parameters, no statistically significant differences were observed. This table also shows a slight female predominance in our study and patients in both groups are either overweight or obese. The majority ethnic groups were the Ditamari, the Bariba, the Dendi.

The genetic profile reveals that 50.75% of DT2 patients are homozygote 1/1; 26.51% heterozygote1/2 and 14.51% homozygote 2/2.

BMI is significantly associated with glycemic status. The higher the BMI, the higher the diabetic hyperglycemia. Ethnicity is significantly correlated with the glycemic status of the subjects. The ethnicity-glycemic status association is stronger in the Ditamari than in the Dendi, and even stronger than in the Bariba. In addition, there is no correlation between the use of these two drugs and glycemic status (Table 2).

With respect to genetic profile, results in Ditamari indicated that allele 1 is a risk factor OR= 3.53 (1.16 - 10.79), p = 0.04. On the other hand, this allele appears to be a protective factor in Bariba and Dendi; OR= 0.85(0.21 - 3.32) and 0.41(0.08 - 2.00) respectively (Table 3).

Educational attainment and BMI are the two most important factors in subjects' glycemic status. Subjects with higher levels of education and those with lower BMI are more likely to change from diabetic to normoglycemia (Table 4).

Table 1. Characteristics of DT2 patients and subjects with normal blood glucose

Characteristics	T2DM	Subjects with normal blood sugar	P-value[a]
Total number (F / M)	132(76/56)	68(45/ 23)	0.28[b]
Age (years)	55.86 ±10.83	56.17±11.44	0.85
Weight (kg)	74.14±16.21	73.82±16.40	0.89
BMI (kg/m^2)	27.95±5.65	24.83-±5.34	0.02*
Waist	95.85±11.67	96.19±11.83	0.84
Ethnic group			0.22[b]
Nominated (%)	47(35.61)	23(33.82)	
Bariba (%)	19(14.39)	15(22.05)	
Dendi (%)	16(12.12)	10(14.71)	
Other (%)	50(37.88)	20(29.41)	
Level of education			0.85[b]
Illiterate (%)	36(27.37)	15(22.05)	
Primary (%)	41(31.06)	21(30.88)	
Secondary (%)	46(34.84)	27(39.71)	
Superior (%)	9(6.81)	5(7.35)	
Physical activity (walking)	92(69.69)	44(64.71)	0.56[b]
Tobacco (%)	6(4.54)	3(4.41)	0.59[b]
Alcohol (%)	15(11.36)	5(7.35)	0.29[b]
Systolic (mmHg)	135.80±21.09	136.04±18.26	0.93
Diastolic (mmHg)	80.98±14.41	81.32±12.82	0.86
Medication			
Metformin	129(97.72)	63(92.64)	0.17[b]
Glibenclamide	63(47.72)	34(50)	
Genetic profile			0.66[b]
Homozygote 1/1 (%)	67(50.75)	34(50)	
Heterozygous 1/2 (%)	35(26.51)	21(30.88)	
Homozygote 2/2 (%)	19(14.39)	6(8.82)	

Data expressed as SD mean or frequency (%); * statistically significant; [a] Student t test; [b] Fisher exact test.

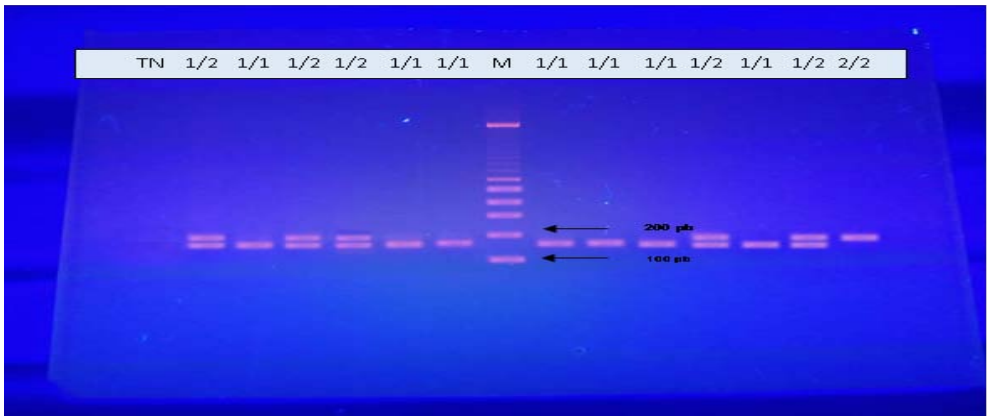

Figure 1. Migration photography of polymorhism SNP19 of CAPN 10 gene (M : ladder (molecular weight marker) ; TN : negative sample; 1/1 : 155 pb ; 1/2 : 155 et 187 ; 2/2 : 187 pb).

Table 2. Correlation between characteristics and glycemic status of subjects

	Glycemic status	
	R	P-value
Sex	0.08	0.24
Age	−0.01	0.85
Weight	0.01	0.89
BMI	−0.09	0.03*
Waist size	0.20	0.07
Ethnic group	0.60	0.04*
Level of education	−0.05	0.41
Physical activity	−0.03	0.61
Tobacco	0.01	0.99
Alcohol	0.06	0.39
Systolic (mmHg)	−0.01	0.93
Diastolic (mmHg)	−0.01	0.87
metformin	0.04	0.52
glibenclamide	0.01	0.97
Genetic profile	0.02	0.72

* Statistically significant.

Table 3. Genotypic frequency of the calpain 10 gene in the population and in the three major ethnic groups

	Genotype, n (%)					Allele, n (%)			
	1/1	1/2	2/2	OR (95% CI)	P	1	2	OR (95% CI)	P
Population, diabetic N = 121	67(55.37)	35(28.92)	19(15.70)	1.18 (0.59 - 2.33)	0.75	112(67.46)	54(32.54)	1.02 (0.58 - 1.78)	0.93
Population, normoglycemia N = 61	34(55.73)	21(34.42)	6(9.83)			55(67.07)	27(32.93)		
Diabetic Ditamari, N = 43	25(58.13)	11(25.58)	7(16.27)	2.27 (0.67 – 7.60)	0.30	36(66.67)	18(33.33)	3.53 (1.16 - 10.79)	0.04*
Ditamari normoglycemia, N = 19	8(42.1)	8(42.1)	3(15.78)			16(59.25)	11(40.75)		
Diabetics Bariba, N = 17	9(52.9)	6(35.3)	2(11.8)	0.85 (0.17 - 4.26)	0.98	15(65.21)	8(34.79)	0.85 (0.21 - 3.32)	0.91
Bariba normoglycemia, N = 12	7(58.33)	4(33.33)	1(8.33)			11(68.75)	5(31.25)		
Diabetics Dendi, N = 15	7(46.66)	4(26.66)	4(26.66)	0.75 (0.12 - 4.66)	0.97	11(57.89)	8(42.11)	0.41 (0.08 - 2.00)	0.45
Normoglycaemia Dendi, N = 10	7(70)	3(30)	0(0)			10(76.92)	3(23.08)		

* Statistically significant.

Table 4. Logistic Regression by Glycemic Status as a Dependent Variable

	β	SE	P
T2DM versus normoglycémie			
Age	−0.01	0.10	0.39
Sex (male)	0.32	0.41	0.43
Ethnic group			
Ditamari	−0.21	0.41	0.60
Bariba	−0.61	0.48	0.20
Dendi	−0.46	0.52	0.37
Education level (higher)	−0.72	0.73	0.03*
Weight	0.02	0.02	0.33
BMI	0.15	0.07	0.04*
Waist	0.02	0.03	0.38
Physical activity (walking)	−0.4	0.5	0.42
Tobacco	1.50	1.61	0.35
Alcohol	0.38	0.58	0.51
Genetic profile			
Homozygote 1/1 (%)	−0.02	0.64	0.97
Heterozygous 1/2 (%)	−0.11	0.67	0.86
Homozygote 2/2 (%)	0.40	0.78	0.60

* Statistically significant..

4. Discussion

According to the literature, Ditamari in northern Benin in the Atacora department are at increased risk of developing type 2 diabetes (6.8% versus 2.6% nationally). However, no studies are being conducted to investigate the genetic basis of type 2 diabetes in this population. Separate studies conducted on CAPN10 have demonstrated its role in insulin resistance phenotypes [8,9]. The purpose of this work was to study the impact of SNP 19 polymorphism in the CAPN10 gene in diabetic subjects followed at CHD-Atacora and the factors associated with treatment response or not.

The results show a female predominance of around 65%. This female predominance was also found in a population of Black Americans compared to Caucasian women, at a rate of one to two [10]. On the other hand, these results are to some extent in contrast to the one described in France in the Obepi study. Indeed, since 1997, the Obepi study evaluates every 3 years the prevalence of overweight and obesity in France, through a self-administered questionnaire sent to 20.000 households. In 2012, 25.714 subjects over the age of 18 responded to this questionnaire. Based on data collected in this study, if subjects with type 2 diabetes are more frequently male (55% of subjects), women are more likely to be obese (severe or very severe), hypertensive or dyslipidemic [11].

The results of the SNP 19 polymorphism of the CAPN10 gene showed in our study that treatment response or not does not depend on the genotype. However, allelic frequency results show that allele 1 is a risk factor in Ditamari and appears to be a protective element in Dendi and Bariba. This result shows a different ethnic association between the marker studied and susceptibility to type 2 diabetes. Our results are similar to those of [12] who have shown that there is a difference between ethnicities in genetic predisposition to type 2 diabetes. Our results are also consistent with that of [13] which states that allele 1 is a risk factor for type 2 diabetes in the Tunisian population in the Arab subgroup of Djerba Island.

Within our study population, a strong correlation between ethnicity and glycemic status was observed. Indeed, the ethnicity-glycemic status association is stronger in the Ditamari than in the Dendi, and even stronger than in the Bariba; these results could probably be explained by the fact that each ethnic group adopts a specific dietary habit that may or may not compromise its glycemic status. Our results are superimposed on those obtained by [5,7]. These studies have shown that the risk of obesity and DT2 is 6 times higher among people from South Asia (Indians, Pakistanis and Bangladeshi) and up to 3 times higher among people of African-compared to white people of European origin (Caucasians).

We showed in our study that body mass index (BMI) was associated with glycemic status and that the higher the BMI, the higher the diabetic hyperglycemia. Therefore, BMI has predictive power in glycemic pathology. Our results are consistent with the cross-sectional study conducted in Spain which showed that the prevalence of diabetes mellitus in overweight or obese patients was 23.6% and the higher the BMI, the higher the prevalence of diabetes [14]. However, a survey in 49 developing countries showed that not only overweight, but also underweight could be implicated in the pathogenesis of diabetes [15].

We also showed that education was a determining factor in glycemic status and that the higher level of education could contribute to move more patients from diabetic status to normoglycemia. This could probably be explained by the fact that a high level of education would be a good asset in the observance of dietary advice, physical exercise, regular taking of medications; things that contribute to good glycemic control. Our results are

consistent with those achieved by [16,17,18] which showed that low educational status was associated with a negative effect on glycemic control. In addition, other studies have shown that educational status has no effect on glycemic control [19,20].

5. Conclusion

The present study showed an association of polymorphism of the CAPN10 gene in diabetic subjects followed at CHD- Atacora within the Ditamari ethnicity of our sample and that allele 1 would be a risk factor in the occurrence of type 2 diabetes for this ethnicity. On the other hand this allele seems to be a protective element in the Dendi and Bariba. This then shows an ethnic association between the marker studied and susceptibility to type 2 diabetes. Factors associated with glycemic status were BMI, education level and ethnicity.

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