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The Association Between the -11377C>G Polymorphism in the Adiponectin Gene and the Predisposition to Type 2 Diabetes in the Moroccan Population

Fatima-Zahrae Aboubakr[1], Youssef Nouhi[1], Otmane El Brini[2], Bouchra Benazzouz[1], Younes Filali-Zegzouti[3], and Omar Akhouayri[1]*

[1]*Laboratory of Biology and Health, Department of Biology, Faculty of Sciences, IBN TOFAIL University, BP 133, 14000 Kenitra, Morocco*

[2]*Diagnostic Center-Moulay Youssef Hospital, Avenue Sidi Mohamed Ben Abdallah, BP 10050, Rabat, Morocco*

[3]*Laboratory of Biology, Environment, and Health, Moulay Ismail University, BP 298, Meknes, Morocco*

Abstract

Adiponectin plays a crucial role in the development of type 2 diabetes and previous studies investigating the relationship between adiponectin polymorphisms and type 2 diabetes risk produced inconsistent results. In this inquiry, we analysed the effect of the promoter variation -11377C>G of the adiponectin gene as a possible risk factor for type 2 diabetes in Moroccan population. DNA taken and underwent genotyping using polymerase chain reaction and restriction fragment length polymorphism, from blood samples of both controls and patients. A successful genotyping was performed for 181 type 2 diabetes patients and 149 controls. The outcomes indicated a strong association, both in terms of genotype (p=0.004) and allele (p=0.01), between the variant and diabetes patients when compared to controls. This suggests a considerable function of the adiponectin gene in multiplying the risk of diabetes, particularly among the Moroccan population.

Keywords: Adiponectin; -11377C>G polymorphism; Diabetes; Morocco

Full-length article **Corresponding Author, e-mail: fatima.zahrae.aboubakr@uit.ac.ma*

1. Introduction

Diabetes mellitus, once ranked as the eighth leading cause of global mortality, has now ascended to the fifth position, trailing behind communicable illnesses, cardiovascular disease, cancer, and traumas [1]. Of significant concern is type 2 diabetes (T2D), which constitutes 90% of all diabetes cases worldwide. The aetiology of T2D involves a multifactorial interplay, encompassing genetic predisposition [2], elevated body weight, sedentary lifestyle, and suboptimal dietary habits. Presently, approximately 2.5 million individuals in Morocco are afflicted with diabetes [3]. Given the escalating prevalence, it becomes imperative to meticulously discern genetic variations and other risk factors that contribute to an increased susceptibility to T2D. Such insights are fundamental for formulating targeted strategies in diabetes prevention and management.

As per the International Diabetes Federation (IDF), the current global count of individuals with diabetes is estimated to be approximately 537 million, and this number is expected to increase to 783 million by the year 2045 [4]. T2D imposes substantial morbidity and mortality, primarily attributed to its associated complications, including renal failure, amputations, cardiovascular disease (CVD), and cerebrovascular events [5]. Adiponectin (ADIPOQ), a protein secreted by adipocytes, plays a crucial role in the regulation of blood glucose levels, insulin sensitivity, and lipid metabolism [6]. Its levels exhibit variation based on age, gender, and body mass index, tending to be lower in individuals with obesity [7]. In individuals with T2D, the ADIPOQ levels are typically reduced [8], with a correlation with insulin insensitivity level [9]. Therefore, reduced plasma ADIPOQ levels may contribute to the development of insulin resistance and T2D, suggesting a potential involvement of the ADIPOQ gene in T2D susceptibility [10]. This association ADIPOQ/T2D displayed variability across different populations, with certain associations being confirmed in

multiple studies, while others did not replicate consistently [11].

Multiple research studies have revealed the presence of 683 single nucleotide polymorphisms (SNPs) within the adiponectin locus in humans. Among these genetic variants, one common one is rs266729, situated in the promoter region of ADIPOQ. This variant involves a C>G substitution at position -11377. Notably, rs266729 has been proposed to be linked with T2D in Asian and Arab populations, exhibiting a significant association with the condition [12]. In this current study, we investigated the potential role of the ADIPOQ polymorphism -11377C>G as a risk factor for individuals with diabetes in Morocco.

2. Materials and Methods

2.1. *Study subjects*

The study gathered data from patients seeking consultation at the diagnostic center in Rabat-Morocco, with each patient providing informed oral consent. The study included a total of 330 subjects, comprising individuals of both genders, 181 diabetic patients, and 149 controls. A total of 330 subjects from both genders were enrolled. Anthropometric measurements, blood pressure assessments, and the analysis of biochemical parameters including age, waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), glycemia, triglycerides (TG), cholesterol (CHO), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were conducted for each participant.

In this study, the diagnosis of diabetes followed the guidelines outlined by the World Health Organization (WHO). According to these criteria, individuals were categorized as having diabetes if their fasting glucose level surpassed 126mg/dl (7.0mmol/l). Conversely, controls were designated as non-diabetic using the same criteria.

2.2. *Genotyping*

DNA was extracted using the high ionic protein recharging technique, serving as the amplification template for polymerase chain reaction (PCR) [13]. The method involves centrifuging the blood sample, collecting the leukocyte cloud, and treating the cell pellet with K proteinase to digest cellular proteins. A lysis solution, consisting of EDTA-Na2 (10mM), SDS (0.2%), Tris-HCl (10mM, pH 7.5), and NaCl (50mM), is introduced to lyse the white blood cells. The remaining components are then precipitated using the ionic force of NaCl (6M). Finally, a cold ethanol solution (95% concentration) is added to precipitate the genomic DNA.

The genotyping of ADIPOQ -11377C>G involved the use of specific primers, forward 5′GCTCTGTGTGGACTGTGGAG-3′ and reverse 5′CTGCCACCCACTTAGGTGTT-3′. Genomic amplifications were carried out in a thermal cycler, with each PCR reaction consisting of 5x reaction buffer supplied with My TaqTM Polymerase (5mM dNTP, 15mM $MgCl_2$, stabilizers, and enhancers) and 0.1U Taq DNA polymerase. The process involved an initial denaturation step of 5 minutes at 95°C, followed by 35 cycles, including 30 seconds at 94°C, 30 seconds at 56°C, and 30 seconds at 72°C. The thermal cycling concluded with a final extension step of 10 minutes at 72°C.

The resulting PCR products, with a fragment size of 278 base pairs, underwent electrophoresis on a 2% agarose gel and were visualized under UV light after ethidium bromide staining to verify accurate sizing. Subsequently, the PCR products were subjected to digestion using 5 units of HinP1I enzyme at 36,5°C. The resulting of restriction fragment length polymorphism (RFLP) method were separated by electrophoresis on a 2% agarose gel, and the genotypes were documented. Each experimental run included negative controls for quality assurance. Distinct genotypes were identified based on the presence or absence of the HinP1I restriction site. The CC homozygote wild type, lacking the HinP1I restriction site, displayed fragments of 278 base pairs. The GG mutant homozygote, characterized by the presence of the HinP1I restriction site, exhibited fragments of 179 and 99 base pairs. The CG heterozygote showcased all three fragments, measuring 278, 179, and 99 base pairs (see Fig. 1).

2.3. Statistical analysis

The statistical analyses conducted in this research utilized the Statistical Package for Social Sciences (SPSS). Descriptive statistics such as mean and standard deviation (SD) were reported for continuous data, and Student t-tests were employed to compare continuous variables between different groups. A significant level of $P<0.05$ was adopted to address potential multiple comparisons. The genotype distributions were examined for adherence to the Hardy-Weinberg Equilibrium (HWE) using chi-square analysis. Additionally, chi-square (χ^2) tests were employed to assess genotype and allele frequencies between the cases and controls. To estimate the strength of associations, odds ratios (ORs) were calculated along with their corresponding 95% confidence intervals (CIs). These analyses were essential for evaluating the relationships between variables and determining potential associations in the study population.

3. Results

Table 1 presents a summary of the metabolic and anthropometric features observed in the study population. The mean values for Glycemia, SBP, DBP, WC, CHO, and TG were notably elevated, and the differences were statistically significant ($P<0.0001$), The significance level for LDL is moderate ($P=0.02$) in patients with diabetes compared to those without diabetes. Conversely, HDL does not show statistical significance ($P=0.18$) between the two groups. Upon analyzing the genotype frequencies for the ADIPOQ -11377C>G polymorphism, a deviation from HWE was observed in both T2D patients and controls. Moreover, the cases and controls showed a notable disparity in genotype distribution. ($P<0.0001$) (Table 2).

This investigation involved 181 Moroccan individuals with diabetes and 149 control participants. Genotype frequencies adhered to HWE in both diabetic and control cohorts. Notably, the predominant genotype in both groups was the wild-type homozygous CC, constituting 59.6% in controls and 58.9% in diabetic patients, as illustrated in Table 1. Conversely, the less prevalent mutant homozygous genotype GG was observed in diabetic patients (8.29%) and controls (0.67%). The heterozygous mutant genotype CG displayed frequencies of 34.25% in controls and 32.89% in diabetic patients.

Table 1. Anthropometric and metabolic characteristics of the study population.

Parameters	Controls Patients (mean ± SD)	T2D Patients (mean ± SD)	P value
Age	49,53 ± 13,34	55,56 ± 10,96	< 0.0001
Glycemia (g/l)	0.88 ± 0.09	1.90 ± 0.60	< 0.0001
WC (cm)	82.15 ± 6.81	91.16 ± 10.62	< 0.0001
SBP (mmHg)	128.30 ± 11.39	141.72 ± 18.59	< 0.0001
DBP (mmHg)	72.30 ± 6.17	78.17 ± 11.82	< 0.0001
TG (g/l)	0.97 ± 0.55	1.52 ± 0.83	< 0.0001
TC (g/l)	1.80 ± 0.40	1.98 ± 0.41	< 0.0001
LDL (g/l)	1.04± 0.36	1.14 ± 0.37	0.02
HDL (g/l)	0.57 ± 0.19	0.54 ± 0.24	0,18
The data are presented in Forms: Mean±SD. SD, standard deviation; T2D, type 2 diabetes; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.			

Table 2. Genotype and allele frequencies of ADIPOQ in control and diabetic patients.

ADIPOQ Genotype	T2D Patients (n=181)	Control Patients (n=149)	OR (95% CI)	P-value
CC	104 (57.46%)	99 (66.44%)	1.00 (reference)	
GC	62 (34.25%)	49 (32.89%)	1.2 (0.75 to 1.91)	P = 0.4
GG	15 (8.29%)	1 (0.67%)	14.27(1.85 to 110.13)	P = 0.01
			χ^2= 10.89	**P = 0.004**
G	92 (25.41%)	51 (17.11%)	1,6503 (1,12-2,42)	0,01
C	270 (74.59%)	247 (82.89%)		
T2D, Type 2 diabetes; OR, odd ratio; CI, confidence interval				

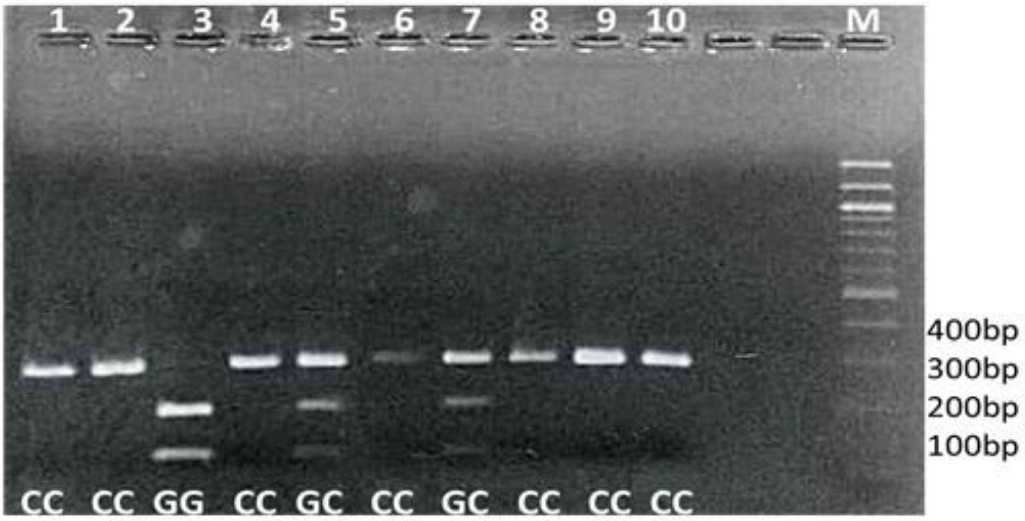

Fig.1. PCR-RFLP digestion for the ADIPOQ -11377C>G polymorphism on an agarose gel electrophoresis (2%). Lanes 1, 2, 4, 6, 8, 9 and 10 represent the CC genotype; lanes 5 and 7 represent the GC genotype; and lane 3 represent the GG genotype. M represent ladder.

Significantly different genotype frequencies were evident between diabetic patients and controls (p=0.004). Furthermore, the frequency of allele G was 25.41% in diabetic patients and 17.11% in controls. These findings underscore a substantial distinction in the G allele frequency between diabetic patients and controls (p=0.01), as outlined in Table 2.

4. Discussion

In this current study, we investigated the association between rs266729 (-11377C > G) polymorphisms of the adiponectin gene and T2D in the Moroccan population. The results revealed a significant association between the rs266729 polymorphism and T2D susceptibility, particularly noting that the C to G SNP in rs266729 is significantly linked to an increased risk of T2D. The ADIPOQ gene is in a region identified as a susceptible locus for T2D [14, 15], Numerous studies have explored the relationship between different SNPs in the adiponectin gene and T2D, notably rs266729, presenting divergent and inconclusive findings regarding the correlation between ADIPOQ gene polymorphisms, specifically rs266729, and T2D susceptibility [16–18]. Our findings are aligning with previous studies on this specific polymorphism [10, 19–21], including a study in French and Iranian populations that found a positive association of rs266729 with T2D [21, 12].

Ramya et al.'s research, which includes studies on Finnish Diabetes Prevention and the Iraqi population, demonstrated a noteworthy association between the rs266729 polymorphism and an increased risk of developing T2D, particularly with allele C in Finnish Diabetes Prevention Studies [12, 23–24]. Hara et al. discovered that the G allele, as opposed to the C allele, in the rs266729 polymorphism, might be linked to an increased risk of T2D [25]. The collective findings from these studies identify rs266729 of the ADIPOQ gene as a potential factor in the development of T2D in the Moroccan population. Nevertheless, certain investigations have presented inconclusive findings, reporting no discernible association of the rs266729 with the risk of T2D [19, 21, 25]. Furthermore, a meta-analysis, incorporating a substantial cohort of 11,963 cases and 15,527 controls, illustrated that the genotypes (CC, CG, and GG) of rs266729 within the ADIPOQ gene exhibited no significant association with the risk of T2D.

Several plausible explanations may account for this inconsistency. Firstly, it could be attributed to the limited sample size and the constrained number of studies encompassed in the meta-analysis conducted by Hara et al., which stands in contrast to the scope of our study. Secondly, the rs266729 polymorphism within the ADIPOQ gene may not represent a significant SNP across the entire population. Thirdly, T2D is a multifaceted ailment influenced by an array of environmental factors, lifestyle choices, socioeconomic conditions, and individual susceptibility [27, 28]. In summary, our study indicates a significant association between rs266729 of the ADIPOQ gene and the risk of T2D in the Moroccan population. Consequently, large-scale studies are required to further validate the association of this specific.

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