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Association of combined -344T/C and K173R polymorphisms in aldosterone synthase gene with type 2 diabetes mellitus in the Moroccan population

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Abstract

Background: Aldosterone synthase (CYP11B2) is crucial for aldosterone production, and variations in its gene may influence type 2 diabetes mellitus (T2DM) development. This study explores the link between two single nucleotide polymorphisms (SNPs) in the CYP11B2 gene - -344T/C and K173R and T2DM in the Moroccan population.

Methods: The research involved 86 individuals with T2DM and 75 control subjects. Genotyping for the -344T/C and K173R SNPs was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Result: Results indicated significant differences in the genotype and allelic distribution of the CYP11B2 K173R polymorphism between T2DM patients and control subjects, with P-values of 0.02 and 0.04, respectively. The -344T/C polymorphism showed no significant genomic level differences, but its allelic variations were statistically significant (P=0.01), indicating a notable association between the C allele and T2DM. Furthermore, the K173R polymorphism was found to significantly increase T2DM risk, with a 2.34-fold higher risk in individuals carrying the KR genotype. The study also examined the combined effect of these SNPs. The dominant model analysis (TT vs. TC+CC and KK vs. KR+RR) showed significant differences between T2DM patients and controls for both SNPs. Additionally, a haplotype-based analysis revealed that the C-R haplotype was associated with an increased risk of T2DM.

Conclusion: Our study suggests a significant association between the CYP11B2-K173R polymorphism and T2DM in the Moroccan population. Conversely, while the CYP11B2 -344T/C polymorphism exhibits a significant difference in allelic distribution, no significant difference is observed at the genomic level.

Keywords: Aldosterone synthase; Type 2 Diabetes Mellitus; SNPs; RFLP; Morocco.

What is new? What is important?

• First investigation conducted in Morocco exploring the association of the CYP11B2 gene polymorphisms (-344T/C and K173R) with the T2DM susceptibility.
• A significant association of CYP11B2-K173R polymorphism with T2DM risk in Moroccans, showing the importance of genetic factors in the pathogenesis of T2DM and may inform and specifies interventions for therapeutic strategies.

INTRODUCTION

Diabetes mellitus (DM), characterized by elevated blood glucose levels, is a global health concern with a rising prevalence. According to the International Diabetes Federation (IDF), approximately 537 million people worldwide were diagnosed with diabetes in 2021, and this number is projected to surge to 783 million by 2045 [1]. The Eastern Mediterranean region, including Morocco, is particularly affected, with a reported diabetes prevalence rate of 12.4% in the adult population [2]. Diabetes-related complications contribute significantly to mortality, with over 12,000 deaths annually in Morocco attributed to this metabolic disorder [2].

Type 2 Diabetes Mellitus (T2DM), the most prevalent form of DM, is a multifactorial disease

influenced by genetics, including genes, genetic susceptibility, genetic polymorphism, and environmental factors [3–5]. Understanding the genetic basis of T2DM is essential for devising effective preventive strategies.

A pivotal element in the pathophysiology of T2DM is aldosterone, a principal effector of the renin-angiotensin aldosterone system (RAAS) [6]. Implicated in T2DM, aldosterone's synthesis is governed by the aldosterone synthase (CYP11B2), encoded by the CYP11B2 gene located on human chromosome 8, plays a crucial role in aldosterone production, achieved through a series of enzymatic reactions wherein deoxycorticosterone is converted into aldosterone [7]. This enzyme influences glucose metabolism and insulin sensitivity [8], by regulating potassium fluxes [9].

One prominent and extensively researched polymorphism is the -344C/T variant located in the 5′ promoter region of the CYP11B2 gene. Within the position -344 in this genetic locus, the two alternative nucleotide bases are thymine (T) and cytosine (C) [10]. Additional noteworthy single nucleotide polymorphisms (SNPs) linked to heightened aldosterone production have been documented, such as a missense mutation in exon 3, resulting in the substitution of lysine (K) with arginine (R) (K173R).

This current study aims to consolidate our findings and delve deeper into the genetic landscape of T2DM in Morocco by examining the -344T/C and K173R SNPs of the CYP11B2 gene. By elucidating the interplay between these genetic variants and T2DM susceptibility, we aim to contribute valuable insights that may illuminate the pathogenesis of the disease and inform targeted interventions and therapeutic approaches.

MATERIALS AND METHODS

SAMPLE COLLECTION

In this study, we enrolled 161 participants, including 86 patients with T2DM and 75 control subjects.

The participants were individuals consulting at the Diagnostic Center-Moulay Youssef Hospital in Rabat. Blood samples were collected as part of routine tests requested by their attending physicians. It's important to note that for our research purposes, only 1ml of blood was retained from each participant for genomic DNA extractions.

Regarding ethical considerations, we confirm that all aspects of our research were conducted in accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants prior to their inclusion in the study. The blood is used to extract genomic DNA using the high-ionic protein recharging method [11].

Patients diagnosed with T2DM were selected based on their medical records and confirmed diagnosis by their attending physicians. The control group consisted of individuals without a history of T2DM or any other relevant medical conditions, and they were selected from the same population pool as the patients, ensuring demographic comparability.

The control group was matched with the patient group based on age and gender to minimize potential confounding variables.

ASSESSMENT OF CLINICAL AND ANTHROPOMETRIC CHARACTERISTICS

We obtained various clinical and anthropometric characteristics, including blood glucose levels, waist circumference (WC), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), systolic blood pressure (SBP), and diastolic blood pressure (DBP), measured using standardized protocols within the Diagnostic Center-Moulay Youssef Hospital in Rabat. The results of these assessments we were provided with the 1ml of blood sample and used as the basis for the subsequent analyses and interpretations in this study.

GENOTYPING -344T>C AND K173R

– Polymorphism -344T>C

To detect the -344T/C polymorphism within the CYP11B2 gene, we employed specific forward (5' GTG TCA GGG CAG GGG GTA 3') and reverse (5' AGG CGT GGG GTC TGG ACT 3') primers. Utilizing the Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) method, as described previously by [12]. The PCR amplification encompassed 35 cycles, involving denaturation at 94°C for 30sec, annealing at 60°C for 30sec, and extension at 72°C for 1 minute.

Subsequently, the resulting 228bp PCR products underwent digestion with Hae III at 37°C for 13h. Electrophoresis of the digested samples on a 2% agarose gel unveiled distinctive patterns: The CT genotype exhibited four distinct fragments upon digestion, measuring 175, 106, 69, and 53bp. In contrast, the CC genotype manifested three fragments, measuring 106, 69, and 53bp, while the TT genotype presented two fragments, measuring 175 and 53bp. This methodology facilitated the precise identification of the -344T/C polymorphism and allowed for the differentiation of genotypes based on fragment size variations.

– Polymorphism K173R

We determined the K173R polymorphism by PCR-RFLP using the primer pair: sense (5' GAA AAG GCT GCA GCT CGA ACA CAA 3') and antisense (5' GCA TGG CCC ACA CCT TCT A 3'). PCR method was conducted following the same protocol than the -344T/C polymorphism [12]. The amplification process consisted of an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension step at 72°C for 5 minutes. Following PCR amplification, the products were subjected to digestion with 5U of Bsu36I restriction enzyme at 37°C overnight. Electrophoresis of the digested products on 2% agarose gels revealed genotypes based on the presence or absence of the restriction site resulting from the K173R substitution. The 173K allele produced a fragment of 371bp, while the 173R allele generated two fragments.

STATISTICS

We performed statistical analysis using the Statistical Package for the Social Sciences (SPSS 24.0). We represented continuous variables as mean±SD and compared them between groups using Student's t-test (Table 1). We examined categorical variables, assessed Hardy–Weinberg equilibrium (HWE), and evaluated genotype/allelic distributions utilizing the χ^2 test (Table 2). Logistic regression analysis was then employed to determine the risk of T2DM associated with the -344T/C and K173R SNPs within the CYP11B2 gene. We also calculate adjusted odds ratios (AOR) with 95% confidence intervals (CI) for confounding parameters (age and gender) (Table 3). We determined linkage disequilibrium (LD) and haplotype analysis using the online version of SNPStats (available at: https://www.snpstats.net/start.htm). We considered results statistically significant at $P \leq 0.05$.

Table 1

Baseline Characteristics of the Study Population

Clinical parameters	**T2DM patients (n = 86)**	**Controls (n = 75)**	**P-value**
Age	53,56±10,65	48,12±13,82	0.005
Gendre (Men (%) /women (%))	35 (41%) /51 (59%)	40 (59%) /35 (47%)	0.111
Blood glucose (g/l)	1,93±0,63	0,88±0,88	< 0.001
WC (cm)	89,55±7,65	82,66±6,43	< 0.001
TC (g/l)	1.96±0.45	1,78±0.40	0.011
HDL (g/l)	0.52±0.23	0.57±0.17	0.158
LDL (g/l)	1.11±0.43	1.03±0.36	0.217
TG (g/l)	1.55±1.03	0.83±0.31	< 0.001
SBP (mmHg)	139.77±17.27	128.56±9.30	< 0.001
DBP (mmHg)	77.30±10.37	72.16±5.57	< 0.001

T2DM, type 2 diabetes mellitus; **WC**, waist circumference; **TC**, total cholesterol; **HDL**, high-density lipoprotein cholesterol; **LDL**, low-density lipoprotein cholesterol; **TG**, triglyceride; **SBP**, systolic blood pressure; **DBP**, diastolic blood pressure. All variables' differences were calculated by a χ^2 test and Student t-test. All data were reported as mean ± standard deviation (SD), except for gender, which was indicated by n (%). $P \leq 0.05$ was considered significant.

Table 2

Genotype distribution of -344T/C and K173R SNPs of CYP11B2 gene in the Study Population

Genotype/allele	T2D patients (n = 86)	Controls (n = 75)	χ^2/ P-value
-344T/C			
TT	41 (47.7%)	49 (65.3%)	5.85/ 0.53
TC	36 (41.9%)	23 (30.7%)	
CC	9 (10.5%)	3 (4%)	
Dominant TT	41 (47.7%)	49 (65.3%)	5.06/ 0.02
TC+CC	45 (52.3%)	26 (34.7%)	
Recessive CC	9 (10.5%)	3 (4.0%)	2.42/ 0.12
TT+TC	77 (89.5%)	72 (96.0%)	
T	118 (69%)	121 (81%)	6.10/ 0.01
C	54 (31%)	29 (19%)	
K173R			
KK	35 (40.7%)	46 (61.3%)	7.63/ 0.02
KR	43 (50%)	22 (29.3%)	
RR	8 (9.3%)	7 (9.3%)	
Dominant KK	35 (40.1%)	46 (61.3%)	6.82/ 0.01
KR+RR	51 (59.9%)	29 (38.7%)	
Recessive RR	8 (9.3%)	7 (9.3%)	0.00/ 0.99
KK+KR	78 (90.7%)	68 (90.7%)	
K	113 (66%)	114 (76%)	4.09/ 0.04
R	59 (34%)	36 (24%)	

P≤0.05 was considered statistically significant.

Table 3

Association between Genetic SNPs of CYP11B2 gene (-344T/C and K173R) and T2DM Risk

Genotypes	T2DM patients (n = 86)	Controls (n = 75)	AOR (95% CI)	P-value
-344C>T				
TT	41 (47.7%)	49 (65.3%)	1.00 (reference)	
TC	36 (41.9%)	23 (30.7%)	3.08 (0.75-12.58)	0.12
CC	9 (10.5%)	3 (4%)	0.32 (0.08-1.32)	0.07
K173R				
KK	35 (40.7%)	46 (61.3%)	1.00 (reference)	
KR	43 (50%)	22 (29.3%)	2.34 (1.17-4.68)	0.01
RR	8 (9.3%)	7 (9.3%)	1.31 (0.42-4.11)	0.64

AOR, Adjusted odds ratio (for age and gender); **CI**, Confidence interval.
P≤0.05 was considered significant.

RESULTS

The study cohort comprised individuals diagnosed with T2DM and a control group, with mean ages of 53.56 and 48.12 years, respectively. A statistically significant age difference was observed between T2DM patients and controls (P=0.005). Gender distribution revealed a higher proportion of women in both T2DM cases (59%) and controls (47%). Nevertheless, no statistically significant differences in sex distribution were noted between the two groups (P=0.111).

Table 1 shows that individuals with T2DM had significantly higher levels of blood glucose (P<0.001), WC (P<0.001), TG (P<0.001), SBP (P<0.001), DBP (P<0.001), and total cholesterol (P=0.011) compared to the control group.

Table 2 shows the frequencies of alleles and genotypes for two different genetic variations of CYP11B2 gene, -344T/C and K173R. The genotype distribution for both variations was found to be in accordance with the Hardy-Weinberg equilibrium for both the cases and controls, indicating that the selected population was a representative sample.

The allelic distribution of the -344T/C variation was significantly different between T2DM patients and controls (P=0.01), but the genomic

distribution was not significantly different (P=0.53). However, the genotypic and allelic distributions of the K173R variation were significantly different between T2DM patients and controls (P=0.02 and P=0.04, respectively). We also found that the dominant genotype for both variations (TT vs. TC+CC and KK vs. KR+RR) was significantly different between the case and control groups (P=0.02 and P=0.01, respectively).

For the CYP11B2 -344T>C variation, controls had a higher frequency of the T allele compared to cases (81% vs. 69%), while the frequency of the C allele in cases was 31% and in controls it was 19%. For the CYP11B2 K173R variation, the frequency of the K allele was 66% in cases and 76% in controls, while the frequency of the R allele was 34% in cases and 24% in controls. After adjusting the odds ratio for confounding factors (age and gender), only the heterozygote KR genotype showed a significant difference (AOR=2.34, 95% CI: 1.17-4.68, P=0.01) between T2DM patients (50%) and controls (29.3%) (Table 3).

Haplotypes were generated for the two variations (-344T/C and K173R) in the CYP11B2 gene that were studied, and SNPStats website was utilized to ascertain their cumulative effect. The degree of LD between variants was measured using the disequilibrium coefficient (D') and the correlation coefficient (r^2). The findings indicate a positive LD between the -344T>C and K173R polymorphisms of the CYP11B2 gene, but this LD is relatively weak (D'=0.11 and r^2=0.01).

Table 4 shows the results from four possible haplotypes formed from the two SNPs. The AOR from haplotype analysis for these two variations and their relationship with T2DM was (AOR=0.24, 95% IC=0.07-0.75) for haplotype CR vs TK allele combinations. The global haplotype analysis for all two variations studied and their association with T2DM was statistically significant (P=0.036).

Table 4

Haplotype distribution of CYP11B2 SNPs (-344T/C and K173R) in T2DM patients and control group

-344T>C	K173R	T2D diabetic	control	AOR (95% CI)	P-value
T	K	0.4793	0.6136	1.00	
T	R	0.2068	0.1931	0.88 (0.46 - 1.68)	0.69
C	K	0.1777	0.1464	0.76 (0.37 - 1.54)	0.45
C	R	0.1363	0.0469	0.24 (0.07 - 0.75)	0.016
Global haplotype association p-value: 0.036. **AOR**, Adjusted odds ratio (for age and gender); **CI**, Confidence interval. P≤0.05 was considered significant.					

DISCUSSION

Aldosterone, a hormone critical in regulating electrolyte balance and blood pressure, plays a pivotal role in the RAAS. Its synthesis is tightly controlled by the aldosterone synthase enzyme, encoded by the CYP11B2 gene. The majority of studies conducted on the CYP11B2 gene have investigated its association with hypertension [8,13–15], cardiovascular diseases [16,17], and exhibits a correlation, albeit to a lesser degree, with T2DM and its associated complication [5,9,18].

Building on the significance of aldosterone in T2DM, the National Center for Biotechnology Information (NCBI) database reports the existence of approximately 227 SNPs of the CYP11B2 gene, across different populations globally. The purpose of this study was to investigate the link between the -344T/C and K173R polymorphisms in CYP11B2 gene and the susceptibility to T2DM in the Moroccan population.

The wild-type allele for the CYP11B2 -344T/C polymorphism is T at codon 344 in the gene's promoter, with variant allele C. This genetic variation assumes a pivotal role by localizing within the putative binding site for the Steroidogenic factor 1 (SF-1), a transcription factor exerts regulatory control over the expression of multiple enzymes essential to the steroid biosynthesis framework within the adrenal cortex [10]. The study found no significant difference between diabetic and healthy groups when comparing co-dominant genotypes of -344T/C polymorphism (χ^2=5.85, P=0.53). However, significant differences were observed when using the dominant model (TT vs. TC+CC) with a χ^2=5.06 and a P=0.02. Allelic frequencies analysis also showed a significant difference (χ^2=6.10, P=0.01), suggesting a potential association between the T allele of the -344T/C

polymorphism and T2DM predisposition. While previous studies have reported negative results [5], others have identified a positive association between this polymorphism and diabetes [9,19]. Gang Jee Ko and colleagues reached the conclusion that SNPs within the CYP11B2 gene, particularly the -344T/C polymorphism, are not linked to diabetic nephropathy [20]. However, they identified an association with the progression of hypertension in individuals T2DM. In contrast, other researchers, such as [9], reported an association of the -344T/C polymorphism with plasma glucose levels and the Metabolic syndrome. Moreover, Larysa Sydorchuk and collaborators found evidence suggesting that the genetic variant -344T/C may have an impact on aldosterone secretion, particularly in individuals at intermediate or higher risk of hypertension when coexisting with T2DM [21]. These diverse findings underscore the complexity of the relationships between CYP11B2 gene polymorphisms and various clinical parameters, highlighting the need for further research to elucidate the precise mechanisms and implications of these genetic variations in the context of diabetes, nephropathy, and hypertension.

In contrast, the K173R variant was significantly associated with an increased risk of T2DM and had an aggravating effect (χ^2=7.63; P=0.02). Notably, the diabetic cohort exhibited a higher prevalence of the KR genotype (50%) compared to the healthier group (29.3%), suggesting a potential link between this polymorphism and T2DM susceptibility (AOR=2.34, 95% CI: 1.17-4.68, P=0.01). The allelic distribution also demonstrated significant differences (χ^2=4.09, P=0.04), with a greater prevalence of the K allele in diabetics (66%) than in healthy subjects (76%). Studies examining the association of the K173R polymorphism with diabetes are scarce. However, Ranade et al. reported a notably significant correlation between fasting plasma glucose levels and the K173R polymorphism. Specifically, individuals with the RR homozygous genotype demonstrated higher mean fasting glucose levels compared to those with KR or RR genotypes [22]. In a study doing in Malysia the frequency of the variant R allele of K173R polymorphism was seen very less in hypertensive patients with T2DM (24.62%) as compared to control subjects (26%) [18].

Finally, we investigated in this study the interaction between the two SNPs -344T/C and K173R of CYP11B2 gene with the susceptibility to T2DM in the Moroccan population. We discovered that the C-R haplotype aggravated T2DM and was linked to its incidence. This finding corroborates the impact of multiple genetic variables on the vulnerability to T2DM. To the best of our knowledge, this is the first study investigate the relation of haplotype of these two polymorphisms of CYP11B2 to T2DM. The major haplotype study trait other disease, in particularly Hypertension [12,23,24].

Previous studies have found K173R and -344T/C in strong LD [25–27]. In our case we found that the two polymorphisms are in LD but not significantly, this is inevitably due to the small size of our sample.

Nevertheless, it is important to acknowledge the limitations of the present study. Initially, we did not investigate additional SNPs in several genes associated with the RAAS, such as SNPs in the angiotensin I converting enzyme gene (ACE I/D). In addition, it is important to note that the sample size of the current study was limited. To gain a more comprehensive understanding of the relationship between genes in the CYP11B2 and the occurrence of T2DM in our patient population, future studies should employ a larger sample size. Similarly, it would have been beneficial to investigate the levels of aldosterone synthase in our patients to ascertain the functional consequences of these genetic variations on the activity of the two genes.

CONCLUSION

Overall, the findings of this study indicate that the CYP11B2 -344T/C and K173R polymorphisms contribute to the occurrence of T2DM in Moroccan individuals. Furthermore, the examination of haplotypes using the two SNPs found in the two genes demonstrated that the C-R haplotype was linked to an increased risk of T2DM. This research also verified that these genetic variations worsen the progression of this disease. In addition, our research revealed a correlation between these two genetic markers and the vulnerability to T2DM. Therefore, future research is imperative to study additional SNPs in various genes within the RAAS. This investigation is essential for comprehensively evaluating the interplay between gene–gene interactions within the RAAS and the occurrence of T2DM.

Introducere: Aldosteron sintaza (CYP11B2) este crucială pentru producția de aldosteron, iar variațiile sale genice pot influența dezvoltarea diabetului zaharat de tip 2 (T2DM). Acest studiu explorează legătura dintre două polimorfisme nucleotidice (SNP-uri) în gena CYP11B2 - -344T/C și K173R și T2DM în populația marocană.

Metode: Cercetarea a implicat 86 de indivizi cu T2DM și 75 martori. Genotiparea pentru SNP-urile -344T/C și K173R a fost realizată folosind analiza PCR-RFLP.

Rezultate: Rezultatele au indicat diferențe semnificative în distribuția genotipurilor polimorfismului CYP11B2 K173R între pacienții cu T2DM și subiecții de control, cu valori P de 0.02 și, respectiv, 0.04. Polimorfismul -344T/C nu a arătat diferențe semnificative la nivel genomic, dar variațiile sale alelice au fost statistic semnificative (P=0.01), indicând o asociere notabilă între alela C și T2DM. În plus, s-a constatat că polimorfismul K173R crește semnificativ riscul de T2DM, cu un risc de 2.34 ori mai mare la indivizii care poartă genotipul KR. Studiul a examinat de asemenea efectul combinat al acestor SNP-uri. Analiza modelului dominant (TT vs. TC+CC și KK vs. KR+RR) a arătat diferențe semnificative între pacienții cu T2DM și controale pentru ambele SNP-uri. În plus, o analiză bazată pe haplotip a relevat că haplotipul C-R este asociat cu un risc crescut de T2DM.

Concluzie: Studiul nostru sugerează o asociere semnificativă între polimorfismul CYP11B2-K173R și T2DM în populația marocană. În schimb, deși polimorfismul CYP11B2 -344T/C prezintă o diferență semnificativă în distribuția alelică, nu se observă nicio diferență semnificativă la nivel genomic.

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