Pathologie Biologie 63 (2015) 243–247

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Original article

Association analysis of *APOA5* rs662799 and rs3135506 polymorphisms with obesity in Moroccan patients

Analyse d'association des polymorphismes rs662799 et rs3135506 du gène APOA5 *avec l'obésité chez des patients marocains*

F. Lakbakbi el Yaagoubi [a,b], H. Charoute [a], A. Bakhchane [a], M. Ajjemami [a], H. Benrahma [a], A. Errouagui [a], M. Kandil [b], H. Rouba [a], A. Barakat [a,*]

[a] *Institut Pasteur, laboratoire de génétique moléculaire humaine, Casablanca, Morocco*
[b] *Équipe d'anthropogénétique et biotechnologies, faculté des sciences, université Chouaïb Doukkali, El Jadida, Morocco*

ARTICLE INFO

Article history:
Received 6 June 2015
Accepted 29 September 2015

Keywords:
APOA5
Polymorphisms
Haplotypes
Obesity
Morocco

ABSTRACT

The aim of the present study is to explore the association between the *APOA5* polymorphisms and haplotypes with obesity in Moroccan patients. The study was performed in 459 subjects, Obese ($n = 164$) and non-obese ($n = 295$). All subjects were genotyped for the *APOA5* -1131T > C (rs662799) and c.56C > G (rs3135506) polymorphisms. The contribution of *APOA5* polymorphisms and haplotypes in the increased risk of obesity were explored using logistic regression analyses. The -1131T > C and c.56C > G polymorphisms were significantly associated with obesity. Both polymorphisms were strongly associated with increased BMI. Analysis of constructed haplotypes showed a significant association between CG haplotype and susceptibility to obesity (OR [95%CI] = 3.09 [1.93–4.97]; $P < 0.001$). These results support a potential role for *APOA5* common variants and related haplotypes as risk factors for obesity.

Mots clés :
APOA5
Polymorphismes
Haplotypes
Obésité
Maroc

RÉSUMÉ

L'objectif de cette étude est d'analyser l'association entre les polymorphismes -1131 T > C (rs662799) et c.56C > G (rs3135506) dans le gène *APOA5* et le risque d'apparition de l'obésité chez une population marocaine. Cette étude a porté sur 459 sujets dont 164 personnes obèses et 295 personnes non obèses marocains. Tous les sujets ont été génotypés pour les polymorphismes -1131 T > C et c.56C > G du gène *APOA5* avec la méthode PCR-RFLP. La contribution des polymorphismes et des haplotypes du gène *APOA5* dans le risque accru d'obésité ont été analysés par la régression logistique. Les analyses statistiques montrent que les polymorphismes -1131T > C et c.56C > G du gène *APOA5* ont été significativement associés avec l'obésité. Les deux polymorphismes sont fortement associés à l'augmentation de l'indice de masse corporelle (IMC). L'analyse des haplotypes a montré une association significative entre l'haplotype CG et la susceptibilité à l'obésité (OR [IC 95 %] = 3,09 [1,93 à 4,97] ; $p < 0,001$). Ces résultats confirment le rôle potentiel des polymorphismes du gène *APOA5* comme des facteurs de risque de l'obésité.

* Corresponding author.
E-mail address: hamid.barakat@pasteur.ma (A. Barakat).

http://dx.doi.org/10.1016/j.patbio.2015.09.002
0369-8114/

1. Introduction

Obesity has become a worldwide pandemic. According to the World Health Organization (WHO), the overweight and the obesity are defined as an abnormal or excessive accumulation of fat which represents a serious risk to health [1]. The Body Mass Index (BMI) is a simple way to measure the obesity in the population: it corresponds to the weight of the person (in kilograms) divided by the square of his or her height (in meters). A person with a BMI of 30 or more is generally considered obese. A person whose BMI is equal or upper to 25 is considered overweight [1].

The overweight concerns more than 2.16 billion persons, and 1.12 billion obese individuals by 2030 [2]. According to the WHO statistics: "In 2014, more than 1.9 billion adults (18 years and older) were overweight. Of these over 600 million were obese." [3]. In 2011, the High Planning Commission (HPC) in Morocco, reported that near the third of the Moroccan population (about 10 million persons) had problems of overweight, and 3.6 million individuals suffer from obesity [4].

Obesity increases the risk of many diseases like type 2 diabetes (T2D), cardiovascular disease, hypertension, atherosclerosis, stroke, cancer, inflammation, and dyslipidemia [5]. The energy imbalance is the major cause of obesity: the calories consumed are more than those utilized. Obesity is a multifactorial disease characterized by interaction between genetic and environmental factors. Gene-Environment interaction studies showed that healthy lifestyle such as physical activity or healthy diet can attenuate the effect of some genetics factors on obesity [6]. Studies of related individuals suggest that genetic factors play a major role in the determination of BMI [7]. Twin studies estimated that genetic factors could explain from 50 to 90% of the variance in BMI heritability [8].

The contribution of genetic factors in the etiology of obesity was investigated by Genome Wide Association Studies (GWAS). Recently, several genes associated with obesity have been identified by GWAS studies such as: *FTO, MC4R, POMC, SH2B1, BDNF, NRXN3 and NEGRI* [9]. In addition, more than 50 loci were strongly associated with obesity parameters such as BMI, waist-to-hip ratio and body fat percentage [6].

So far, various studies suggested a potential role of the Apolipoprotein A5 gene in obesity [10,11]. The *APOA5* is located on chromosome 11q23 next to *APOA1/APOC3/APOA4* gene cluster. This gene consists of 4 exons and encodes a protein of 366-amino acids [12]. Apolipoproteins, synthesized in the liver or the intestine, play a role of mediator in various biochemical processes involved in the lipid metabolism [13,14].

Genetic variations in the *APOA5* gene have significant effects on lipid parameters in various populations [15]. The *APOA5* polymorphisms -1131 T > C and c.56C > G have been reported to be associated with dyslipidemia, triglyceride levels and increased risk of metabolic syndrome [12,16,17].

In the present study, we analysed the association of *APOA5* polymorphisms and haplotypes with the risk of obesity in Moroccan patients.

2. Materials and methods

2.1. Study population

In this case-control study, a total of 164 unrelated obese individuals and 295 unrelated non-obese controls were recruited. According to the World Health Organization recommendations, the study participants were classified based on the BMI (kg/m^2), obese subjects had BMI $\geq 30\ kg/m^2$ and non-obese subjects had BMI $< 30\ kg/m^2$.

During this study in the Medical Biology Center of Pasteur Institute of Morocco in Casablanca, we filled out the questionnaire containing clinical and biochemical parameters. All the subjects were from various ethnic groups.

2.2. Ethics statement

The protocol of this study was approved by local Research Ethics Committee of Pasteur Institute of Morocco. Informed consents were collected from all participants before including them in this study.

2.3. Biochemical measurements

Blood samples were collected from individuals after overnight fasting (12 h), for immediate measurements of Glycemia, Total Cholesterol (TC), Triglycerides (TG) and High-Density Lipoprotein Cholesterol (HDL-C) levels using the VITROS (5.1 FS Chemistry System). Low-density Lipoprotein Cholesterol (LDL-C) level was calculated according to the Friedwald's formula. All biochemical measurements were performed in the Biochemistry Laboratory of the Medical Biology Center in Pasteur Institute of Morocco.

2.4. Isolation of DNA

Total genomic DNA was extracted from blood using the standard phenol-chloroform method. DNA was quantified by Qubit v3.

2.5. Genotyping APOA5 polymorphisms

Genotyping of -1131 T > C and 56C > G polymorphisms was performed using the PCR-RFLP analysis by using a thermal cycler and Taq Polymerase (Bioline). c.56C > G polymorphism fragment was amplified using two oligonucleotides, forward: 5′- GGC TCT TCT TTC AGG TGG GTC TCCG -3′reverse: 5′- GCC TTT CCG TGC CTG GGT GGT -3′ [18] at 96 °C for 5 min followed by 30 cycles of 96 °C for 30 s, 64 °C for 30 s, 72 °C for 45 s, and a final extension of 72 °C for 10 min. The PCR products were digested for 2 h at 65 °C with TaqI restriction enzyme.

And the -1131 T > C polymorphism was genotyped using the following primers: Forward: 5′- CCC CAG GAA CTG GAG CGA AA TT-3′, reverse 5′- TTC AAG CAG AGG GAA GCC TGTA-3′ at 96 °C for 5 min, followed by 32 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 10 min. The PCR products were digested with Mse I.

2.6. Statistical analysis

Clinical and biochemical data were expressed as means ± standard deviation (SD) or median [interquartile range, 25–75%]. Student's *t* test was used to compare quantitative parameters that follow a normal distribution. Otherwise, Mann-Whitney U test was applied for non-normally distributed quantitative trait. Logistic regression analysis was performed to analyse the association of *APOA5* genotypes and haplotypes with obesity. *P*-values less than 0.05 were considered as statistically significant. All statistical analyses were performed using STATA software, version 11.0. Testing for deviations from Hardy–Weinberg equilibrium (HWE) was carried out using a fast exact test [19], and the significance level of departure from HWE among controls was set at $\alpha = 10^{-3}$ [20]. The PLINK software v1.07 was used for haplotypes construction and analyses. Linkage disequilibrium between *APOA5* variants was estimated using Haploview software, version 4.2.

3. Results

3.1. Clinical and biochemical characteristics of study participants

The 459 subjects were classified according to the BMI as obese ($n = 164$) and non-obese ($n = 295$). Clinical and biochemical parameters of obese and non-obese subjects are presented in

Table 1
Comparisons of major clinical parameters between obese and non-obese subjects.

	Non-Obese ($n = 295$) Median [1st–3rd]	Obese ($n = 164$) Median [1st–3rd]	*P*-value
Age	51.00 [45.00–57.00]	52.00 [47.00–57.00]	0.1638
Systolic blood pressure	121.00 [112.00–128.00]	130.00 [120.00–150.00]	< 0.0001
Diastolic blood pressure	80.00 [74.00–84.00]	83.00 [77.00–90.00]	< 0.0001
Triglycerides	1.08 [0.74–1.40]	1.35 [0.95–1.78]	< 0.0001
Total cholesterol	1.89 [1.67–2.12]	2.01 [1.80–2.28]	0.0009
LDL-cholesterol	1.17 [0.96–1.36]	1.26 [1.03–1.44]	0.0152
HDL-cholesterol	0.49 [0.41–0.60]	0.47 [0.40–0.55]	0.1483
Fasting plasma glucose	0.90 [0.82–1.01]	1.03 [0.89–1.36]	< 0.0001