ISSN 2250-3153

Association between polymorphism in APOC3, and Metabolic Syndrome in the Moroccan Population

Maria Ajjemami[1], Houria Rhaissi[2], Houda Benrahma[1], Hicham Charoute[1,*], Errouagui Abdeltif[1], Fouzia Lakbakbi el yaagoubi[1], Mostafa Kandil[3], Abdelhamid Barakat[1], Hassan Rouba[1]

[1]. Laboratoire de Génétique Moléculaire Humaine, Département de Recherche Scientifique, Institut Pasteur du Maroc, 1, Place Louis Pasteur, 20360 Casablanca, Morocco.
[2]. Laboratoire de Physiologie et Génétique Moléculaire, Faculté des sciences Ben M'Sik, Université Hassan II, Mohammedia, Morocco.
[3]. Equipe d'Anthropogénétique et biotechnologies, Faculté des Sciences, Université Chouaïb Doukkali, El Jadida, Morocco

Abstract- Metabolic syndrome (MS) is regarded as a real public health problem its prevalence rises each year as well as its morbidity. It is a multi factorial disease and besides environmental factors and genetic factors also contribute to the pathogenesis of MS. In several studies the SstI (3238C> G) polymorphism of APOC3 gene is associated with increased plasma concentrations of triglyceride (TG) and hypertriglyceridemia. The aim of the present study was to determine the association between polymorphism 3238C> G in APOC3, and Metabolic Syndrome in the Moroccan Population. Statistical analysis has revealed an association of polymorphism APOC3 3238C>G susceptibility with the metabolic syndrome in two models, codominant 1 [OR = 4.21 [1.66-10.68], p = 0.0008] and dominant [OR = 3.83 [1.59-9.19] p = 0.0010]. The variant APOC3 3238G were associated with elevated TG levels (P = 0.0146) and LDL-C (p = 0.0068) compared to patients with MS and controls non-carriers of this variant.

Index Terms- Metabolic Syndrome, APOC3 gene, Polymorphisms, TG.

I. Introduction

Metabolic syndrome (MS), as the name suggests is not a specific disease but a syndrome. A syndrome is a recognized set of symptoms with no obvious cause. The components of the syndrome coexist regularly enough that their appearance is not randomly assigned. When the cause is clearly defined, the syndrome becomes disease [1]. MS thus refers to a combination of metabolic abnormalities linked together, the clinical significance remains controversial and accurate.

The definitions of MS the most famous are those of the World Health Organization (WHO), European Study of Insulin Resistance (EGIR), National Cholesterol Education Program – Adult Treatment Panel III (NCEP-ATPIII), American Association of Clinical Endocrinologists (AACE),American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) and the International Diabetes Federation (IDF) .[2-6].

The IDF requires as a mandatory criterion the presence of abdominal obesity plus 2 other criteria: High levels of TG> 1.7 mmol / L or treatment, reduced HDL-C <1 mmol / L in men and <1.3mmol / L in women or treatment, high systolic and diastolic blood pressure ≥ 130/85mmHg or treatment, high fasting plasma glucose ≥ 100 mg / dL or treatment of type 2 diabetes.

MS is regarded as a real public health problem, its prevalence rises each year as well as its morbidity. In Morocco as defined by the NCEP-ATPIII, the prevalence of MS was 16.3% according to a study of 249 Saharawi women in southern Morocco **[**7**],** according to the study of El Ayachi et al. the prevalence of MS was 17.8% [8] and recently, another study on a population of 640 cardiac patients with a male predominance shows that the prevalence of MS according to the IDF is about 12.18%. [9].

The prevalence of MS is age-dependent [10,11]. Numerous studies have shown that MS is associated with an increased risk for Cardiovascular disease [12,13], type 2 diabetes [14], myocardial infarction and stroke [15]. It is a multi factorial disease and besides environmental factors such as cigarette smoking, obesity, lack of exercise and bad nutrition habits, genetic factors also contribute to the pathogenesis of MS [16,17**].** The APOC3 gene codes for apoCIII, several polymorphic sites have been detected within and around the APOC3 gene. The most extensively studied has been the SstI polymorphism, resulting from the substitution of cytosine by guanine at nucleotide 3238 [3238C> G] in the 3'UTR region of exon 4 of the gene [18]. In the literature, the SstI polymorphism is associated with increased plasma concentrations of TG and hypertriglyceridemia [19].

The aim of the present study was to determine the association of known functional SstI polymorphism [3238C> G] in APOC3 gene in the Moroccan population with and without the MS as defined by the International Federation of Diabetes (IDF).

II. Materials and Methods

This is a case-control study in a Moroccan population of 283 subjects recruited at the Pasteur Institute of Morocco Casablanca, aged between 20 and 60 years,

We have 176 patients with metabolic syndrome according to the criteria of the International Federation of Diabetes (IDF) and105 controls subjects according to the criteria of IDF.

All participants signed informed consent forms, and the study protocol was approved by local Committee on Research Ethics of Pasteur Institut of Morroco.

Systolic and diastolic blood pressures were measured using a sphygmomanometer after 5 minutes of rest in a sitting position at

least. Weight and height were measured to determine body mass index (BMI). BMI was calculated by the following formula: weight in kilograms (kg) / height in square meters (m) ².Waist and hip circumference were also measured.

Fasting glucose, triglyceride (TG), total cholesterol (Total-C), LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) were measured after at least 8 hours of fasting. All assays were performed using an automatic (VITROS) by the biochemistry laboratory IPM Casablanca

Genomic DNA was extracted from whole blood by using conventional phenol-chloroform-isoamyl alcohol [20]. To detect the SstI (APOC3-3238C>G) (rs5128) polymorphism, polymerase chain reaction (PCR) conditions and restriction fragment- length polymorphism analyses were performed according to previous published protocols [21].

III. Statistical Analysis

Statistical analyses were performed using STATA software, version 11.0. Quantitative data were expressed as means ± standard deviation (SD). Student test was used to compare quantitative parameters. Manne-Whitney test was used to compare parameters not normally distributed. Chi-square test was applied to examine differences in genotype distributions between cases and controls. Odds ratios (ORs) and their 95% confidence intervals (CIs) were computed to assess strength of association.

IV. Results

Clinical and biochemical characteristics of MS patients and control subjects are shown in Table 1. Serum triglycerides, HDL-Cholesterol and fasting plasma glucose levels, BMI, waist circumference, hip circumference, Systolic and diastolic blood pressure values were significantly elevated in the MS group compared to the controls. But no significant association in total cholesterol, LDL-Cholesterol in the MS group compared to the controls.

Table 1: Anthropometric, clinical and biological characteristics of the patients with metabolic syndrome and control subjects (means ± SD)

	Controls (n=69)	**Patients (n=116)**	**P-value**
Systolic blood pressure (mm Hg)	11.49±1.21	12.93±1.84	**<0.001**
Diastolic blood pressure (mm Hg)	7.71±0.87	8.39±1.25	**0.0001**
Total cholesterol (mg/dl)	1.89±0.37	1.95±0.44	0.3773
Triglycerides (mg/dl)	0.99±0.32	1.47±0.69	**<0.001**
LDL-cholesterol (mg/dl)	1.16±0.33	1.24±0.36	0.1316
HDL-cholesterol (mg/dl)	0.55±0.12	0.48±0.17	**<0.001**
Fasting plasma glucose (mg/dl)	0.85±0.09	1.31±0.54	**<0.001**
Body mass index (Kg/m²)	25.04±3.04	30.98±5.12	**<0.001**
Waist circumference (cm)	84.64±10.43	100.84±11.91	**<0.001**
Hip circumference (cm)	104.04±11.54	114.41±11.30	**<0.001**

(means ± SD).HDL, high-density lipoprotein. LDL, low-density lipoprotein

Statistical analysis of genotype distribution models

Statistical analysis of different models of genotype distribution was studied; Table 2 summarizes the results of this study. All genotypic distributions are in Hardy-Weinberg equilibrium. (APOC3-3238C>G / T=0,090 and p= 0,510).

Statistical analysis has revealed an association of polymorphism APOC3 3238C>G susceptibility with the metabolic syndrome in two models, codominant 1 [OR = 4.21 [1.66-10.68], p = 0.0008] and dominant [OR = 3.83 [1.59-9.19] p = 0.0010]. But no association was found in two recessive codominant model for all SNPs 3238C> G of APOC3.

Table 2: Distribution of APOC3 genotype among Metabolic Syndrome subjects and controls

Polymorphism	Genotype	Controls	Patients	Model	OR [95%CI]	P-value
APOC3 3238C>G	C/C	62 [89.9%]	81 [69.8%]	Codominant 1 [CC vs CG]	**4.21 [1.66-10.68]**	**0.0008**
	C/G	6 [8.7%]	33 [28.4%]	Codominant 2 [CC vs GG]	1.53 [0.14-17.27]	0.7254
	G/G	1 [1.4%]	2 [1.7%]	Dominant [CC vs CG+GG]	**3.83 [1.59-9.19]**	**0.0010**
				Recessive [CC+CG vs GG]	1.19 [0.11-13.40]	0.8853

Association study between clinical and biochemical parameters and genotypes of polymorphism 3238C> G gene APOC3

We grouped the rare allele carriers for SNP 3238C> G, gene APOC3, and we compared their frequency with the common allele for all parameters of the metabolic syndrome [Table 3].

Holders of the variant APOC3 3238G were associated with elevated TG levels (P = 0.0146) and LDL-C (p = 0.0068) compared to patients with MS and controls non-carriers of this variant.

Table 3: Subjects characteristics according to the APOC3 3238C>G genotypes

	APOC3 3238C>G		
	CC	CG+GG	P-value
Systolic blood pressure	12.37±1.77	12.45±1.80	0.7879
Diastolic blood pressure	8.13±1.12	8.15±1.32	0.9894
Total cholesterol	1.92±0.37	1.94±0.53	0.8581
Triglycerides	1.22±0.56	1.52±0.77	**0.0068**
LDL-cholesterol	1.18±0.33	1.33±0.42	**0.0146**
HDL-cholesterol	0.50±0.13	0.54±0.24	0.1746
Fasting plasma glucose	1.11±0.49	1.23±0.47	0.0605
BMI	28.46±5.05	29.79±6.02	0.3096
Waist circumference	93.85±12.49	98.02±17.36	0.0847

V. Discussion

During the last years, the rapid increase in the prevalence of MS in industrialized countries associated with devastating complications for human health, mainly due to a higher risk of developing cardiovascular disease [16]. In vivo apoCIII modulates the postprandial management of the TG [22] and inhibits the hepatic uptake of VLDL remnants [23]. The genetically determined deficiency of apoCIII in humans has been shown to increase the rate of TG clearance from plasma by 6- to 7-fold [24]. Associated with an increased risk of diabetes and CVD risk, SM is now considered one of the most important public health problems of our time.

To our knowledge, this is the first study to test such an association in the Moroccan population. Our results show an association of polymorphism APOC3 3238C>G susceptibility with the metabolic syndrome in two models, codominant 1 and dominant. But no association was found in two recessive codominant model for all SNPs 3238C> G of APOC3. Moreover the variant APOC3 3238G were associated with elevated TG levels and LDL-C compared to patients with MS and controls non-carriers of this variant.

Several polymorphic sites were detected in APOC3 gene. The most studied is the SstI polymorphism, resulting from the substitution of a guanine with cytosine at nucleotide 3238(3238C>G) in the 3'UTR region of exon 4 of the gene [18]. Alleles of this transversion are: S1 and S2. The frequency of the rare allele (S2) varies among different ethnic groups [25,26]. Several study suggests an association between the rare allele S2 and total cholesterol and high cardiovascular risk [27,28]. In several case-control study, the SstI polymorphism was associated with HTG [25,29]

Acknowledgments

The authors would like to thank all families for their cooperation. This work was supported by Pasteur Institute of Morocco.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Last JM, ed. A Dictionary of Epidemiology, 3rd edn. New York : Oxford UniversityPress 1995: 180.

[2] Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15:539-553, 1998

[3] Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of InsulinResistance[EGIR]. Diabet. Med. 1999 Mai; 16[5]:442-443.

[4] Einhorn D, Reaven GM, Cobin RH, Ford E, Ganda OP et al. American College of Endocrinology position statement on the insulinresistance syndrome. Endocr.Pract. 2003; 9: 237-252.

[5] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005 Oct 25;112[17]:2735-52

[6] Alberti K.G.M.M., FRCP; Robert H. Eckel, MD, FAHA; Scott M. Grundy, MD, PhD, FAHA; Paul Z. Zimmet, MD, PhD, FRACP; James I. Cleeman, MD; Karen A. Donato, SM; Jean-Charles Fruchart, PharmD, PhD; W. Philip T. James, MD; Catherine M. Loria, PhD, MS, MA, FAHA; Sidney C. Smith Jr, MD, FAHA. Harmonizing the Metabolic Syndrome Circulation. 2009; 120: 1640-1645

[7] Rguibi M and Belahsen R. Metabolic syndrome amongMoroccan Sahraoui adultWomen. American journal of humanbiology : the official journal of the HumanBiology Council 2004 ; 16 : 598-601.

[8] El Ayachi M, Mziwira M, Denis L, Rekia B. Prevalence of parameterindicators of obesity and itsrelationshipwithmetabolic syndrome in urbanMoroccanwomen. American journal of humanbiology : the official journal of the HumanBiology Council 2008 ; 20 : 484-6.

[9] Bertal Filali K, Labaq a, Jalal H, Arioua S, Khatouri a. P53 Prévalence et caractéristiques du syndrome métabolique chez les cardiaques. Diabetes & Metabolism 2010 ; 36 : A52-A52.

[10] Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findingsfrom the third National Health and Nutrition Examination Survey. JAMA. 2002; 287: 356-359.

[11] Azizi F, Salehi P, Etemadi A, Zahedi-Asl S. Prevalence of metabolic syndrome in an urban population: TehranLipid and Glucose Study. DiabetesRes. Clin. Pract. 2003 Jul; 61[1]:29-37.

[12] Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S .Diabetes Care. 2005; 28: 2745-2749.

[13] Gami, A. S., Witt, B. J., Howard, D. E., Erwin, P. J., Gami, L. A., Somers, V. K., and Montori, V. M. [2007]. Metabolic syndrome and risk of incident cardiovascular events and death: asystematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol 49, 403-414.

[14] Grundy, S. M., Hansen, B., Smith, S. C., Jr., Cleeman, J. I., and Kahn, R. A. [2004]. Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. Circulation 109, 551-556.

[15] Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS.Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey.Circulation. 2004;109:42-46.

[16] Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988- 1994. Arch Intern Med 2003;163:427e36.

[17] Grundy SM. Metabolic syndrome: a multiplex cardiovascular risk factor. J Clin Endocrinol Metab 2007;92:399e404

[18] Talmud PJ and Humphries SE. Apolipoprotein C-III gene variation and dyslipidaemia. Curr Opin Lipid vol 1997;8:154–8.

[19] Groenendijk M. Cantor RM, de Bruin TW. Et al. The apoAI-CIII-AIV gene cluster. Atherosclerosis 2001. Vol. 157, n°1, pp. 1-11.

[20] Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, Vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

[21] Chhabra. S, R Narang, LR Krishnan, S Vasisht, DP Agarwal, LM Srivastava, SC Manchanda and N Das, Apolipoprotein C3 SstI polymorphism and triglyceride levels in Asian Indians, BMC Genetics 2002, 3, 1-6

[22] Maeda N, Li H, Lee D, Oliver P, Quarfordt SH, Osada J: Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. J Biol Chem 1994, 269:23610-23616

[23] Windler E, Havel RJ: Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. J Lipid Res 1985, 26:556-565

[24] Ginsberg HN, Le NA, Goldberg IJ, Gibson JC, Rubinstein A, Wang-Iverson P, Norum R, Brown WV: Apolipoprotein B metabolism in subjects with deficiency of Apolipoprotein CIII and A. J Clin Invest 1986, 78:1287-1295

[25] Rees A, Stocks J, Sharpe CR, Vella MA, Shoulders CC, Katz J, Jowett NI, Barelle FE, Galton DJ: Deoxyribonucleic acid polymorphism in the apolipoprotein A-1-C-III gene cluster. J Clin Invest 1985, 76:1090-1095

[26] Rees A, Stocks J, Paul-Hayase H, Ohuchi Y, Galton D: Haplotypes identified by DNA polymorphisms at the apolipoprotein A-I and C-III loci and hypertriglyceridemia: a study in a Japanese population. Hum Genet 1986, 72:168-171

[27] J.M. Ordovas, F. Civeira, J. Genest Jr., S. Craig, A.H. Robbins, T. Meade, M. Pocovi, P.M. Frossard, U. Masharan, P.W.F. Wilson, D.N. Salem, R.H. Ward, E.J. Schaefer. Restriction fragment length polymorphisms of the apolipoprotein A-I, C-III, A-IV gene locus Relationships with lipids, apolipoproteins, and premature coronary artery disease. Atherosclerosis. Volume 87, Issue 1, Pages 75–86, March 1991

[28] DE Lorenzo F, Monticelli A, Cocozza S, DE Simone B, Rubba P: Extracoronary atherosclerosis and genetic variants of apolipoprotein AI-CIII cluster in myocardial infarction survivors from southern Italy. Clin Invest. 72: 435-441, 1994.

[29] Tas S: Strong association of a single nucleotide substitution in the 3'-untranslated region of the apolipoprotein C-III gene with common hypertriglyceridemia in Arabs. Clin Chem 1989, 35:256-259

AUTHORS

First Author – Maria Ajjemami, PhD student, Institut Pasteur du Maroc, ajj-maria@hotmail.com
Second Author – Houria Rhaissi, PhD, Faculté des sciences Ben M'Sik, houriarhaissi@hotmail.com.
Third Author – Houda Benrahma, PhD, Institut Pasteur du Maroc, benrahmahouda@yahoo.fr

Correspondence Author – Hicham Charoute, hcharoute@gmail.com, Tel: +212 522 43 44 50; Fax: +212 522 26 09 57