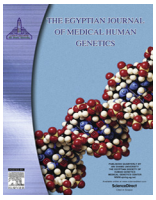
The Egyptian Journal of Medical Human Genetics 19 (2018) 43–48

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com



Original article

Association of genetic polymorphisms of *PON1* and *CETP* with the presence of metabolic syndrome; the effects of genotypes on their serum activity and concentrations

Behdokht Fathi Dizaji [a], Mahdi Rivandi [b,c], Ali Javandoost [d], Maryam Saberi Karimian [b], Atena Raei [d], Amirhossein Sahebkar [e], Gordon Ferns [f], Majid Ghayour Mobarhan [g,*], Alireza Pasdar [b,h,i,*]

[a] Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Department of Modern Sciences & Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Student Research Committee, Department of Modern Sciences & Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Department of Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[e] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[f] Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK
[g] Biochemistry of Nutrition Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[h] Division of Applied Medicine, Medical School, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK
[i] Medical Genetics Research Centre, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 8 November 2017
Accepted 24 December 2017
Available online 17 January 2018

Keywords:
Metabolic syndrome
PON1 gene
CETP gene
Single nucleotide polymorphism (SNP)

ABSTRACT

Background: The Metabolic syndrome (MetS) is associated with an increased risk of cardiovascular disease and type 2 diabetes. *PON1* and *CETP* genes may be involved in the pathogenesis of lipid metabolism and thus MetS. Several single nucleotide polymorphisms of genes were demonstrated to affect their function. Curcumin (diferuloylmethane) is a yellow pigment of turmeric that has shown numerous pharmacological activity against obesity and related conditions through anti-oxidant/anti-inflammatory properties.
Objective: We aimed to assess the association of these polymorphisms with metabolic syndrome and to investigate if these genetic variants were associated with an altered activity of PON1 and the protein levels of CETP at base line and after Curcumin supplementation.
Methods: The genotypes of *PON1* and *CETP* polymorphisms were determined in 81 patients with MetS and 100 healthy individuals using ARMS-PCR and PCR-RFLP techniques.
Results: Individuals with different genotypes of the *PON1 rs662*, rs854560 and rs705379 polymorphisms did not differ with paraoxonase activity and *CETP* serum protein concentrations, either at baseline, or after intervention. Individuals with different *PON1 rs854560* genotypes differ significantly in serum arylesterase activities (p = .037). There were statistically significant differences in genotype frequencies between cases and controls for *CETP rs5882* genotypes (p-value = .034) but not in genotype frequencies and haplotypes for *PON1* studied polymorphisms (p-value < .05). The odds ratio for *CETP rs5882* was statistically significant using a dominant model. OR (95% CI) = 0.48 (025–0.92), p-value = .029.
Conclusions: There were no associations between the *PON1* polymorphisms, or haplotypes with MetS. There was an association between *CETPrs5882* and metabolic syndrome. AA genotype of *CETPrs5882* appeared to be protective against MetS in our studied population. There were no association between the *PON1* and *CETP* polymorphisms with PON1enzymatic activities and CETP protein levels at base line and after curcumin supplementation.

1. Introduction

Metabolic syndrome (MetS) is defined by a clustering of cardiovascular risk factors including: central obesity, insulin resistance, hypertension and atherogenic dyslipidemia, and is associated with a greater risk of type 2 diabetes and cardiovascular disease (CVD) [1,2].

Peer review under responsibility of Ain Shams University.
* Corresponding authors at: Molecular Medicine Group, Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran (A. Pasdar). Cardiovascular Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran (M.G. Mobarhan).
E-mail addresses: GhayourM@mums.ac.ir (M.G. Mobarhan), a.pasdar@abdn.ac.uk, Pasdara@mums.ac.ir (A. Pasdar).

https://doi.org/10.1016/j.ejmhg.2017.12.001
1110-8630/

The prevalence of (MetS) is increasing globally, and in Iran it has one of the highest occurrences worldwide. In the adult population, a prevalence of 30% in Tehran and 45% in Khorasan province has been reported [3].

MetS is a multifactorial disorder in which environmental factors as well as genetic factors play a role [4]. The heritability of MetS has been estimated to be 24–30% [5,6].

Two genes, *PON1* and *CETP* take part in pathways that may be involved in the pathogenesis of MetS [7,8].

Paraoxonase 1 (PON1) is involved in the antioxidant defence system and while it has major catalytic paraoxonase/arylesterase activities, its physiological substrates are lactones, oxidized-LDL and homocysteine thiolactone, and hence it may protect against cardiovascular diseases (CVD) [7].

The paraoxonase gene cluster comprises three members *PON1-PON2-PON3* that are located on chromosome 7q21.3 [9]. More than 160 polymorphisms (SNPs) have been reported for the *PON1* gene [10], of which the Q192R, L55M, -108C/T have been shown to be associated with serum enzyme protein concentrations and activity. The Q192R and L55M SNPs are in coding regions and cause an amino acid substitution. −108C/T is in the promoter of the gene and affects its expression [7,11,12].

Studies have demonstrated that the serum activity and concentrations of PON1 are reduced in conditions in which oxidative stress may be a contributory factor, such as CVD, Alzheimer, MetS, aging and higher levels of PON1 may be protective against these diseases [13].

Cholesterol ester transfer protein (CETP) is a plasma glycoprotein expressed in the liver and secreted into the plasma. The *CETP* gene is located on the chromosome 16q13, has 16 exons and is 26 kb long[14].

CETP is involved in the exchange of TG on LDL with cholesteryl esters on HDL, and results in HDL particles that can be degraded by hepatic lipase. CETP is also involved in reverse cholesterol transport (RCT) and therefore it is not completely clear whether increasing CETP activity would be pro- or anti-atherogenic [8,15–17].

Several SNPs of the CETP gene have been identified which the I405V causes an isoleusine for valine substitution. In genome wide association studies (GWAS) it has been found that variation at the *CETP* gene locus is associated with low levels of HDL-c and is related to CVD [18].

Curcumin is a polyphenolic compound derived from turmeric that has anti-inflammatory and antioxidant properties. It has poor bioavailability when given orally, but this can be improved by using a phytosomal complex [19,20]. Phytosomes are chemical complexes which are synthesized by one or two moles of synthetic or natural phospholipids mainly phosphatidylcoline, and one mole of botanical extracts [21].

The aim of this study was to investigate the association between *PON1* and *CETP* genetic polymorphisms and the presence of MetS. In addition, the associations of *PON1* and *CETP* genetic polymorphisms with PON1 enzymatic activities and the serum concentrations of CETP protein in response to curcumin supplementation was studied in the context of a clinical trial in MetS patients.

2. Subjects and methods

Blood samples of individuals with MetS were obtained from subjects who were randomised for a clinical trial study with registration number of IRCT2014052014521N3 that examined the efficacy of phytosomal curcumin in MetS patients. Volunteers referred to the Nutrition Clinic of Qaem hospital in Mashhad city between September to November 2015. A diagnosis of MetS was made using IDF criteria [1]. All participants gave their written informed consent to participate in this study, which received the approval of the Research Council of the Mashhad University of Medical Science. Patients with kidney disease, systemic lupus erythematosous (SLE), pregnant women and patients taking anti hyperlipidemic, or anti hyperglycaemic drugs during the previous 6 months were excluded from the study. The subjects were randomly allocated to three subgroups receiving either: 1-phytosomal Curcumin, 2-curcumin and 3-placebo. Then for this study, groups 1 and 3 were selected as cases (n = 81 of 120 subjects) of the randomised clinical trial study. The work has been carried out in accordance of the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans with IR.MUMS.REC.1394.209 reference number. In addition, 100 healthy individuals, sex and age matched with cases were selected as the control group to conduct this case control study. MetS patients were treated either with phytosomal curcumin (1mg per day; group1)/plain curcumin (1g per day-group2) or placebo (group3) for six weeks. Twenty ml of blood was taken before and after the intervention period. Anthropometric, demographic and biochemical parameters were recorded for each subject before and after the intervention period. The paraoxonase/arylestrase activity of paraoxonase 1 enzyme and levels of CETP protein before and after intervention were assessed in all MetS patients. The relationship between the *PON1 rs662, rs854560* and *rs705379* genotypes and serum enzymes paraoxonase/arylestrase activity, and the *CETP5882* genotypes with serum protein concentrations were determined before and after intervention.

2.1. DNA extraction

DNA was extracted using a DNA extraction kit (Pars tous, Iran) according to the manufacturer's instruction and the integrity of extracted DNA were examined by electrophoresis technique.

2.2. Genotyping

ARMS-PCR and PCR-RFLP were used to identify the various SNP genotypes, and sequencing was performed to confirm the validity of these techniques.

2.2.1. ARMS-PCR

ARMS-PCR with 2 different sets of primers was designed to determine genotypes of *PON1 rs662* and *CETP rs5882* in patients and controls. Three primers were used to determine the alleles of polymorphism and a pair of primers to amplify an internal control band. Primers to amplify *PON1 rs662* were 5′-ACTATTTTCTT GACCCCTACTTATG-3′ and 5′-CTATTTTCTTGACCCCTACTTTCA-3′ and 5′-AGTTCACATACTTGCCATCGG-3′ with an annealing temperature of 62^{0c} for 35 cycles yielding a 158 bp fragment for allele A and 159 bp for allele G. Primers to amplify *CETPrs5882* were 5′-GCAGAGCAGCTCCGAGTACG -3′, 5′-GCAGAGCAGCTCCGAGTACA -3′ and 5′-CCGCGGGGTGGCAAAGATAA-3′ with an annealing temperature of 63^{0c} for 35 cycles yielding a 311 bp fragment for both alleles A and G. We also designed a pair of primers to replicate a 408 bp of *HBB* gene as an internal control band to check the accuracy of PCR reactions. The primers were 5′-GAAGTCTGCCGTTACTGCCC-3′ and 5′-GATCCACGTGCAGCTTGTCA-3′. The PCR reaction was carried out for *PON1* rs662 in a total volume of 15 μl using Taq DNA Polymerase 2x Master Mix Red (Ampliqon) and 0.5 pmol/μl of allele specific primers and 0.3 pmol/μl of internal control primers and for *CETPrs5882* the total volume was 15 μl and the same PCR conditions as *PON1* rs662 but with a greater concentration of template DNA.

2.2.2. PCR-RFLP

The *PON1 rs854560* and rs705379 SNPs each was genotyped separately by PCR amplification and restriction digest. Primers