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Paraoxonase 1 genotype–phenotype correlation in patients with metabolic syndrome

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Abstract

The aim of the study was to investigate the influence of three single nucleotide polymorphisms (SNPs) (-108C>T, -162A>G and -909G>C) from the promoter region of paraoxonase 1 (PON1) gene on the enzyme activity, in patients with metabolic syndrome (MS). The study group consisted of 61 individuals with MS and the control group of 73 individuals without MS, matched for age and gender. For each individual, clinical and genetic parameters with possible influence on PON1 activities (paraoxonase, arylesterase and lactonase) were measured. PON1 genotyping was performed with PCR-RFLP, using specific primers and restriction enzymes. We found no differences for distribution of PON1 -108C>T, -162A>G and -909G>C polymorphisms, between the two groups (p-NS). The -108C>T and -909G>C polymorphisms were associated with paraoxonase (p=0.03, p=0.006, respectively), arylesterase (p<0.001, p<0.001, respectively) and lactonase (p<0.001, p<0.001, respectively) activities. The -162A>G polymorphism was not associated with paraoxonase (p-NS) or lactonase (p-NS) activities, but influenced the arylesterase activity (p=0.03). PON1 activities were influenced by all three polymorphisms, regardless of the presence of MS.

Keywords: paraoxonase, arylesterase, lactonase, activity, genotype, metabolic syndrome.

Introduction

Metabolic syndrome (MS) is a major health problem because of its increasing prevalence around the world [1].

Along with two other members of the paraoxonase (PON) gene family, paraoxonase 1 gene (PON1) is located on chromosome 7q21.3–22.1 and, in serum, the enzyme is associated with high density lipoproteins (HDLs) particles containing apolipoprotein A1 (apoA1) and apolipoprotein J (clusterin). The resulting PON1-HDL complex has the ability to protect low-density lipoproteins (LDLs) and HDLs against oxidation [2, 3].

In general population, there is a wide interindividual variation of PON1 concentrations and activities; diseases involving oxidative stress and lipid peroxidation have been associated with low levels of this enzyme [4].

The interindividual variability in serum PON1 activity is partly produced by certain PON1 gene polymorphisms [5]. Approximately 184 single nucleotide polymorphisms (SNPs) associated with PON1 gene have been described so far. Five of them, three in the promoter region (-909G>C or rs854572, -162A>G or rs705381 and -108C>T or rs705379) and two in the coding region (L55M or rs854560 and Q192R or rs662), are known to influence serum PON1 activity [3, 6–8]. Apparently, polymorphisms in the -909 and -162 positions do not alter gene expression but act as potential transcription factor binding loci; thus, polymorphism in the -108 position might induce a two-fold increase in gene expression. When taken together, they do not seem to have a cumulative effect [9].

Data show that SNPs in the regulatory region are in linkage disequilibrium and that the 55L allele might be in linkage disequilibrium with the -108C, -162A and -909G alleles [6].

Except for the -108 polymorphism, frequencies of PON1 alleles have different values depending on the ethnic group [6]. Distribution of the -108T allele for Caucasians in Europe is 0.46 [5].

The strongest effect on arylesterase (A-ase) activity seems to be triggered by the -108 polymorphism (the C allele) [3, 5], followed by -162 and -909 polymorphisms [6]. Controversial data has been reported regarding the rs854572 polymorphism, which, despite its very low effect on PON1 activity [3, 5, 6], is still in linkage disequilibrium with functional polymorphisms. The -108C and -162A polymorphisms, which are in linkage disequilibrium with each other, are two of the polymorphisms in the regulatory region which can independently increase PON1 expression, the -108C>T polymorphism explaining 22% of the variability in PON1 expression levels [3, 5, 6].

MS reported reduced antioxidant capacity of PON1 [3, 10–13] and low values of the HDL-PON1 complex [3]. However, although some studies have found a limited antioxidant status in obese patients with MS, they have failed to highlight changes in PON1 enzyme system [3, 14]

or to identify any combinations between this enzyme and plasma lipid levels [13, 15].

An accurate assessment of PON1 activity involves measuring all three PON1 activities: arylesterase, paraoxonase and lactonase, as recent studies have shown that PON1 is first of all a lactonase [16].

Moreover, recent studies have emphasized the importance of defining both PON1 activities and functional phenotypes in the assessment of the association between PON1 and MS, by using genotyping of several SNPs and enabling a more accurate estimation of possible associations [5].

The aim of this study was to investigate three SNPs in the regulatory region of PON1 gene, as well as other factors, in order to determine their effect on PON1 activities, in a group of subjects with MS *versus* non-MS subjects.

Materials and Methods

Study participants

The study included 61 patients admitted to the internal medicine ward, for multidisciplinary assessment, and diagnosed with MS. The control group consisted of 73 patients with different pathologies, but without MS, also admitted to the same hospital, in the same period of time. The groups were balanced in terms of age (MS patients 54.21±11.77 *vs.* controls 53.52±13.16, p=0.50) and gender (42.6% male MS patients *vs.* 31.5% male controls, p=0.2).

Each participant signed an informed consent before being included in the study. This, together with the research protocol, was consistent with the World Medical Association Declaration of Helsinki. The study has been approved by the University Ethics Committee.

The inclusion criterion in the study group was the presence of MS. According to the IDF consensus Worldwide definition of the MS, the criteria for the MS were represented by central obesity (defined by waist circumference with ethnicity specific values – for Europeans ≥94 cm in males and ≥80 cm in women), plus any two of the following factors: elevated triglycerides (TG) (≥150 mg/dL or specific treatment for this lipid abnormality), reduced HDL-cholesterol (<40 mg/dL in males, <50 mg/dL in females or specific treatment for this lipid abnormality), elevated blood pressure (BP) (systolic ≥130 or diastolic ≥85 mmHg or treatment of previously diagnosed hypertension) or elevated fasting plasma glucose (FPG) (≥100 mg/dL or previously diagnosed type 2 diabetes). However, since 2009, the world societies for the study of the cardiovascular diseases agreed that there should not be an obligatory component and three abnormal findings out of five (as stated previously by the IDF consensus) would qualify a person for the MS [17]. We applied the consensus from 2009, and patients who had any three abnormal findings out of five (central obesity, elevated triglycerides, reduced HDL-cholesterol, high BP or elevated FPG) were diagnosed with MS. The rest of the patients were included in the control group.

In order to rule out other causes that influence PON1 activity, all subjects with liver diseases (viral hepatitis – acute or chronic, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, alpha-1 antitrypsin deficiency, hemochromatosis, porphyria, Budd–Chiari syndrome, hepatic cirrhosis), as well as subjects who reported alcohol consumption of over 20 g/day in men and over 10 g/day in women in the past six months, have been excluded from the study. For the same reason, we excluded subjects suffering from kidney disease, infections, psychiatric disorders or various malignancies.

Anthropometric measurements and clinical examination

For each participant, we recorded general information: age, gender, area of residence. All patients resided in the same geographical region (Transylvania) and all were Romanians. Clinical and laboratory data have also been recorded for each subject. The existence of any associated diseases (hypertension and type 2 diabetes) has been assessed following a detailed and thorough history and general clinical examination.

Anthropological parameters (height, weight and waist circumference) have been measured. We performed three height measurements with a stadiometer, subjects being placed in an upright position, without wearing shoes. The arithmetic mean of these three measurements was recorded, using the metric system (m).

Waist circumference was also recorded in an upright position, using a flexible measure tape, midway between the iliac crest and the costal grid, at the end of a light exhale. There have been three measurements and the average value, expressed in centimeters (cm), was recorded.

Subjects were weighed dressed in lightweight clothes, without shoes, so that there could be a margin of error of up to ±50 g. Body mass index (BMI, kg/m^2) was calculated for each subject, according to the weight (kg)/height (m^2) ratio. BMI was considered normal for values between 18.5–25 kg/m². Subjects with BMI values between 25 and 30 kg/m^2 were classified as overweight. Subjects with grade 1 obesity had BMI values between 30.1 and 34.9 kg/m^2, those with grade 2 obesity had BMI values between 35 and 40 kg/m^2, and those with grade 3 obesity had BMI values over 40 kg/m^2.

Blood pressure was measured with the subject seated and after adequate rest time. Three measurements were made at 5-minute intervals, recording the highest value. Measurements have been performed by means of a sphygmomanometer and values expressed in mmHg.

Blood tests

Blood samples

Venous blood samples were collected for all subjects in the morning, after 12 hours of fasting, using clot-activator and EDTA vacutainers, which were placed at 4^{0}C after initial processing as stated by the manufacturer. PON1 activities and biochemical markers were assayed on serum samples obtained by centrifugation (3 minutes at 3000 rpm) and stored at -80^{0}C. For genotyping, the blood was processed for isolation and purification of genomic DNA, which was stored at -20^{0}C until analysis.

Blood tests included measurements of total cholesterol levels (normal value of 100–200 mg/dL), HDL-cholesterol levels (normal value of 40–80 mg/dL in men and 50–88 mg/dL in women), TG levels (normal value of 50–150 mg/dL in men and 45–150 mg/L in women) and blood glucose levels (normal values of 70–100 mg/dL).

LDL-cholesterol was calculated with the Friedewald equation: LDL-cholesterol [mg/dL] = Total cholesterol [mg/dL] - (TG [mg/dL]/5 + HDL-cholesterol [mg/dL]).

Serum PON1 activities

Paraoxonase (P-ase) and arylesterase (A-ase) activities of PON1 were measured spectrophotometrically at 25^0C, according to the protocol described by Eckerson *et al.* [18], with minor changes.

P-ase (expressed as U/L) was measured using paraoxon as a substrate (O,O-diethyl-O-*p*-nitrophenyl phosphate, Sigma, UK). Basic sample mixture included 1 mM paraoxon, 2 M NaCl and 1 mM $CaCl_2$ in 50 mM glycine–sodium hydroxide buffer (pH 10.5). The reaction was initiated by addition of serum sample and absorption was monitored at 405 nm for 90 seconds.

A-ase activity (expressed as kU/L) was measured using phenyl acetate as a substrate (Sigma, UK): 1 mM in 20 mM Tris-HCl (pH 8.0) containing 1 mM $CaCl_2$. Hydrolysis of phenyl acetate was determined by monitoring the increase in absorption at 270 nm over a period of 90 seconds. A blank sample containing incubation mixture without serum was run simultaneously to correct spontaneous decomposition of substrate, in both determinations.

All samples were run in duplicate and the mean value was used to calculate the activity by means of molar extinction coefficient of 18.290 $M^{-1}cm^{-1}$ at 412 nm for *p*-nitrophenol and 1310 $M^{-1}cm^{-1}$ at 270 nm for phenol.

Lactonase (L-ase) activity expressed as kU/L, was determined by a pH-sensitive colorimetric assay, with slight variations [19]. Briefly, 3 µL of serum were incubated with 1 mM *δ*-valerolactone in 1.2 mL Bicine buffer 2.5 mM (pH 8.3), containing 0.15 M NaCl, 1 mM $CaCl_2$ and 0.2 mM *m*-Cresol Purple. The bleaching rate, resulting from the carboxylic acid formation, was monitored spectrophotometrically at 577 nm, for one minute. A standard calibration curve was performed with 10 mM acetic acid, for the rate factor.

Genotyping

Genomic DNA was isolated from whole peripheral blood using the Wizard®Genomic DNA Purification kit and protocol (Promega, USA). Genotyping of DNA samples was performed with the polymerase chain reaction (PCR) technique, adapted from a previously published protocol [6] and used the following primers (Invitrogen, USA):

- FW 5'-AACATGTCACTGTGGCATATATAATG CTC-3' and RV 5'-TATTATAATATATTATATCATT CACAGTAACAGCAGACAGCAGAGAAAAGA-3' for -909G>C (annealing temperature of 60^0C for 60 seconds; 35 cycles);
- FW 5'-GCTATTCTTCAGCAGAGGGT-3' and RV 5'-TGAATCTGTAGCCAGGGCAC-3' for -162A>G (annealing temperature of 56^0C for 40 seconds; 30 cycles);
- FW 5'-GACCGCAAGCCACGCCTTCTGTGCAC C-3' and RV 5'-TATATTTAATTGCAGCCGCAGCC CTGCTGGGGCAGCGCCGATTGGCCCGCCGC-3' for -108C>T (annealing temperature of 63^0C for 60 seconds; 25 cycles).

PCR products were digested with Bsh1236I (BstUI) (for -162 and -108 positions) and BsmAI (Alw26I) (for -909 position) (10 U/µL, Thermo Scientific) at 37^0C for three hours and then migrated on 2% agarose (Top Vision™ LE GQ Agarose) gel. The resulting fragments were as follows: 256 bp for -909CC and 50 bp and 206 bp for -909GG; 1210 bp for -162AA and 674 bp and 536 bp for -162GG; 119 bp for -108TT and 52 bp and 67 bp for -108CC.

Statistical analysis was performed using the SPSS Software Version 21.

Nominal variables were described using frequencies. Continuous variables were tested for normality of distribution using Kolmogorov–Smirnov test and were described by mean and standard deviation, or by median and percentiles (25–75%), depending of the situation. A *Chi*-square test was used in order to compare the frequencies of nominal variables. Difference between groups regarding continuous variables were examined using *t*-test, Mann–Whitney test, ANOVA or Kruskal–Wallis when appropriate. The correlation between two continuous variables was assessed using Pearson's correlation or Spearman's *rho*, depending on the normality of the distribution. Deviations of allelic frequencies from Hardy–Weinberg equilibrium were calculated using a *chi*-square test.

Multivariate analysis was carried out using linear regressions. We used as dependent variable the PON1 activities: P-ase (*log*-transformed), A-ase and L-ase. We included as independent variables the parameters that achieved the criterion of significance at $p<0.2$ in univariate analysis. We used the Bonferroni's correction in order to account for multiple comparisons. The level of statistical significance was set at $p<0.05$.

Results

Clinical and demographic characteristics and laboratory tests in the two groups of patients are presented in Table 1.

Table 1 – ***Clinical and demographic characteristics and laboratory tests in the two groups of patients***

Feature		Patients without MS (*n*=73)	Patients with MS (*n*=61)	*P*
Age [years]*		53.52±13.16	54.21±11.77	NS
Gender	Men**	23 (31.5%)	26 (42.6%)	NS
	Women**	50 (68.5%)	35 (57.4%)	
DM or hyperglycemia		8 (11%)	39 (63.9%)	<0.001
Arterial hypertension		17 (23.3%)	47 (77%)	<0.001
BMI [kg/m^2]*		25.27±4.87	31.32±4.16	<0.001
Waist circumference for men [cm]***		89.7 (84; 104)	112 (104.5; 117.5)	<0.001
Waist circumference for women [cm]***		80.9 (76.5; 97.25)	109 (100; 118)	<0.001
High abdominal circumference		40 (54.8%)	58 (95.1%)	<0.001
Total cholesterol [mg/dL]*		213.96±44.64	212.77±54.74	NS
HDL-cholesterol [mg/dL]*		52.58±11.44	47.61±9.93	<0.001
Triglycerides [mg/dL]***		111 (87.5; 138.5)	174 (111; 217)	<0.001
LDL-cholesterol [mg/dL]*		136.84±39.09	130.64±53.16	NS
Blood glucose level [mg/dL]***		88 (83.5; 95)	103 (90; 110)	<0.001
Insulin levels [µU/mL]		3.3 (2; 6.39)	9.2 (4.27; 14.15)	<0.001
HOMA index (absolute value)		0.74 (0.45; 1.42)	2.15 (1.09; 4.81)	<0.001
HOMA index (>2)		8 (11%)	35 (57.4%)	<0.001

Feature	Patients without MS (*n*=73)	Patients with MS (*n*=61)	*P*
A-ase [kU/L]*	69.66±19.56	64.77±16.89	NS
L-ase [kU/L]*	55.35±15.21	58.19±16.1	NS
P-ase [U/L]***	330.5 (185.4; 477.47)	221 (157.75; 383.35)	0.06
-108CC** genotype	13 (17.8%)	7 (11.5%)	
-108CT** genotype	41 (56.2%)	37 (60.7%)	NS
-108TT** genotype	19 (26.0%)	17 (27.9%)	
-909CC** genotype	19 (26.0%)	18 (29.5%)	
-909GC** genotype	39 (53.4%)	31 (50.8%)	NS
-909GG** genotype	15 (20.5%)	12 (19.6%)	
-162AA** genotype	6 (8.2%)	3 (4.9%)	
-162AG** genotype	27 (37.0%)	21 (34.4%)	NS
-162GG** genotype	40 (54.8%)	37 (60.7%)	

*Mean±SD; **Number (%); ***Median (percentile 25%; 75%); A-ase: Arylesterase activity; L-ase: Lactonase activity; P-ase: Paraoxonase activity; MS: Metabolic syndrome; DM: Diabetes mellitus; BMI: Body mass index; SD: Standard deviation; NS: Non-significant.

We did not find a significant difference between the two groups regarding PON1 activities (Table 1). We determined the existence of an inverse correlation between age and P-ase levels (r=-0.177, p=0.04). We found no correlation between age and A-ase or L-ase levels (r=-0.051, p-NS, r=-0.053, p-NS). Patient gender had not a significant influence on P-ase, A-ase and L-ase activities (p-NS). We have established a correlation between HDL-cholesterol level and P-ase, A-ase and L-ase levels (r=0.194, p=0.02, r=0.304, p<0.001, r=0.254, p=0.002).

Patients with -108CC genotype [295.45 (215.12; 756.57) U/L] showed significantly greater P-ase levels compared to those with -108TT genotype [238 (112.72; 375.57) U/L] (p=0.01). -108CC patients showed significantly greater A-ase levels (82.58±16.06 kU/L) compared to those with CT (70.82±15.3 kU/L) or TT (51.85± 15.21 kU/L) genotypes (p=0.008, p<0.001 respectively). -108CT patients also showed significantly greater A-ase levels compared to those with TT genotype (p<0.001). -108CC patients showed significantly greater L-ase levels (69.78±12.76 kU/L) compared to those with CT (58.1± 13.13 kU/L) or TT (44.45±13.95 kU/L) genotype (p=0.004, p<0.001 respectively). -108CT patients showed significantly greater L-ase levels compared to those with the TT genotype (p<0.001).

The rs854572 polymorphism influenced all three PON1 activities. Thus, subjects with the -909GG genotype displayed higher P-ase levels [283.9 (214.6; 708.1) U/L] than those with the CC genotype [203.1 (114.45; 348.2) U/L] (p=0.003), and subjects with the GC genotype displayed higher P-ase levels [328.3 (186.2; 491.17) U/L] than those with the CC genotype (p=0.006). The -909CC patients displayed significantly lower A-ase levels (53.04± 18.7 kU/L) compared to those with GG genotype (79.25± 15.7 kU/L) (p<0.001) or with the CG genotype (70.56± 14.36 kU/L) (p<0.001). The -909GC patients also displayed significantly lower L-ase levels (58.75±11.75 kU/L) compared to those with GG genotype (68.86±14.15 kU/L) (p=0.04). Subjects with the CC genotype displayed lower (43.75±14.08 kU/L) L-ase levels than those with CG genotype (p<0.001) or with the GG genotype (p<0.001). CG heterozygotes showed lower L-ase levels than GG homozygotes (p=0.002).

-162AA patients showed significantly greater A-ase levels (80.96±22.66 kU/L) compared to those with GG genotype (64.73±19.16 kU/L) (p=0.03). The rs705381 polymorphism did not influence P-ase and L-ase levels (p-NS).

To determine the independent influence of various parameters on P-ase levels, we built a model using multiple linear regression (forward method). The following variables have been included: G-909C and C-108T polymorphisms, age, BMI and HDL-cholesterol. The chosen model explained 16.8% of the variability in P-ase activity. The -909CC genotype had the greatest influence on the variability in this activity (R^2=9.1%, p<0.001). The variables succeeded each other in the following order of importance: HDL-cholesterol (R^2=4.9%, p=0.008) and age (R^2=2.8%, p=0.04). When applying correction for multiple measurements, age did not retain its independent influence.

We designed a model, which would explain the variability in A-ase levels, by using multiple linear regression (forward method). We included the following variables: PON1 polymorphisms at positions G-909C, C-108T and A-162G, BMI and HDL-cholesterol. The chosen model has explained 38% of the variability in A-ase activity. The -108TT genotype had the greatest influence on the variability in this activity (R^2=26.6%, p<0.001). The variables succeeded each other in the following order of importance: HDL-cholesterol (R^2=7.6%, p<0.001) and the -108CC genotype (R^2=3.8%, p=0.006).

To determine the independent influence of various parameters on L-ase levels, we designed a model using multiple linear regression (forward method). We included the following variables: PON1 polymorphisms at positions G-909C and C-108T, gender, BMI and HDL-cholesterol. The chosen model explained 39.2% of the variability in L-ase activity. The -909CC genotype had the greatest influence on the variability in this activity (R^2=26.4%, p<0.001). The variables succeeded each other in the following order of importance: the -909CG genotype (R^2=6.1%, p=0.001), BMI values (R^2=4.9%, p=0.001) and HDL-cholesterol (R^2=3.6%, p=0.008).

Discussion

Showing low levels of A-ase, P-ase [20] and L-ase [21] activities and displaying some associations between MS with less active variants of the PON1 gene [3, 22], recent studies have suggested a possible involvement of PON1 in the pathogenesis of MS.

In a representative cohort of subjects with MS, this study aimed to investigate PON1 activities (A-ase, P-ase and L-ase) and the three major SNPs in the regulatory region of the PON1 gene (rs854572, rs705381 and rs705379), in comparison with a control group without MS.

For the first time in Romania, this research provides evidence of the association between the -108C>T and the -909G>C polymorphisms and all three activities of PON1, as well as between the -162A>G polymorphism and the A-ase activity, similar to other foreign studies [5, 7]. Even if there is no other data regarding the distribution of PON1 gene variants in this geographical region, the observed frequencies are consistent with those described for the European Caucasian population [5, 23].

Distribution of the polymorphisms in the two groups showed no statistically significant difference, the data obtained being consistent with other reports [6].

In this study, P-ase activity was not significantly lower in patients with MS (p=0.06) compared to subjects without MS. Although they exhibited lower values in the group of patients with MS, L-ase and A-ase activities have not reached the significance threshold. Probably, the low number of participants in this study did not allow obtaining a final conclusion in this regard. Similar results, which found no changes in these PON1 activities, have been reported [14] for P-ase and A-ase activities in a young population in Turkey with non-diabetic MS and obesity.

As reported by other studies [3, 7, 24], we found a negative influence of age on P-ase activity. However, when examining this activity according to genetic factors, BMI or HDL value, age had no significant influence. Therefore, changes in P-ase activity are most likely due to time progression of oxidative stress, because of age-related alteration of sulfhydryl groups. A-ase and L-ase activities showed no significant age-related changes, similar to other studies [3].

Gender had no significant influence on any of PON1 activities, as in other reports, most likely a consequence of genetic heterogeneity [18].

We observed that each of the three PON1 activities showed a weak direct association with HDL-cholesterol levels. These findings are in agreement with other studies, which demonstrated a weak association between PON1 and HDL in obese patients with MS [14]. It has been shown that PON1 is not distributed to all HDL particles, but only to a subset of HDL particles (*i.e.*, HDL3). These HDL3 particles have poorer lipid content [5] and contain apoJ [2, 15, 25]. The decrease in protein content of HDL structure, that will lead to changes in binding affinity and stability of PON1 [3], could be another explanation for the weak HDL-PON1 association. Obesity in MS may also induce changes in apoA1, due to high amounts of leptin, whose concentration increases gradually with fat mass. In turn, apoA1 is important, not only for PON1 transfer from hepatocytes to HDL, but also for PON1 activity [2, 5, 15, 25]. There are also changes in adipocyte secretion profile, which can turn them into real humoral factors secreting numerous biologically active peptides [15, 26]. The increase in inflammatory mediators that occurs because of this adipokine imbalance will ultimately determine the transformation of the HDL-PON1 complex from anti-atherogenic and anti-inflammatory into pro-atherogenic and proinflammatory [5].

We performed a multivariate analysis showing, similar to another study [6], that the -108TT genotype independently influenced the P-ase activity, being the major determinant of this activity, the CC genotype exhibiting the highest activity. The T variant at -108 position affects the sequence which recognizes the ubiquitous transcription factor (Sp1) and therefore, binding of this factor is weaker in the presence of T allele than in that of C allele. The data obtained is consistent with the findings of other studies [5–7].

Although data regarding the rs854572 polymorphism are controversial [5, 6], usually being considered to have a weak or no effect on PON1 activity, in this study the -909 polymorphism influenced all three PON1 activities, the GG genotype providing the highest antioxidant protection.

The rs705381 polymorphism only influenced A-ase activity, though in a lesser extent than the rs705379 polymorphism, providing low gene protection, probably due to its location in the consensus binding site for nuclear factor 1 (NF-1) [13]. The data is consistent with other previous reports [5, 6].

P-ase activity exhibited the highest levels for -108CC and -909GG genotypes and it has not been influenced by the A-162G polymorphism. Other studies have reported associations between the -162AA genotype and P-ase activity [6, 7]. The -108TT genotype had the greatest influence on this activity and the -108CT and -909GC heterozygotes and the -162GG homozygotes were the most frequent in the MS group. Since the -162AA genotype did not influence this activity in this current report, we may conclude that -108TT and the -909CC genotypes could be risk factors associated with low levels of P-ase activity of the PON1 gene.

A-ase activity presented the highest levels for -108CC, -909GG and -162AA genotypes, the latter being the only one affecting this activity in our study. The -108TT genotype had the greatest influence on this activity and the -108CT and -909CG heterozygotes and the -162AA homozygotes were the most representative in the group with MS. Under these circumstances, we might state that the -108TT, -909CC and 162GG genotypes are associated with low A-ase activities.

L-ase activity exhibited the highest levels for -108CC and -909GG genotypes, with the greatest influence coming from the -909CC genotype. Given that -162GG homozygotes and -108CT and -909GC heterozygotes were the most frequent in the MS group and that the -162AA genotype does not affect this activity, we might conclude that -108TT and -909CC genotypes are associated with low L-ase activities.

At the same time, several reports have suggested that serum A-ase level is an indicator of the expression of the PON1 gene. Given that all three polymorphisms in the regulatory region influence this activity, but their individual cumulative effect is not a strictly additive one, but may depend on the context, genotyping for -108, -909 and -162 positions could be one of the screening tests for the assessment of PON1 activity and indirectly, for the oxidative state, the presence of these polymorphisms being able to modulate the severity of oxidative stress.

The limitations of this study would be the following: it is a cross-sectional study, with everything that this type of study implies, and much more information could be obtained by tracking the dynamics of PON1 status.

In other order of ideas, we did not analyze the possible environmental influences on the genetic spectrum, typical for this geographical area (smoking, diet, physical activity and so on).

We did not study the cumulative effect of these three polymorphisms and we did not analyze the linkage disequilibrium between the three polymorphisms studied and the polymorphisms in the coding region. The study also lacks an analysis of the phase polymorphism or of the influence of environmental factors on PON1 activity. Further studies are required so as to quantify this data in order to obtain a more accurate characterization of PON1.

Conclusions

We showed that PON1 activities were influenced by all three polymorphisms, regardless of the presence of MS. Also, the HDL-cholesterol levels were correlated with all PON1 activities. A future analysis of other PON1 gene polymorphisms also seems of great importance, alongside with a functional genomics analysis, in order to accurately characterize the molecular mechanisms for the regulation of PON1, as well as the functions and the importance of this enzyme.

Conflict of interests

The authors declare that they have no conflict of interests.

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