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Abstract: Background. Oxidative stress is associated with HIV-infection. Paraoxonase-1 (PON1) is an antioxidant enzyme bound to high-density lipoproteins (HDL). We evaluated whether PON1 gene haplotypes influence the metabolic disturbances, presence of sub-clinical atherosclerosis, and virologic outcome of the infection.
Methods. DNA from blood samples of HIV-infected patients (n=234) and healthy control subjects (n=633) had single nucleotide polymorphisms of PON1192, PON155, PON1-162, PON1-832, PON1-909, PON1 -1076, and PON1-1741 analyzed using the Iplex Gold MassArrayTM method and, subsequently, their influence on measured biochemical and clinical variables was assessed.
Results. We observed significant differences in the haplotype distribution between the control group and the HIV-infected patients. Haplotype H10 (GTCCGTC) was more prevalent in the patients (6.41% vs. 0.64%; P = 2.502 x 10-6) and the haplotype H5 (GACCGTC) was less prevalent in the patients (27.7% vs. 42.9%; P = .001). In HIV-patients, haplotype H7 (AATTCCT) was associated with a better CD4+ cell count recovery, higher levels of HDL-cholesterol (P = .048) and apolipoprotein A-I (P = .019), lower levels of triglycerides (P = .004) and lower rates of sub-clinical arteriosclerosis (P < .001).
Conclusions. PON1 haplotypes segregate with HIV infection, HDL metabolism, the presence of sub-clinical atherosclerosis, and CD4+ cell recovery following treatment.

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Paraoxonase-1 gene haplotypes are related to metabolic disturbances, atherosclerosis and immunologic outcome in HIV-infected patients

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ABSTRACT

Background. Oxidative stress is associated with HIV-infection. Paraoxonase-1 (PON1) is an antioxidant enzyme bound to high-density lipoproteins (HDL). We evaluated whether *PON1* gene haplotypes influence the metabolic disturbances, presence of sub-clinical atherosclerosis, and virologic outcome of the infection.

Methods. DNA from blood samples of HIV-infected patients (n=234) and healthy control subjects (n=633) had single nucleotide polymorphisms of $PON1_{192}$, $PON1_{55}$, $PON1_{-162}$, $PON1_{-832}$, $PON1_{-909}$, $PON1_{-1076}$, and $PON1_{-1741}$ analyzed using the Iplex Gold MassArray™ method and, subsequently, their influence on measured biochemical and clinical variables was assessed.

Results. We observed significant differences in the haplotype distribution between the control group and the HIV-infected patients. Haplotype H10 (GTCCGTC) was more prevalent in the patients (6.41% *vs.* 0.64%; $P = 2.502 \times 10^{-6}$) and the haplotype H5 (GACCGTC) was less prevalent in the patients (27.7% *vs.* 42.9%; $P = .001$). In HIV-patients, haplotype H7 (AATTCCT) was associated with a better CD4+ cell count recovery, higher levels of HDL-cholesterol ($P = .048$) and apolipoprotein A-I ($P = .019$), lower levels of triglycerides ($P = .004$) and lower rates of sub-clinical arteriosclerosis ($P < .001$).

Conclusions. *PON1* haplotypes segregate with HIV infection, HDL metabolism, the presence of sub-clinical atherosclerosis, and CD4+ cell recovery following treatment.

Keywords: HDL-cholesterol; HIV; Oxidative stress; Paraoxonase-1; Sub-clinical atherosclerosis

The spread of HIV-1 infection continues to increase, while the survival of these patients is considerably extended by new, and more effective, anti-retroviral therapy. Hence, it is likely that long-term consequences of treatment and infection will become increasingly common. These consequences involve not only the immuno-compromised status of the patient but also metabolic derangements, including lipoprotein disorders that may lead to cardiovascular disease [1]. One of these problems is an increased oxidative stress due to the infection itself, or to the secondary effects of treatments [2]. Paraoxonase-1 (PON1) is an enzyme with antioxidant properties. PON1 is an esterase and lactonase that catalyzes the hydrolysis of oxidized phospholipids and lipophylic lactones [3]. In the general population and in diabetic patients, PON1 preserves high-density lipoproteins (HDL) and low-density lipoproteins (LDL) from peroxidation and, as such, has been associated with a protective role against the development of atherosclerosis [4-8]. *PON1* knockout mice have increased macrophage oxidative stress and are more susceptible to atherosclerosis [9], and human *PON1* transgenic mice show a decrease in atherosclerosis formation and lipoprotein oxidation [10]. PON1 attenuates *in vitro* the production of monocyte chemoattractant protein-1 (MCP-1) by monocytes. MCP-1 is a pro-inflammatory chemokine involved in the initial step in the formation of the atheromatous plaque [11]. Previous studies from our group showed an increased plasma MCP-1 concentration in HIV-infected patients, and that the polymorphisms in the *MCP-1*$_{-2518}$ allele are associated with the presence of sub-clinical atherosclerosis [12]. We have also reported previously that PON1 status is influenced by the course of HIV infection, and results in a decrease in PON1 activity [13].

The hypothesis of the present study is that the relationship between oxidative stress, HIV infection and atherosclerosis is such that *PON1* gene polymorphisms could

be related to the metabolic disturbances associated with the infection, as well as to the immunological, virological and clinical course of this disease.

METHODS

Study population and design. In an initial study, we performed case-control comparisons to assess the differences in genotype distributions of the PON1 genes in HIV-infected patients compared to that in the general population. The participants used as the control group participated in a population-based study conducted in our area. Details of this study have been published previously [14]. Briefly, the participants were ostensibly healthy individuals (n = 633; 339 women, 294 men, mean age: 45 years, range 18 to 81) of Caucasian ethnic origin from the Mediterranean region of Catalunya. The HIV-infected patients studied (n=234; 72 women, 162 men, mean age: 39 years, range 20 to 66) were from among those attending our outpatient AIDS clinic, and were of the same ethnic origin as that of the control participants in the study. The only exclusion criterion was an age <18 years.

Following on from the initial study, we performed a case-control assessment with the HIV-infected patients receiving antiretroviral treatment. The purpose was to evaluate the associations of *PON1* gene haplotypes in relation to the metabolic, immunological and virological variables measured. We defined as 'cases', in this second study, those HIV patients with lipodystrophy, metabolic syndrome, dyslipidemia (according to the National Cholesterol Education Program Adult Treatment Panel III: total cholesterol >5.0 mmol/L or LDL-cholesterol >3.0 mmol/L, HDL-cholesterol <1.0 mmol/L in men and <1.2 in women or triglycerides >1.7mmol/L), a positive cardiovascular disease risk assessed with the Framingham risk score, and the presence of atherosclerosis measured as the intima-media thickness (IMT) in the carotid artery.

Although IMT is a continuous variable, and we have reported the data in this form, we also defined sub-clinical atherosclerosis as a categorical (dichotomized) variable of an IMT >0.8 mm, or the presence of an atheromatous plaque in the analyzed zones of the arteries.

We used previously-established definitions of case/control in relation to the magnitude of the CD4+ cell increase; i.e. we re-assigned the HIV-patients as "case" or "control" on the basis of response or non-response, respectively, to anti-HIV treatment. Patients who did not have an increase >50cells/mm^3 in the CD4+ cell count after 12 months of treatment follow-up were considered as 'non-responders' [15]. In terms of virological variables, we consider as 'cases' those patients who had a rebound in the viral load during the course of the 12-month observational period of the study. Patients who abandoned the treatment and those who completed the 12-month observational period with negative viral load were censored from the present statistical analyses.

All the participants provided fully informed consent. The data were coded to ensure anonymity. The study was approved by the Ethics Committee of the *Hospital Universitari de Sant Joan de Reus*. All procedures were according to Declaration of Helsinki principles and of Good Clinical Practice.

Biochemical measurements. A sample of fasting venous blood was obtained during the clinical examination. Plasma viral load was measured with the Cobas® TaqMan HIV-1 assay (Roche, Basel, Switzerland) and CD4+ T-cells were determined by flow cytometry (Beckman-Coulter, Fullerton, CA, USA). HDL-cholesterol was analyzed by a homogeneous method (Beckman-Coulter). LDL-cholesterol was calculated using the Friedewald formula [16]. Serum total cholesterol and triglycerides concentrations were measured by standard methods (Beckman-Coulter). Serum PON1 esterase activity was

measured as the rate of hydrolysis of paraoxon at 410 nm and 37ºC in 0.05mmol/L glycine buffer (pH=10.5) with 1 mmol/L $CaCl_2$ [17]. Activities were expressed as U/L (1 U = 1μmol of paraoxon hydrolyzed per minute). Serum PON1 lactonase activity was measured in an assay reagent containing 1 mmol/L $CaCl_2$, 0.25 mmol/L 5-(thiobutyl)-butyrolactone (TBBL) and 0.5 mmol/L 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) in 0.05 mmol/L Tris-HCL buffer (pH=8.0). The increase in absorbance was monitored at 412 nm [18,19]. Activities were expressed as U/L (1 U = 1mmol of TBBL hydrolyzed per minute). Serum PON1 concentration was determined by enzyme linked immunosorbent assay (ELISA) using an antibody raised against a peptide derived from the sequence of mature PON1 [6]. Serum concentration of oxidized LDL (ox-LDL) was measured by ELISA (Mercodia, Uppsala, Sweden). Serum apolipoprotein (apo) A-I and A-II concentrations were determined by immunoturbidimetry (Beckman-Coulter and Dialab Gmbh, Vienna, Austria, respectively). The serum concentration of C-reactive protein (CRP) was measured using a high sensitivity method (Beckman-Coulter). The plasma concentration of MCP-1 was measured by ELISA (Human MCP-1 ELISA Development Kit, Prepotech, London, UK).

Genotyping. Genomic DNA was obtained from leukocytes (Puregene DNA Isolation reagent set, Gentra Systems Inc., Minneapolis, MN). *PON1*$_{192}$, *PON1*$_{55}$, *PON1*$_{-162}$, *PON1*$_{-832}$, *PON1*$_{-909}$, *PON1* $_{-1076}$, *PON1*$_{-1741}$, and *MCP-1*$_{-2518}$ single nucleotide polymorphisms (SNP) were analyzed by the Iplex Gold MassArray™ method (Sequenom Inc., San Diego, CA) at the Spanish National Genotyping Center (*Centro Nacional de Genotipado*, of the *Universitat Pompeu Fabra*, Barcelona, Spain).

Arterial IMT measurement. We performed carotid and femoral ultrasound measurements in 183 HIV-infected patients. These measurements were conducted using the identical protocol by the same investigators [12] who were blinded with respect to the results of the other variables studied. We used a GE Logiq 700 with an ultrasound probe of 7-10 MHz. We identified 3 segments in the carotid arteries on which to conduct the measurements: the common carotid artery (1cm proximal to the bifurcation), the carotid bulb (in the bifurcation), and the internal carotid artery (1cm distal to the bifurcation). We evaluated the common femoral artery 1cm proximal to the bifurcation. The far wall IMT images were obtained and digitalized for each participant.

Statistical analysis. We used the Chi-square test to assess the degree of association between categorical variables. The ANOVA test or the Student t-student test was employed for the continuous variables that followed a normal distribution. The Mann-Whitney U and Wilcoxon rank sum tests were employed for non-parametric variables. Results are presented as means and standard deviation in parenthesis for parametric variables, and median and range in parenthesis for non-parametric variables. Single nucleotide polymorphisms (SNP) were tested for Hardy-Weinberg equilibrium using Haploview 4.0 software [20]. Estimates of linkage disequilibrium between SNP were calculated using D' and r^2. Haplotype estimations were performed with the PHASE software [21] with default settings and the SNPator package [22]. Linear or logistic regression models were used to identify the haplotypes that predicted the dependent variables adjusted for potential confounding factors such as age, gender, dyslipidemia, smoking habit, hypertension, fasting glucose, body mass index, hepatitis C virus co-infection, lipid-lowering treatment, duration of each antiretroviral treatment scheme, and basal CD4+ cell count. The Kaplan-Meyer hazard model was used to determine the

association between the haplotypes and the time to undetectable viral load at a 95% confidence interval (CI). All statistical analyses were performed with the SPSS 15.0 statistical package.

RESULTS

Genotype distributions in the control subjects and in HIV-infected patients. The general characteristics of the patients are summarized in table 1. We obtained 25 different haplotypes corresponding to the SNP mutations $PON1_{192}$, $PON1_{55}$, $PON1_{-162}$, $PON1_{-832}$, $PON1_{-909}$, $PON1_{-1076}$, and $PON1_{-1741}$ (table 2). We observed a strong linkage disequilibrium between all the SNP in HIV-infected patients (D' = 43) except for the SNP between the $PON1_{192}$ and $PON1_{55}$ polymorphisms (figure 1). We observed significant differences in the distributions of haplotype H10 (OR: 10.6; 95%CI: 3.52-32.2, $P = 2.502 \times 10^{-6}$) and haplotype H5 (OR: 0.61; 95%CI: 0.46-0.83, $P = .001$) between control subjects and HIV-infected patients (figure 2A).

Relationship between PON1 haplotypes and the immunological and virological outcomes in HIV-infected patients. In a bivariate analysis, we found significant differences in the CD4+ cell counts in HIV-infected patients segregated according to haplotypes. Patients carrying the H7 haplotype presented higher levels of basal CD4+ cells (figure 2B and table 3). Segregated according to CD4+ cell count, we also observed that patients carrying the H7 haplotype had a greater proportion of 'responders' than patients who did not carry this haplotype (72.9% *vs.* 52.7%; $P = .017$).

The probability of maintaining an undetectable viral load while receiving antiretroviral treatment was also significantly related to *PON1* gene haplotypes.

Carrying haplotypes H10 and H5 was associated with a higher probability of maintaining viral suppression (P = .047 and P = .022, respectively).

Relationship between PON1 haplotypes and the presence of lipodystrophy, metabolic syndrome, cardiovascular disease risk and dyslipidemia. We did not observe (using bivariate analysis) any association between *PON1* haplotypes and the presence of lipodystrophy, metabolic syndrome or the 10-year risk of cardiovascular disease as assessed by the Framingham risk score. However, we did find an association between dyslipidemia and the H7 and the H4 haplotypes (P = .025 and P = .006, respectively). Patients carrying the H7 haplotype had lower serum triglyceride concentrations, higher levels of HDL-cholesterol, and higher apo A-I and apo A-II concentrations (table 3).

HIV patients carrying the H4 haplotype also showed lower serum triglyceride concentrations [mean (SD)] [2.16 (2.05) mmol/L *vs.* 3.09 (2.35) mmol/L; P = .042] and higher levels of HDL-cholesterol [1.23 (0.53) mmol/L *vs.* 0.97 (0.25) mmol/L; P = .012].

Levels of HDL-cholesterol (as dependent variable) were predicted in a linear logistic regression model by triglycerides ($\beta = -.075$; 95%CI: −.107 to −.044; P <.001), and the time under the non-nucleoside antiretroviral treatment scheme (β = .009; 95%CI: .004 to .015; P <.001).

Serum apo A-I concentrations were predicted in a linear regression model by the H7 haplotype, (β = .176; 95%CI: .070 to .283; P = .008), the time under antiretroviral treatment (β = .008; 95%CI: .003 to .0012; P < .001) and the hepatitis C virus co-infection ($\beta = -.106$; 95%CI: –.119 to .012; P = .028).

Relationship between PON1 haplotypes and the presence of sub-clinical atherosclerosis in HIV-infected patients. We found an association between the presence of sub-clinical atherosclerosis and the H7 haplotype (figure 2C). Patients who did not carry this haplotype had higher rates of sub-clinical atherosclerosis (83.3% *vs.* 16.7%; *P* <.001). In the logistic regression model, the variables that predicted the presence of atherosclerosis were: age (β = 1.261; 95%CI: 1.136 to 1.401; *P* <.001), the *MCP-1 -2518G* allele (β = .162; 95%CI: .056 to .470; *P* = .001) and the H7 haplotype (β = .381; 95%CI: .151 to .961; *P* = .041).

The absence of the H7 haplotype was also associated with an increased IMT as a continuous variable 0.78 (0.19) *vs*. 0.70 (0.16) mm in patients carrying the H7 haplotype (*P* = .010). In the linear regression model, the variables that predicted the IMT values in the carotid arteries were: age (β = .010; 95%CI: .006 to .014; *P* <.001), the time under antiretroviral treatment (β = .004; 95%CI: .001 to .007; *P* = .005), and the H7 haplotype (β = –.075; 95%CI: –1.141 to .010; *P* = .026).

We did not observe any significant association between serum PON1 activity or concentration and the immunological, virological, metabolic or clinical variables analyzed.

DISCUSSION

Results from the present study show significant differences between the control group and the HIV-infected patients with respect to the genetic distribution of two *PON1* gene haplotypes. The H10 haplotype is more prevalent in HIV-infected patients than in control group, albeit the small number of participants carrying this haplotype makes this conclusion very preliminary. The absence of H10 haplotype is also associated with a higher probability of maintaining a negative viral load. As such, we can suggest that this

haplotype is more prevalent in HIV-patients due to it being associated with an increased patient survival. The H5 haplotype is significantly less prevalent in HIV-infected patients than in the control group, and its absence is also associated with a higher probability of maintaining viral suppression. Perhaps patients with this genotypic background show less capability of controlling viral replication when receiving antiretroviral treatment, and may be less protected in the first stages of the HIV transmission.

The strong linkage disequilibrium observed between the SNPS of *PON1* also allowed us to detect candidate SNP with functional and clinical implications that warrant further investigation. $PON1_{192}$ codes for isoenzymes that show different functional antioxidant activity, as has been reported previously [5,23]. The H10 and the H5 haplotype contain the R allele at position 192, and which is the allele that is less effective in the protection against lipid peroxidation [7]. The H7 haplotype appears to have an important role with respect to the lipid profile, the presence of atherosclerosis and the levels of CD4+ cells. We observed that patients carrying this haplotype had higher levels of HDL-cholesterol and apo A-I, as well as lower triglyceride concentrations. The H7 haplotype was also associated with lower rates of sub-clinical atherosclerosis. In the multivariate regression model, not carrying the haplotype predicted the presence of atherosclerosis, after adjustment for other classical cardiovascular disease risk factors. Also in the regression model, the adjusted levels of apo A-I, but not the triglyceride levels, were predicted by the H7 haplotype. That apo A-I and HDL levels are influenced by the *PON1* polymorphisms can be hypothesized as an effect related to conformational changes in the PON1 isoform which confers a more stable binding to apo A-I [24]. The H7 carries the Q allele at $PON1_{192}$ position, and this

allele is known to confer better antioxidant properties and better protection against the development of atherosclerosis [5].

Of note is that the H7 haplotype was also associated with higher levels of basal CD4+ cells and better CD4+ recovery with treatment. Basal level of CD4+ cells is an independent risk factor for atherosclerosis in HIV-infected patients [25,26]. As such, it is possible that the *PON1* genotypic background modulates the lipid profile and also confers a better oxidative status which, in turn, lowers CD4+ cell apoptosis and, consequently, lowers the predisposition to atherosclerosis related to the pro-inflammatory and pro-oxidative status of this patient population.

A caveat on the present study is that, despite *PON1* gene haplotypes being clearly associated with severe complications of HIV infection, we did not observe any significant association between these derangements and PON1 circulating levels, either with respect to the activity or to the concentration of the enzyme. The explanation for this phenomenon cannot be ascertained from the present study, but a possible underlying mechanism could be related to the observation commented-upon above i.e. that $PON1_{192}$ polymorphism influences PON1 binding to apo A-I and HDL causing differences in enzyme stability and modifying HDL function independently of PON1 levels [24]. Our results showing that patients carrying H7 haplotype (which contains $PON1_{192}$ polymorphism) have higher HDL-cholesterol and apo A-I concentrations would tend to support this hypothesis. Another limitation of the present study is its cross-sectional design which makes any conclusions about the relationships to disease progression and pathogenesis very preliminary. Prospective, longitudinal studies, investigating the influence of *PON1* haplotypes on seroconversion and patient survival would be necessary to describe more clearly the role of these haplotypes in disease progression.

In conclusion, *PON1* haplotypes are related to HDL and apo A1 levels, the presence of atherosclerosis, the immunological status, and the virological outcome following treatment. The clinical implication is that novel therapeutic strategies may need to take into account the *PON1* genotypic background of the patient especially if the therapeutic option is to modulate serum PON1 activity and HDL or apoA-I levels [27,28]. Our results suggest that the PON1 - HDL complex may play a role in the homeostasis of atherosclerosis and HIV infection i.e. the HDL part of the complex is involved in the reverse cholesterol transport and catabolism while the PON1 part modulates inflammation, oxidative status and the immunological system. This concept warrants further research since it is an aspect that may provide beneficial consequences for these patients for whom, currently, effective treatment options with less toxic effects are not very extensive.

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Table 1. General characteristics of the HIV-infected patients

Characteristics	Value
Age, years (n = 234)	38.7 (6.8)
Gender; % Male (n = 234)	162 (69.3)
Conventional cardiovascular disease risk factors (n = 234)	
Current smoker	184 (78.6)
Hypertension	21 (8.9)
Abnormal fasting glucose	20 (8.5)
BMI (Kg/m^2)	23.1 (3.2)
Dyslipidemia	85 (36.3)
Risk factors for HIV infection (n = 234)	
Intravenous drug use	133 (56.8)
Male homosexual contact	29 (12.3)
Heterosexual contact	68 (29.1)
Years since HIV diagnosis (n = 234)	5.4 (3.3)
Baseline CD4+ count; U/mm^3 (n = 185)	359.9 (297.3)
Viral load <200 copies/mL; % (n = 234)	92 (39.3)
AIDS-related disease; % (n = 234)	77 (32.9)
Hepatitis C virus co-infection; % (n = 234)	142 (60.6)
Lipodystrophy; % (n = 234)	51 (21.7)
Previous antiretroviral therapy; months (n = 234)	
Nucleoside analogues	103.3 (62.7)
Protease inhibitor	30.2 (27.3)
Non-nucleoside analogues	8.1 (10.4)
Treated with statins; % (n = 234)	6 (2.6)
Treated with fibrates; % (n = 234)	20 (8.5)
Lipid Profile; mmol/L	
Cholesterol (n = 186)	4.9 (1.3)
HDL-cholesterol (n = 202)	1.2 (.5)
LDL-cholesterol (n = 171)	2.8 (1.0)
Triglycerides (n = 184)	2.3 (2.1)
Apolipoprotein A-I; g/L (n = 146)	1.4 (.3)
Apolipoprotein A-II; g/L (n = 196)	.33 (.06)

Quantitative variables are reported as means and SD (in parentheses). Qualitative variables are reported as n and % (in parentheses).

Table 2. Differences between the haplotypes distribution in the control group and the HIV-infected patients

	Haplotype	Control group n (%)	HIV-infected patients n (%)	*P*
H1*	AACCGTC	111(17.5%)	55(23.5%)	.054
H2*	ATCCGTC	563(88.9%)	199(85.04%)	.760
H3*	GACTCTT	57(9.0%)	16(6.8%)	.419
H4*	AACCCTT	96(15.1%)	41(17.5%)	.368
H5*	GACCGTC	272(42.9%)	65(27.7%)	.001
H6	ATTTCCT	11(1.7%)	3(1.3%)	1.000
H7*	AATTCCT	240(37.9%)	81(34.61%)	.631
H8*	GACCCTT	80(12.6%)	31(13.2%)	.740
H9	ATCCGTT	1(.15%)	0(0)	1.000
H10*	GTCCGTC	4(.63%)	15(6.41%)	2.502×10^{-6}
H11*	AATTCCC	47(7.42%)	15(6.41%)	.771
H12*	GATTCCT	48(7.58%)	21(8.97%)	.488
H13	GACCTGG	4(.63%)	0(0)	.578
H14	ATCCCTT	32(5.05%)	8(3.41%)	.469
H15	GTTCCTT	1(.15%)	0(0%)	1.000
H16	AACTCTT	9(1.42%)	9(3.84%)	.031
H17	GTCCCTT	12(1.89%)	9(3.84%)	.131
H18	AACCCTC	4(.63%)	3(1.28%)	.390
H19	AACTCCT	1(.15%)	0(0%)	1.000
H20	ATTCCTT	1(.15%)	0(%)	1.000
H21	ATTTCCC	1(.15%)	0(%)	1.000
H22	GTTTCCT	1(.15%)	0(%)	.265
H23	GATCCTT	1(.15%)	0(%)	.265
H24	AATCCTT	1(.15%)	0(%)	.265
H25	GACCCCT	1(.15%)	0(%)	.265

Haplotypes with a frequency > 5% in HIV-infected patients are highlighted with an asterisk

Table 3. Differences in the lipid profile, inflammatory and oxidative markers, PON1 levels and CD4+ cell count in HIV-infected patients segregated with respect to presence of the H7 haplotype

	H7 haplotype carriers	n	Average *	*P*
Cholesterol; mmol/L	Yes	49	5.05 (1.45)	.260
	No	137	4.89 (.94)	.260
HDL-cholesterol; mmol/L	Yes	56	1.28 (.45)	.048
	No	146	1.14 (.44)	
LDL-cholesterol; mmol/L	Yes	47	2.81 (.79)	.832
	No	124	2.77 (1.07)	
Apolipoprotein A-I; g/L	Yes	53	1.49 (.28)	.019
	No	93	1.35 (7.0)	
Apolipoprotein A-II; g/L	Yes	51	.35 (.07)	.030
	No	145	.32 (.06)	
Triglycerides; mmol/L	Yes	49	1.36 (.6-5.6)	< .001
	No	135	2.77 (1.07)	
PON1 esterase activity; U/L	Yes	57	258.6 (115.1-667.0)	.090
	No	153	306.8 (75.9-818.1)	
PON1 lactonase activity; U/L	Yes	56	5.10 (1.58)	.702
	No	158	5.19 (1.69)	
PON1 concentration; U/L	Yes	56	126.1 (107.6)	.509
	No	151	140.9 (166.1)	
Ox-LDL; U/L	Yes	57	82.14 (38.3)	.329
	No	158	87.4 (33.01)	
CRP; mg/L	Yes	57	4.48 (7.97)	.528
	No	152	3.90 (4.07)	
MCP-1; ng/L	Yes	47	73.65 (41.65)	.718
	No	125	76.48 (47.20)	
Basal CD4+; cells/mm^3	Yes	49	425.9 (320.9)	.035
	No	136	318.1 (282.5)	
Age; years	Yes	49	37.2 (7.1)	.056
	No	136	39.7 (7.3)	

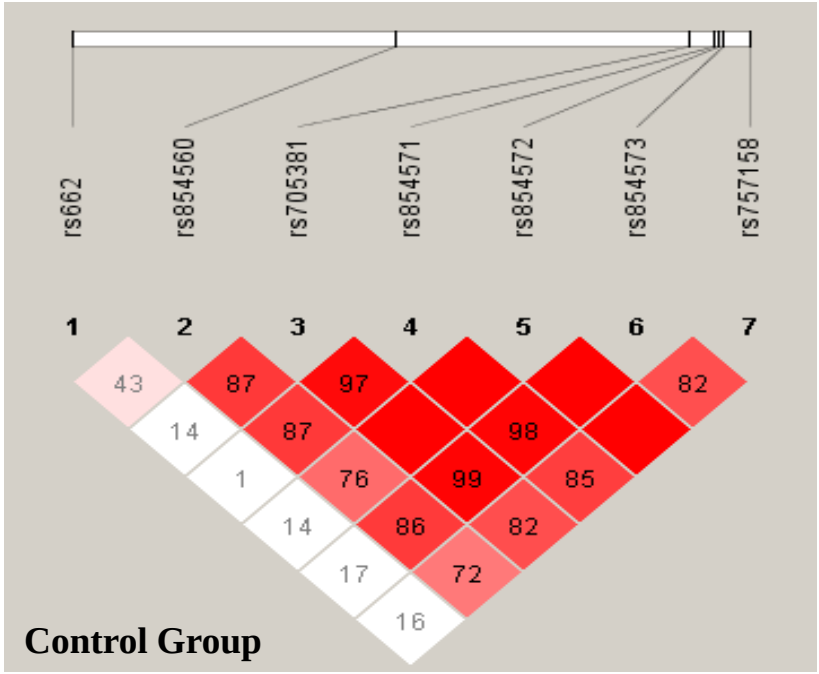
* Results are shown as means (SD) for variables with parametric distributions, and as medians (range) for variables with non-parametric distributions.

FIGURE LEGENDS

Figure 1. Linkage disequilibrium structure of *PON1* gene in the control population (A) and in the HIV-infected patients (B). Linkage disequilibrium structure for 7 common single nucleotide polymorphisms labeled by their RS numbers vertically. Note that SNP number 1 is $PON1_{192}$, followed by $PON1_{55}$, $PON1_{-162}$, $PON1_{-832}$, $PON1_{-909}$, $PON1_{-1076}$ and $PON1_{-1741}$. Each square denotes strength and significance of linkage disequilibrium between pairs of markers in the region. Red indicates no (or minimal) evidence of historical recombination. Numbers of squares indicates 100XD' (a statistical measure of linkage disequilibrium) with missing values indicating result of 100.

Figure 2. (A) Differences between the haplotype distributions in control subjects and HIV-infected patients. (B) Differences in the proportion of CD4+ responder HIV patients in relation to the presence of absence of haplotype H7. (C) Differences in the proportion of HIV-infected patients with sub-clinical atherosclerosis in relation to the presence of absence of haplotype H7.

Figure 1.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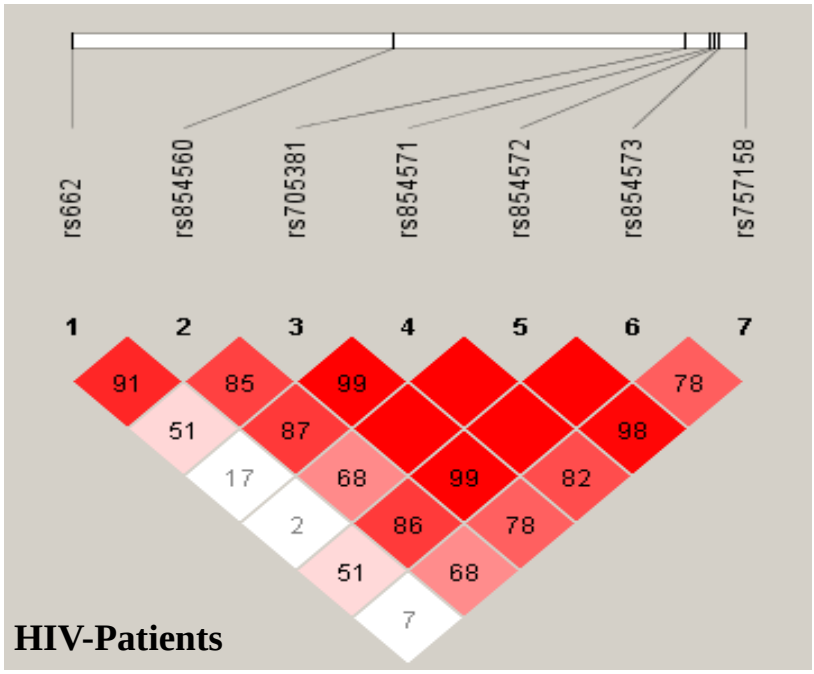
43
87 97
14 87 98
1 76 99 85
14 86 82
17 72
16
Control Group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1 2 3 4 5 6 7
91
85 99 78
51 87 98
17 68 99 82
2 86 78
51 68
7
HIV-Patients

Figure 2

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