Enzymatic and genetic polymorphisms of paraoxonase-1 in the Gabonese population: the relation to lipid parameters in patients with diabetes

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Abstract

Background: The objective was to study the relationship between the paraoxonase-1 activity, genetic polymorphisms and lipid parameters in a black patient population.

Method: This study investigates patients with type 2 diabetes and hypertension for which concentrations of total high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, apolipoprotein AI, apolipoprotein B100, the latency time of oxidation of small and dense LDL, arylesterase activity and genetic polymorphism of paraoxonase-1 at positions T(-107)C, L55M and Q192R were determined and compared to healthy subjects.

Results: Concerning the T(-107C) polymorphism, the -107T allele is higher in healthy subjects (0.325) than in those with diabetes (0.660). This was the same for the 55M allele, whereas the -107C allele was lower in healthy patients (0.675) than in patients with diabetes (0.340). Paraoxonase-1 activity was lower in patients with diabetes than in healthy subjects, irrespective of genotype. The -107CC genotypes had higher HDL cholesterol AI apolipoprotein I concentrations than -107TT in the control group (p-value = 0.0001), patients with diabetes (p-value = 0.002), and patients with hyptertension (p-value = 0.001). This result was also obtained with the 55LL genotype.

Conclusion: The prevalence of paraoxonase-1 alleles associated with lesser enzyme activities was found to be more common in patients with diabetes than in the control group. Possibly, this genetic distribution contributes to the high cardiovascular risk that is observed in certain black patients, compared to that in white patients.

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Introduction

Cardiovascular disease is a major cause of morbidity and mortality in developed countries.[1] In developing countries, particularly in Africa, there is an upsurge of metabolic diseases such as obesity, type 2 diabetes and atherosclerosis, and its cardiovascular complications. All these entities partially share a common basis, namely the development of a state of imbalance between pro-oxidant and antioxidant[2] factors. Paraoxonase-1 is a member of the three-gene family, PON 1, PON 2 and PON 3. PON 1, the best studied, is a calcium-dependent esterase and can hydrolyse organophosphates.[3] This enzyme circulates bound to the high-density lipoproteins (HDLs) and prevents oxidation of HDLs and low-density lipoproteins (LDLs). The gene coding for PON 1 has three major polymorphisms that account for the variation of the enzymatic activity. Two of them are located in the coding region; a Gln (Q) Arg(R) substitution at position 192, and a Leu (L)/Met (M) substitution at position 55. Another important polymorphism is located in the promoter region, at position -107, called T(-107)C.[4] It has been suggested that low-serum paraoxonase-1 activities are characteristic of metabolic syndrome, and may influence the metabolic-syndrome-related risk of coronary artery disease.[5] Thus, paraoxonase-1 may slow the evolution of the early stages of atherosclerosis, and may participate in the reduction of cardiovascular risk through its activities, depending on genetic and environmental factors.[6] These properties make paraoxonase-1 important in the assessment of cardiovascular risk.[7] However, international standards only require the inclusion of quantitative changes of plasma lipoproteins in the primary or secondary prevention of this risk,[8] regardless of the oxidation effect. This study focuses on the relationship between the enzymatic activities of paraoxonase-1 and the lipid parameters of cardiovascular risk, in order to highlight the importance of these oxidative phenomena on the cardiovascular risk in a black patient population.

Method

Population

This prospective survey was conducted from 30 October 2010 to 1 March 2011. The study was carried out in the biochemistry laboratory of the Faculty of

Medicine, in conjunction with the Department of Cardiology and Endocrinology of the Hospital Centre of Libreville, Gabon. Patients who consecutively consulted for diabetes and/or high blood pressure in these structures for the duration of this study were used as cases. Healthy subjects, free of disease, were recruited from the biochemistry laboratory of the Faculty of Medicine. Patients with type 1 diabetes, acute or chronic renal disease, who had had a stroke or coronary event in the last three months and who were taking lipid-lowering drugs, or estrogen or progesterone were excluded from the study, as were patients who had consumed alcohol the night before the sampling. This protocol took into account the ethical principles of research involving human subjects, enacted in the Declaration of Helsinki of 1964 and revised in 2004.[9] All subjects signed the free informed consent forms, and the protocol was approved by the Gabonese national ethics committee.

A questionnaire was administered to assess personal and family history, cardiovascular disease, smoking habits, diabetes and hypertension. Two blood pressure measurements were performed on each arm of the patient, after a rest of at least 15 minutes, using a usual sphygmomanometer. The two measurements were averaged. If the two readings differed (diastolic over 15 mmHg systolic or more than 25 mmHg), a third reading was taken. The average of the two closest measurements was used subsequently. Patients in whom either the systolic or diastolic was equal to, or exceeded 140 and 90 mmHg respectively, or who were taking antihypertensive therapy, were considered to be hypertensive.[10] Subjects whose fasting glucose was above 7 mmol/l and those taking hypoglycaemic drugs were considered to be patients with diabetes.[11] Samples were taken from patients who fasted for at least 10 hours, and were collected in tubes containing ethylene diamine tetraacetic acid (EDTA), as well as fluoride-oxalate tubes and plain tubes. After centrifugation at 3 000 revolutions per minute (rpm) for five minutes, plasma and sera were collected and stored at -70° C until assayed.

Conventional assays

The determination of total cholesterol and triglycerides was performed using standard enzymatic techniques based on samples taken in tubes containing EDTA. Glucose was assayed by the glucose oxidase method on the plasma obtained from samples taken in tubes containing potassium fluoride and sodium oxalate. LDL cholesterol was obtained by direct measurement from the serum, using a method based on the use of two detergents.[12] HDL cholesterol was assayed by a method called Ultra Direct HDL Cholesterol, or Daïchi's method, on a serum sample by selective detergent methodology accelerator.[13] Apolipoprotein AI (ApoA1) and B100 (ApoB100) were measured by immunoprecipitation in the homogeneous phase with the reagent kit supplied by Orion Diagnostica® analyzer Mindray BS-200®. The intra- and interassay coefficients of variation were 2.7% and 4.4% for ApoAI, and 3.1% and 3.9% for ApoB100, respectively.

Extraction and oxidation of low-density lipoprotein and small dense low-density lipoprotein

The precipitation reagent (0.1 ml) containing 150 U/ml of sodium heparin and 90 mmol/l $MgCl_2$ was added to 0.1 ml of a test serum, and the mixture was incubated at 37°C for 10 minutes. Samples were transferred to an ice bath for 15 minutes, then centrifuged at 15 000 rpm for 15 minutes at 4°C. The clear supernatant contained the fraction of small dense low-density lipoprotein.[14] Thirty-two microlitres of $CuCl_2$ (1 mmol/l) were added to 10 µl of this supernatant. The appearance of the reaction products of oxidation, conjugated dienes, was continuously registered at 37°C, using a spectrophotometer at 234 nm.[15]

Assessing the arylesterase activity of paraoxonase-1

A volume of 0.1 ml of serum was added in a final volume of 250 µl containing 1 mmol/l of phenylacetate and 2 mmol/l of $CaCl_2$ in Tris-HCl 20 mmol/l pH 8.0. The initial rate of hydrolysis was determined spectrophotometrically at 270 nm.[14]

DNA extraction and genotyping

Deoxyribonucleic acid (DNA) was obtained from whole blood and extracted with phenol-chloroform.[16] Genotype determinations were carried out by an amplification reaction [polymerase chain reaction (PCR)] in an Eppendorf® thermocycler, followed by analysis of the fragments through enzymes restriction. Sixty pmol of oligonucleotide primers, KCl 62.5 µmol/l, $MgCl_2$ 15 mmol/l, 50 µmol/l of deoxynucleoside triphosphate, 1.5 U of Taq polymerase (Amersham Pharmacia Biotech) in buffer Tris-HCl 12.5 mmol/l and pH = 8.3 (all in a final volume of 50 µl), was added to 10 µg of DNA extract. T(-107)C polymorphism was determined by the method known as amplification refractory mutation system, a simple method for genotyping single nucleotide polymorphism.[17] Two first external sequences have been used, i.e: 5'-GACGCAAGGACCGGATGGCACAAAGTGAGTG-3' andTGGGCGCAGACACCGACGGGCTAGGAGGCTCT-3' -andtwofirstspecificinternaltotwoallelesof-107C(5'-attg-TAGCTGCGGACCCGGCGGGGAGGAGC-3') and107T (5'-attgTAGCTGCGGACCCGGCGGGGAGGAGT-3') to distinguish between T and C alleles at position -107.

These internal primers contained a mismatch voluntary sequence, three bases upstream of the end 3' (G → A) to minimise non-specific binding of primers 3' which were non-complementary. A 5'(attg) sequence was also synthesised to prevent the reverse elongation (3' → 5 ') chain. The amplification protocol was established on the basis of the mixture previously

mentioned, a mixture to which dimethyl sulfoxide 100 ml/l was added. External primers (30 pmol each) were added to each mixture's reaction, followed by 30 amplification cycle, performed at 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for one minute, followed by a final extension at 72°C for six minutes. The PCR fragments were analysed on an agarose gel 2% with ethidium bromide staining and ultraviolet (UV) transillumination. For the PON 1 192 polymorphism, the PCR products were subjected to the action of the restriction enzyme *AlwI* for four hours at 37°C, then separated by electrophoresis on 3% agarose gel for 75 minutes at 60 V with ethidium bromide staining and UV transillumination. PON 1 55 polymorphism was determined after the action of the restriction enzyme *Hsp92II* of the amplification products, followed by electrophoresis under the same conditions as before.

Statistical method

Continuous variables were expressed as mean and standard deviation, while qualitative variables were expressed as frequency. The comparison of quantitative variables was performed using the nonparametric Kruskal Wallis test. The chi-square Mantel-Haenszel was used to compare allele frequencies between groups. Two regression models were performed, namely a linear regression model including all the variables influencing PON 1 activity, and another regression carried out step by step, to select the most relevant variables based on their importance. The null hypothesis was rejected for p-values < 0.05. The statistical analysis of data was performed using Statistica 8® US Windows for StatSoft France.

Results

The epidemiological data of the studied cases are summarised in Table I. The study population was predominantly male (64.2%), with an average age of 62.7 ± 12 years. Of the patients, 31.7% were patients with hypertension, 25.8% were patients with diabetes, 15.8% were patients with diabetes and hyptertension, and 25.7% were considered to be healthy.

In this entire cohort, genotyping at position -107 showed a predominance of -107TC heterozygotes (0.440), followed by -107TT and -107CC homozygotes (Table II), corresponding to frequency of 0.540 for -107 T allele and 0.460 for -107C allele. At positions 55 and 192, we obtained a predominance of 55LL genotypes (0.510) and 192RR (0.460), corresponding to frequencies of 0.695 and 0.635 for 55L and 192R alleles, against 0.365 and 0.305 for alleles 55M and 192Q, respectively. By contrast, in the control group, we obtained a prevalence of genotype -107CC (0.560) for a frequency of 0.675 for the -107C allele, and 0.325 for -107T allele, respectively. In subjects with diabetes, the predominant genotype was -107TT, with an allele frequency of 0.660 and 0.695 -107T respectively, as they were simply diabetic or hypertensive and diabetic (vs. 0.340 and 0.305 for allele C) at -107 position.

Table I: Epidemiological parameters of subjects setting numbers (%)

Parameter	Number (%) or medium ± SD
Subjects	600
Gender	
Female	215 (35.8)
Male	385 (64.2)
Tobacco	
Yes	75 (12.5)
No	525 (87.5)
Age (years)	62.7 ± 12.0
Height (m)	1.62 ± 0.09
Body mass index (kg/m²)	27.4 ± 5.1
Male waist circumference (cm)	104 ± 12
Women waist circumference (cm)	86 ± 8
Alcohol consumption	
Yes	163 (27.1)
No	437 (72.9)
Sport practice	320 (53.3)
Clinical category	
Control group	160 (26.7)
Patients with diabetes	155 (25.8)
Patients with hypertension	190 (31.7)
Patients with hypertension and diabetes	95 (15.8)
Average weight (kg)	71.9 ± 9.7
Average systolic blood pressure (mmHg)	124 ± 6
Average diastolic blood pressure (mmHg)	81 ± 3

SD = standard deviation

In the coding region at position 55, genotype 55LL was found in 66% of controls and 51% of patients with simple hypertension. This corresponded to the allele 55L frequencies of 0.745 and 0.630 in controls and hypertensive subjects, respectively (vs. 0.255 and 0.370 for allele 55M). However, in patients with diabetes, the frequency of the genotype 55LL dropped to 0.420 and 0.340 in patients with diabetes and patients with diabetes and hypertension respectively, with frequencies of 0.470 and 0.420 for allele 55L in both groups, against 0.530 and 0.580 for allele 55 M.

The Q192R polymorphism was characterised by relative constancy in the distribution of alleles 192Q and 192R in the four identified groups. Indeed, the 192Q allele was found at a frequency of 0.290 in the control group and in subjects with hypertension. Its frequency was 0.244 in subjects with diabetes and 0.230 in subjects with

Table II: Genotype frequencies according to clinical category and in the entire cohort

Number of subjects	Control group (160)	Patients with hypertension (190)	Patients with diabetes (155)	Patients with hypertension and diabetes (95)	Entire cohort (600)
PON 1 -107					
TT	0.21	0.19 (0.127)	0.57 (0.0001)	0.61 (0.001)	0.32
TC	0.23	0.21 (0.131)	0.18 (0.002)	0.17 (0.002)	0.44
CC	0.56	0.60 (0.231)	0.25 (0.0001)	0.22 (0.0001)	0.24
PON 1 55					
LL	0.66	0.51 (0.01)	0.42 (0.0001)	0.34 (0.0001)	0.51
LM	0.17	0.24 (0.021)	0.10 (0.001)	0.16 (0.234)	0.37
MM	0.17	0.25 (0.001)	0.48 (0.0001)	0.50 (0.0001)	0.12
PON 1 192					
QQ	0.16	0.21 (0.067)	0.11 (0.078)	0.11 (0.076)	0.19
QR	0.26	0.16 (0.052)	0.27 (0.467)	0.24 (0.413)	0.35
RR	0.48	0.53 (0.050)	0.62 (0.051)	0.65 (0.003)	0.46

p-values for comparison with the control group in brackets

Table III: PON 1 activity according to according to clinical category and in the entire cohort

Number of subjects	Control group (160)	Patients with hypertension (190)	Patients with diabetes (155)	Patients with hypertension and diabetes (95)	Entire cohort (600)
PON 1 -107 (U/ml)					
TT	65 ± 7	60 ± 5 (0.134)	50 ± 9 (0.043)	45 ± 12 (0.037)	56 ± 8
TC	79 ± 6	75 ± 6 (0.087)	60 ± 7 (0.047)	58 ± 6 (0.0001)	70 ± 7
CC	87 ± 8	81 ± 7 (0.057)	72 ± 13 (0.0021)	67 ± 12 (0.0001)	78 ± 10
PON 1 55 (U/ml)					
LL	92 ± 7	81 ± 5 (0.046)	75 ± 4 (0.013)	64 ± 7 (0.0001)	80 ± 6
LM	76 ± 10	71 ± 10 (0.0634)	64 ± 9 (0.048)	55 ± 8 (0.0002)	68 ± 9
MM	65 ± 9	59 ± 7 (0.032)	52 ± 5 (0.0001)	47 ± 6 (0.0001)	57 ± 6
PON 1 192 (U/ml)					
QQ	103 ± 17	81 ± 15 (0.0021)	71 ± 17 (0.0001)	58 ± 15 (0.0001)	81 ± 16
QR	95 ± 22	83 ± 11 (0.043)	75 ± 10 (0.0001)	63 ± 12 (0.0001)	81 ± 14
RR	98 ± 16	86 ± 12 (0.0235)	68 ± 18 (0.0001)	60 ± 10 (0.0001)	80 ± 14

p-values for comparison with the control group in brackets

diabetes and hypertension (p-value = 0.432). Similarly, the 192R allele had a frequency of 0.710 in controls and subjects with hypertension. This frequency was 0.755 in subjects with diabetes and 0.770 in subjects with diabetes and hypertension (p-value = 0.652).

The distribution of enzyme activities, based on clinical category and genotypes, is summarised in Table III. Genotypes associated with increased enzyme activity were -107CC and 55LL. However, the PON 1 activity was relatively constant, regardless of the polymorphism at position 192. On the other hand, a gradual decline of enzyme activity from the control group to that of patients with diabetes and hypertension, was noticed whatever the site of polymorphism concerned.

The relationship between polymorphisms whose genotypes had an effect on enzyme activities and lipid parameters is summarised in Tables IV and V. Among the parameters studied, and irrespective of the group of people, there was no association between LDL cholesterol concentrations and the different genotypes involved in the T(-107C) polymorphism. There was a relationship between the concentrations of HDL, ApoAI and the lag time and genotypes of the promoter region studied. This was regardless of patient category. A particular decrease in concentrations of HDL cholesterol and ApoAI in the same direction was observed, from the -107CC genotype to the -107TT, with heterozygotes CT occupying an intermediate position. Vertical analysis of Table IV showed the concentration of HDL cholesterol and a high lag period, homozygous -107CC in the control group. Both parameters decreased with the onset of the allele T. Regarding the L55M polymorphism, in the control group, 55LL

Table IV: Impact of genotype on lipid parameters according to -107CT polymorphisms

	CC	CT	TT	p-value
Control group				
HDL cholesterol (mmol/l)	1.45 ± 0.04	1.24 ± 0.03	1.24 ± 0.04	0.0001
LDL cholesterol (mmol/l)	3.42 ± 0.04	3.40 ± 0.02	3.51 ± 0.04	0.851
ApoAI (g/l)	1.14 ± 0.03	1.00 ± 0.02	1.01 ± 0.03	0.005
Lag time (seconds)	90 ± 2	80 ± 5	75 ± 2	0.0001
Patients with diabetes				
HDL cholesterol (mmol/l)	1.20 ± 0.01	0.91 ± 0.07	0.88 ± 0.06	0.002
LDL cholesterol (mmol/l)	4.25 ± 0.07	4.34 ± 0.05	4.42 ± 0.04	0.843
ApoAI (g/l)	0.92 ± 0.02	0.84 ± 0.03	0.82 ± 0.02	0.006
Lag time (seconds)	75 ± 4	60 ± 2	54 ± 4	0.0001
Patients with hypertension				
HDL cholesterol (mmol/l)	1.40 ± 0.06	1.22 ± 0.04	1.20 ± 0.02	0.001
LDL cholesterol (mmol/l)	4.01 ± 0.04	3.95 ± 0.06	4.10 ± 0.05	0.765
ApoAI (g/l)	1.20 ± 0.02	0.95 ± 0.05	0.90 ± 0.04	0.001
Lag time (seconds)	80 ± 2	75 ± 4	70 ± 2	0.0001
Patients with hypertension and diabetes				
HDL cholesterol (mmol/l)	0.90 ± 0.02	0.75 ± 0.03	0.72 ± 0.04	0.002
LDL cholesterol (mmol/l)	4.63 ± 0.07	4.57 ± 0.06	4.62 ± 0.08	0.742
ApoAI (g/l)	0.84 ± 0.03	0.69 ± 0.02	0.70 ± 0.03	0.006
Lag time (seconds)	62 ± 4	53 ± 3	52 ± 4	0.0002

ApoA1: apolipoprotein AI, HDL: high-density lipoprotein, LDL: low-density lipoprotein

Table V: Impact of genotype on lipid parameters according to 55LM polymorphisms

	LL	LM	MM	p-value
Control group				
HDL cholesterol (mmol/l)	1.64 ± 0.04	1.42 ± 0.05	1.13 ± 0.04	0.0001
LDL cholesterol (mmol/l)	3.47 ± 0.06	3.42 ± 0.05	3.38 ± 0.07	0.453
ApoAI (g/l)	1.32 ± 0.07	1.21 ± 0.06	1.10 ± 0.05	0.002
Lag time (seconds)	105 ± 5	95 ± 2	90 ± 4	0.0001
Patients with diabetes				
HDL cholesterol (mmol/l)	0.74 ± 0.05	0.54 ± 0.04	0.58 ± 0.02	0.0001
LDL cholesterol (mmol/l)	4.45 ± 0.05	4.45 ± 0.07	4.43 ± 0.08	0.732
ApoAI (g/l)	1.11 ± 0.02	0.84 ± 0.08	0.78 ± 0.04	0.006
Lag time (seconds)	82.5 ± 5	70 ± 4	65 ± 5	0.001
Patients with hypertension				
HDL cholesterol (mmol/l)	0.94 ± 0.06	0.82 ± 0.04	0.80 ± 0.03	0.002
LDL cholesterol (mmol/l)	4.32 ± 0.04	4.28 ± 0.06	4.31 ± 0.07	0.326
ApoAI (g/l)	0.85 ± 0.05	0.72 ± 0.03	0.69 ± 0.02	0.004
Lag time (seconds)	92 ± 4	83 ± 2	75 ± 2	0.001
Patients with hypertension and diabetes				
HDL cholesterol (mmol/l)	0.65 ± 0.05	0.49 ± 0.04	0.44 ± 0.02	0.004
LDL cholesterol (mmol/l)	4.19 ± 0.08	4.02 ± 0.05	4.20 ± 0.08	0.321
ApoAI (g/l)	0.92 ± 0.02	0.54 ± 0.03	0.51 ± 0.02	0.0001
Lag time (seconds)	72 ± 3	62 ± 2	57 ± 3	0.0001

homozygotes had a higher concentration of HDL, of 1.64 ± 0.04 mmol/l. This concentration decreased to 1.13 ± 0.04 mmol/l in 55MM homozygotes. This decrease was observed in other patient groups, with a greater depletion of HDL in patients with hypertension and diabetes. Furthermore, the lag time of subjects in the control group and 55LL homozygotes, was 105 ± 5 seconds against 90 ± 4 seconds in homozygous 55MM in the same group, with a significant difference (p-value = 0.0001). The decrease of lag time with the appearance of the M allele was obtained with all groups of patients.

The results of the step-by-step regression analysis that was performed using all factors involved in the variability of PON 1 activity are shown in Table VI. The considered variables were those that were correlated with PON 1 in multivariate analysis. The main dependent variables that determined the enzyme activity were ApoAI and lag time. The non-modifiable variables that had an influence on the arylesterase activity were age (r^2 = 3.9, p-value < 0.00001), gender (r^2 = 0.38, p-value < 0.006), genotype at position 55 (r^2 = 3.0, p-value < 0.0001), and position -107 (r^2 = 1.84, p-value < 0.0005), and body mass index (r^2 = 0.55, p-value = 0.003).

Table VI: Step-by-step regression analysis of PON 1 determinants

Dependent parameters	r^2	p-value
Apolipoprotein AI	8.4	< 0.0001
HDL cholesterol	0.7	< 0.026
Lag time	9.1	< 0.0001
Age	3.0	< 0.0001
Genotype 55	2.7	< 0.0001
Genotype 107	1.7	< 0.0005
Triglycerides	0.60	0.035
Tobacco	0.59	0.038
Body mass index	0.55	0.003
Sport practice	0.43	0.005

Discussion

In this paper, we studied the relationship between the arylesterase activity of PON 1 and lipids parameters in a group of black patients with diabetes, compared to a control group. The enzyme activity used was arylesterase activity. Yet, the phenylacetate used as a substrate does not show a difference in reactivity with respect to the Q192R locus, as paraoxon. The only substrate that differentiates the activities of each of these alleles is paraoxon. The R192 isoform hydrolyses paraoxon nine times faster than the allozyme Q192.[18]

Contrary to paraoxon, phenylacetate is easy to use and to obtain. Otherwise, the most commonly used method to measure the susceptibility of LDL to in-vitro oxidation is the determination of the lag time of the formation of conjugated dienes, initiated by a catalytic amount of transition metals.[15] The formation of conjugated diene represents an intermediate stage of the oxidation process. As an inverse relationship between the latency, the severity and the rate of the progression of coronary atherosclerosis, the lag time is considered to be the most discriminating index of the oxidisability of LDL.[19]

However, with this protocol, we showed that in subjects who were considered to be healthy, predominant genotypes in our population were -107CC and 192RR and 55LL. This distribution is similar to that found by Thyagarajan et al[20] in the black patient population of the Coronary Artery Risk Development in Young Adults (CARDIA) study. The comparison of our distribution with that obtained in other populations is shown in Table VII.

Distributions for 55L and 55M alleles were similar to those found in the United States and Brazil.[21] Other distributions, especially for alleles 192Q and 192R, were different. Such a difference could be one of the factors involved in the conflicting results of epidemiological studies that have been conducted in different populations. If this hypothesis is true, then the incidence of diabetes and hypertension should be higher in Gabon, than in Korea or Thailand for example, other causes being equal. The association of PON 1 genotypes, enzymatic activity and parameters of oxidation suggests a particularly high cardiovascular risk in patients with diabetes in Gabon, compared to what could be expected in Asia.

It is now suggested that the incidence of hypertension and cardiovascular mortality is higher in black patients

Table VII: Comparison of the distribution of allele frequencies with that of other populations[19]

Population (number of cases)	PON 1 55			PON 1 192		
	L	M	p-value	Q	R	p-value
Gabon (120)	0.695	0.305	-	0.365	0.635	-
Korea (988)	0.941	0.058	0.007	0.705	0.295	< 0.0001
Thailand (475)	0.955	0.005	0.0001	0.710	0.290	< 0.0001
Iran (132)	0.590	0.410	0.032	0.690	0.310	< 0.0001
United States (2 553)	0.637	0.363	0.131	0.720	0.280	< 0.0001
Brazil (376)	0.640	0.360	0.131	0.660	0.340	< 0.0001
Italy (273)	0.601	0.399	0.061	0.711	0.289	< 0.0001

living in urban zones than in Caucasians.[22,23] Similarly, Hall et al have also shown a particularly significant increase of diabetes in African patients.[24] However, the predominant genotypes in the control group also have the highest enzymatic activity outside the 192 position. These subjects are less likely to develop diabetes. The alleles -107T, 55M and 192R are prevalent in diabetics and are characterised by a low enzyme activity. The importance of these elements probably reflects the susceptibility of these individuals, whose PON1 activity results in a lower predisposition to developing diabetes, as suggested by the work of Martinelli.[5] Therefore, the measurement of the PON 1 activity would provide supplemental information to the cardiovascular risk in this population.

In the same way, Bhattacharyya et al[25] have shown a decreased incidence of cardiovascular events in subjects whose PON 1 concentration is high. In addition, the decrease of PON 1 activity in patients with diabetes is followed by a decrease in HDL concentration. This result proves that it is rather the protective potential of atherogenesis that is defective in this particular situation. LDL is present, as we have demonstrated, but with a decrease in the HDL and PON 1, a more intensive oxidative stress was obtained. This result is confirmed by Mastorikou et al[26] who suggested that non-functional HDL in type 2 diabetes would be an additional factor in increased cardiovascular disease related to type 2 diabetes in metabolising 20% less membrane hydroperoxides than the HDL of controls. This reduction in the antioxidant activity of HDL in diabetes may relate to the glycation of PON 1 in these individuals and the oxidation of ApoAI and LDL. Genotypes and PON 1 activity are independently associated with concentrations of HDL and ApoAI. This relationship depends on the pathophysiological state. One interpretation of these data is that PON 1 determines the concentration of HDL and ApoAI. The generally accepted hypothesis is that PON 1 was the main beneficiary in this association. Results showing the importance of HDL in the secretion and transport of PON 1, agreed with this.[27] However, the relationship between the genotype and the HDL could also reflect an impact of PON 1 on the HDL, and is consistent with the independent associations of genotypes and enzyme activity in multivariate analyses. Indeed, the oxidation of ApoAI that can inhibit PON 1[20] disrupts the ability of the HDL to achieve cholesterol efflux from cells. This result was confirmed by Yildiz et al,[28] who showed that hypertension was accompanied by an increase in the concentration of lipid hydroperoxides, total antioxidant status, and decreased enzymatic activity of arylesterase. Gaillard et al[29] also demonstrated that the nature of the HDL in subjects who did not have diabetes could be responsible for increased cardiovascular events in the black patient population, despite a higher concentration of HDL. Our study relates paraoxonase activity to the polymorphism of its gene and to lipid concentrations in different groups of study.

We have demonstrated a consistently decreasing lag time from the control group to patients with diabetes and hypertension, regardless of the genotype in question, and this diminution related to the PON 1 activity. This result corresponds with that of Nishtha et al.[14] In fact, the oxidation of LDL cholesterol in an endothelial area that PON 1 can inhibit is considered to be as an important step in the development of atherosclerosis.[30]

Smoking is among the parameters that may influence PON 1 activity. This result confirms other work from James et al[31] that has shown that increased oxidative stress is one mechanism by which smoking exerts its deleterious influence on cardiovascular risk, although Sepahvand et al[32] found that smoking did not have an influence on paraoxonase activity. But this author has worked with an Iranian population where smoking habits altered the results. Finally, we have found a decrease in PON 1 activity with aging. This result is consistent with that found by other authors.[33] The relationship between PON 1 activity and oxidative stress could take place according to a reduction-of-oxygen mechanism involving cysteine residues of PON 1 for example, because a reduction in PON 1 activity under oxidative stress conditions has been demonstrated.[33] In this case, the system of regeneration of the reduced form of the enzyme still needs to be determined.

Relations obtained between the genotype of PON 1, PON 1 enzyme activity and other cardiovascular risk factors suggest that the Gabonese population is at particularly high cardiovascular risk. However, it seems necessary to search the haplotype with the higher enzymatic activity, and link this haplotype with the incidence of cardiovascular complications and diabetes in this population before the PON 1 activity is included in the assessment of cardiovascular risk.

Financial interest

The authors declare that they have no competing financial interests.

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**References available on request*