Tecnociencia 2010, Vol. 12, N° 1.

GENETIC POLYMORPHISMS AND ACTIVITIES OF PARAOXONASE IN THE PANAMANIAN POPULATION

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ABSTRACT

Human paraoxonase (PON1) is an enzyme associated to high-density lipoprotein (HDL) particles that protects against oxidative damage to both low-density lipoprotein (LDL) and HDL. The enzyme is also involved in the hydrolysis of highly toxic metabolite from organophosphate pesticides. In the present study, the distribution of the PON1 polymorphisms at position -108 in the promoter region and at positions 55 and 192 in the coding region, in the Panamanian population (n = 187) was determined. The genotype of each individual was determined by PCR-RFLP. The genotype frequencies at position 55 were: LL 0.631, LM = 0.331, and MM = 0.037; frequencies at position 192 were: QQ = 0.331, QR = 0.497, and RR = 0.171; and the frequencies at position -108 were: CC = 0.428, CT = 0.428, and TT = 0.117. The highest allele frequencies were: Q = 0.581, L = 0.796 and C = 0.655. However, the Amerindian Panamanian group, Gnöbé Bugle, presented a low frequency for the -108CC genotype (0.125), which might be associated to a higher risk to organophosphate pesticides poisoning. Arylesterase and diazoxonase activities of PON1 were determined in samples of the Caucasian and Black Panamanian population. The most frequent haplotype in the whole population was -108CC/55LL/192QR; this haplotype was the second most frequent in Black Panamanians and the most frequent in Caucasian Panamanians. The arylesterase activities for this haplotype were 116.16 U/mL and 146.30 U/mL in Black and Caucasian Panamanians, respectively. Diazoxonase activities were 10.09 U/mL and 11.25 U/mL in the same groups, respectively. Relationships between the arylesterase activity of PON1 and these polymorphisms are discussed.

KEYWORDS
PON1 polymorphisms, genotype, arylesterase activity, diazoxonase activity.

RESUMEN
La Paraoxonasa (PON1) humana es una enzima asociada a las lipoproteínas de alta densidad (HDL) que protege contra el daño oxidativo tanto a las lipoproteínas de baja densidad (LDL) como a las HDL. La enzima está involucrada en la hidrólisis de metabolitos altamente tóxicos de pesticidas organofosforados. En el presente estudio, se determinó la distribución de los polimorfismos de PON1 en la posición -108 de la región promotora y en las posiciones 55 y 192 de la región codificadora en la población Panameña (n=187). El genotipo de cada individuo fue determinado mediante PCR-RFLP. Las frecuencias genotípicas encontradas en la posición 55 fueron: LL 0.631, LM = 0.331, y MM = 0.037; en la posición 192: QQ = 0.331, QR = 0.497, y RR = 0.171; y en la posición -108: CC = 0.428, CT = 0.428, y TT = 0.117. Las frecuencias alélicas más altas fueron: Q = 0.581, L = 0.796 y C = 0.655. Sin embargo, El grupo indígena Panameño, Gnöbé Bugle, presentó una frecuencia baja para el genotipo -108CC (0.125), lo que podría estar asociado a un mayor riesgo al envenenamiento por pesticidas organofosforados. Las actividades enzimáticas de PON1 arilesterasa y diazoxonasa fueron determinadas en muestras de la población panameña de blancos y negros. El haplotipo más frecuente en la población total fue -108CC/55LL/192QR; éste haplotipo fue el segundo más frecuente en la población Negra Panameña y el más frecuente en la población Blanca Panameña. La actividad arilesterasa para este haplotipo fue de 116.16 U/mL y 146.30 U/mL en Negros y Blancos Panameños, respectivamente. La actividad diazoxonasa fue de 10.09 U/mL y 11.25 U/mL en los grupos antes mencionados. Relaciones entre la actividad arilesterasa y los polimorfismos de PON1 son discutidos en este artículo.

PALABRAS CLAVES
Polimorfismos PON1, genotipo, actividad arilesterasa, actividad diazoxonasa.

INTRODUCTION

Human paraoxonase (PON1) is an enzyme associated to HDL particles which is capable of reducing the oxidation of LDL and HDL components, in addition to hydrolyze highly toxic metabolites derived from organophosphate pesticides (Brophy *et al*., 2000). Several studies have found that PON1 acts as a protector agent against the development of the atherogenic plaque due to its capacity of hydrolyzing oxidized lipids present in the arterial wall, avoiding the oxidation of LDL particles (Durrington *et al*., 2001). It is well known that the accumulation of oxidized LDL particles is one of the factors involved in heart diseases as a consequence of development of

atherosclerosis. The enzyme is a 355 amino acid residues protein encoded by the PON1 gene which is located on chromosome 7q21.3-22.1 (Furlong *et al*., 2006). In humans, the enzymatic activity of PON1 can vary from one individual to another. This fact is due to the presence of a considerable amount of polymorphisms in the PON1 gene in the coding region, as well as in the regulatory region (Furlong *et al*., 2005). Apparently, these polymorphisms explain the differences observed in serum PON1 activity and serum concentration of this enzyme. In the coding region, polymorphisms at positions 55 and 192 are the most common found in individuals and they affect the efficiency of hydrolysis of pesticide metabolites. Polymorphism at position 192 generates two isoforms, Q and R, depending on the presence of a glutamine or arginine residue, respectively. The product of the R allele is more efficient than the Q one in hydrolyzing paraoxon; however, the opposite is true for the hydrolysis of soman and sarin (Furlong *et al*., 2006). Polymorphism at position 55 also generates two isoforms, L and M (leucine or methionine at that position) which is partly responsible for the differential serum levels observed in human PON1. Individuals possessing the M allele present lower levels of this enzyme in blood.

Polymorphisms in the regulatory region at position -108 affect the expression level of PON1 gene. Two alleles are associated to this position, either occurring a C or a T nucleotide. The presence of the allele C produces a two fold higher level of serum PON1 (Costa *et al*., 2003; Furlong *et al*., 2005). Apparently, the -108 polymorphism lies within a binding site for Sp1, a ubiquitous transcription factor common in TATA-less genes such as PON1.

Variations in the allelic frequencies for every polymorphism of PON1 in different populations were previously reported (Castaño *et al*., 2006; Rojas *et al.*, 2005; Brophy *et al*., 2001; Holland *et al*., 2006). However, there are very few studies about the distribution of these polymorphisms in South- and Central-American populations despite the fact that the use of organophosphate pesticides is a common practice in the region.

Taking into account the problems with organophosphate pesticides intoxication besides that the heart diseases are one of the most frequent

causes of death in Panama, we considered important to determine the distribution of PON1 polymorphisms at positions - 108, 55 and 192 in a sample of the Panamanian population.

MATERIALS AND METHODS

Subjects

The population sample consisted of 187 unrelated individuals (adult men and women) from four different racial groups: 50 Caucasian-Panamanians, 40 Black-Panamanians, 49 Mestizos, and 48 Ngöbe-Buglé Amerindians. Only individuals showing the representative phenotype of each racial group were considered. Additional criteria were that both parents would have the same phenotype, so that was no doubt about the major ancestral genetic contribution. Blood samples were collected by venipuncture in 5 ml tubes containing Na-EDTA. Every sample was conveniently identified and kept in an ice-cooled box until they were taken to the lab, in a period no longer than one hour. This research was approved by the Research National Committee of Bioethics (Gorgas Memorial Institute, Panama).

PON1 Genotype

The blood samples were centrifuged at 4000 rpm in a refrigerated centrifuge to separate the red and white blood cells from the plasma. The plasma was used immediately to determine the activities of PON1 in Caucasian and Black-Panamanian subjects. DNA was isolated from the cell fraction either by using the Puregene DNA Purification Kit (Gentra Systems Inc., Minneapolis, Minnesota, USA) according to this manufacturer's instructions, or by following the method described by Sambrook & Russel (2001) and stored at -20^{o} C until the polymorphic analysis. PON1 genotype at positions -108, 55 and 192 was determined by polymerase chain reaction followed by polymorphism specific restriction digestion and electrophoresis. Amplification was performed by using 100-200 ng of DNA, 1 U of *Taq* polymerase (Promega) and the primers (Integrated DNA technologies), in a final volume of 25 μL (Brophy *et al*., 2001; Campo *et al*., 2004).

The Primers GACCGCAAGCCACGCCTTTCTGTGCACC and TGAAAGACTTAAACTGCCAGTC were used to amplify PON1 -108 polymorphic region. The PON1 55 region was amplified with primers CCTGCAATAATATGAAACAACCTG and TGAAAGACTT AAACTGCCAGTC. Primers TATTGTTGCTGTGGGACCTGAG

and GACATACTTGCCATCGGGTGAA were used to amplified PON1 192 polymorphic region. A GeneAmp 2400 thermal cycler (Perkin-Elmer) was used to amplify the variant regions under the following PCR conditions: initial denaturing temperature of 96^{o} C for 5 min, annealing temperature of 63^{o} C for 1 min (except for variant 192 Q/R, where a temperature of 61^{o} C was used), extension temperature of 72^{o} C for 1 min, and then 94^{o} C for 1 min. A final extension temperature of 72^{o} C for 10 min was achieved for every determination. These conditions were repeated for 25, 30, and 30 cycles for the PON1 -108, 55 and 192 polymorphisms, respectively. Aliquots of 15 μL of the amplified products were digested at 37^{o} C for 16 hours by using 1 U of the following restriction endonucleases (New England Biolabs): *BstUI* for the -108 C/T, *NlaIII* for the 55 L/M, and *AlwI* for the PON1 192 Q/R polymorphism**.**

The presence of the -108C allele results in digested bands of 67 bp and 52 bp (instead of an undigested band of 119 bp, which indicates the presence of a T allele). Digested bands (106 bp and 66 bp) indicated the presence of the M allele, whereas absence of digestion indicated the presence of the L allele. The R allele was determined by the presence of two bands; 172 bp and 66 bp and an undigested band of 238 bp indicated the presence of the Q allele (Figure 1).

PON1 Activities

PON 1 enzymatic activities were only assayed in serum from Caucasian- and Black-Panamanian individuals by using phenylacetate and diazoxon as substrates (Rosenblat *et al.*, 2003; Leary & Edwards, 2005). There were no serum samples from Ngöbé and Mestizo groups. Arylesterase activity was measured by phenol production from the hydrolysis of 10 mM phenylacetate using 20 mM Tris-HCl, pH 8.0, 0.9 mM $CaCl_2$, in a total volume of 3 mL. Every determination was done by duplicate in a Shimatzu spectrophotometer by setting the apparatus in the kinetic mode at 25^{o} C. The reaction was started by adding the substrate.

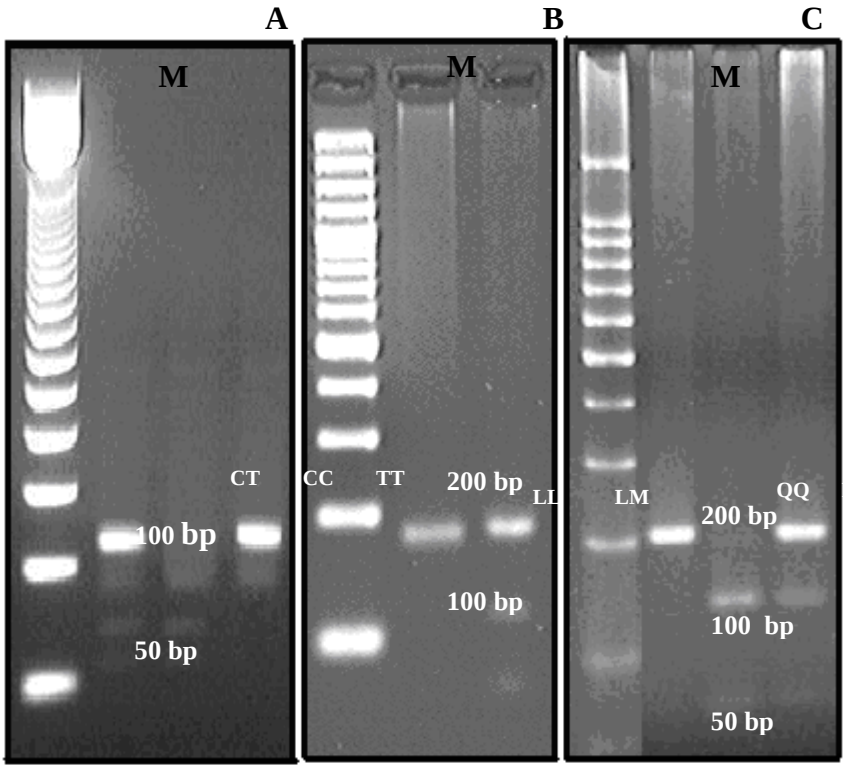

Figure 1. Agarose gels showing the PON1 polymorphisms at positions -108, 55 and 192.

PON1 -108 genotypes (A): M, molecular marker (100 bp DNA Ladder, Fermentas); CT (119, 67 and 52 bp bands); CC (67 and 52 bp bands) and TT (119 bp band). PON1 55 genotypes (B): M, molecular marker (100 bp Ladder, Invitrogen); LL (172 bp band); LM (172, 106 and 66 bp bands); MM (data not shown). PON1 192 genotypes (C): M, molecular marker (100 bp DNA Ladder, Fermentas); QQ (238 bp band); RR (172 and 66 bp bands); and QR (238, 172 and 66 bp bands).

The activity of diazoxonase was measured by pyrimidinol production from the hydrolysis of 1 mM diazoxon using 50 mM Tris-HCl, pH 8.0, 1 mM $CaCl_2$, in a final volume of 3 mL. Every measurement was also done by duplicate in the apparatus under the same kinetic mode at 25^{o} C.

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Statistical Analyses

Data were analyzed by the Epi Info™ 3.3.2 program. Allele and genotype frequencies of PON1 -108, 55 and 192 were obtained by direct counting. The chi-square test was used to evaluate the concordance of genotype frequencies with Hardy-Weinberg's expectations. Differences of PON1 activities among genotypes were evaluated by using the capabilities of the EPIDAT program using Pearson test with Yates corrections.

RESULTS

PON 1 genotype and allele frequencies

Genotype and allele frequencies for the PON1 polymorphisms at positions -108, 55 and 192, in four of the major Panamanian racial groups are summarized in Table 1. A good agreement was found between the observed and expected genotype frequencies according to Hardy-Weinberg equilibrium for the whole population. However, when the population was segregated into racial groups, deviation from Hardy-Weinberg equilibrium (HWE) was observed in Caucasian individuals for PON1 192 genotype ($\chi^2 = 4.6$, $p = 0.032$).

Table 1**.** Genotypes and allele frequencies of PON1 polymorphisms at positions -108, 55 and 192 among 187 Panamanian subjects corresponding to the four major racial groups. Parentheses represent number of individuals or alleles quantities. HWE was calculated using χ^2.

PON1 -108	**Whole population** (n=187)	**Caucasian** (n=50)	**Mestizo** (n=49)	**Black** (n=40)	**Ngöbé** (n=48)
C	0.655	0.730 (73)	0.724 (71)	0.787 (63)	0.396 (38)
T	0.344	0.270 (27)	0.275 (27)	0.213 (17)	0.604 (58)
CC	0.428	0.500 (25)	0.510 (25)	0.600 (24)	0.125 (6)
CT	0.428	0.460 (23)	0.428 (21)	0.375 (15)	0.542 (26)
TT	0.117	0.040 (2)	0.061 (3)	0.025 (1)	0.333 (16)
χ^2	0.0136	1.360	0.296	0.606	0.839
PON1 55					
L	0.796	0.750 (75)	0.796 (78)	0.775 (62)	0.864 (83)
M	0.203	0.250 (25)	0.204 (20)	0.225 (18)	0.135 (13)
LL	0.631	0.600 (30)	0.592 (29)	0.600 (24)	0.729 (35)
LM	0.331	0.300 (15)	0.408 (20)	0.350 (14)	0.270 (13)
MM	0.037	0.100 (5)	0 (0)	0.050 (2)	0 (0)
χ^2	0.293	2.005	3.275	0.002	1.290
PON1 192					
Q	0.581(217)	0.610 (61)	0.561 (55)	0.387 (31)	0.729 (70)
R	0.419(157)	0.390 (39)	0.438 (43)	0.613 (49)	0.270 (26)
QQ	0.331(62)	0.30 0 (15)	0.326 (16)	0.125 (5)	0.542 (26)
QR	0.497(93)	0.620 (31)	0.469 (23)	0.525 (21)	0.375 (18)
RR	0.171(32)	0.080 (4)	0.204 (10)	0.35 (14)	0.083 (4)
χ^2	0.212	4.6	0.137	0.482	0.182

The most frequent PON1 alleles at positions -108, 55 and 192 detected in the whole population were C (0.655), L (0.796) and Q (0.581) respectively. Similarly, the most frequent genotypes were CT (0.428), LL (0.631) and QR (0.497). The most frequent allele at position 55 in each racial group was L. At position 192, Q was the most frequent in Caucasians, Mestizos and Ngöbés but not in Blacks. In Caucasians, Mestizos and Blacks, C allele at position -108, showed a higher frequency than the T allele, in contrast to Ngöbés where T was the most frequent allele.

Allele frequencies among racial groups showed statistical differences for PON1 192 in Caucasians - Blacks (p=0.0048), Mestizos - Blacks (p=0.031), Mestizos-Ngöbés (p=0.0249) and Blacks-Ngöbés (p<0.0001). At position 55, no statistical differences were observed among races. The allele frequency at position -108 in Ngöbés was statistically different from the other groups (p < 0.0001) (Table 2).

Table 2**.** p-values obtained from alleles frequencies pairwaise comparison among different racial group. The p-values correspond to chi square calculated using Pearson test with Yates corrections.

		Caucasian	**Black**	**Mestizo**	**Ngöbé**
PON1 -108	**Caucasian**	--			
	Black	0.4731	--		
	Mestizo	0.9422	0.4268	--	
	Ngöbé	<**0.0001**	<**0.0001**	<**0.0001**	--
PON1 55	**Caucasian**	--			
	Black	0.8298	--		
	Mestizo	0.4588	0.8769	--	
	Ngöbé	0.0698	0.1735	0.2941	--
PON1 192	**Caucasian**	--			
	Black	**0.0048**	--		
	Mestizo	0.5807	**0.0310**	--	
	Ngöbé	0.1164	<**0.0001**	**0.0249**	--

Allele frequencies for the Panamanian population obtained in this study were compared with allele frequencies for other populations previously reported (Table 3).

Table 3**.** Allele frequencies of PON1 -108, 55 and 192 polymorphisms in different World populations compared with those found in the Panamanian population.

		PON1 -108			PON1 55			PON1 192		
Population	**n**	**C**	**T**	***P****	**L**	**M**	***P****	**Q**	**R**	***P****
AMERICA										
Panamanian[a]	187	0.66	0.34		0.80	0.20		0.58	0.42	
African-Americans[b]	117	0.85	0.15	∠ 0.0001				0.37	0.63	∠ 0.0001
Caribbean-Hispanics[b]	203	0.65	0.35	0.9608				0.54	0.46	0.2826
Costa Ricans[b]	518							0.757	0.243	∠ 0.0001
Peruvians[b]	89	0.61	0.39	0.0501				0.539	0.461	0.4454
Mexicans[c]	214	0.45	0.55	0.0650	0.84	0.16	0.1580	0.510	0.490	0.0526
Mexican-Mestizos[b]	182							0.522	0.478	0.1294
Canadians[b]	865	0.48	0.52	0.1898				--	--	--
African-Brazilians[b]	70							0.471	0.529	0.0351
European-Brazilians[b]	101							0.693	0.307	0.0101
Caucasian-Americans[b]	82	0.38	0.62	0.0036				0.730	0.270	0.0012
Americans-USA[b]	376	0.50	0.50	0.5916				0.728	0.272	
Washington[d]	376				0.64	0.36	0.0001			∠ 0.0001
California[e]	260				0.82	0.18	0.5230			
Cayapa Indians[b] (EC)	83							0.211	0.789	∠ 0.0001
Chileans[b] NS	195							0.569	0.431	0.8152
Chileans[b] ES	129							0.663	0.337	0.0441
EUROPE										
Northern Irish[b]	170							0.712	0.288	0.0003
English[b]	282							0.710	0.290	∠ 0.0001
Finnish[b]	169							0.737	0.263	∠ 0.0001
Dutch[b]	250							0.716	0.284	∠ 0.0001
Germans[b]	2784							0.718	0282	∠ 0.0001
Italians[b] (Sardinia)	161							0.752	0.248	∠ 0.0001
French[b]	125							0.765	0.235	∠ 0.0001
Spanish[b]	141	0.38	0.62	0.0005				0.700	0.300	0.0024
Turkish[b]	381							0.692	0.308	0.0003
ASIA										
Japanese[b]	132	0.48	0.52	0.3886				0.402	0.591	∠ 0.0001
Chinese[b]	475	--	--	--				0.352	0.648	∠ 0.0001
Thais[b]	202	0.25	0.75	∠ 0.0001				0.710	0.290	0.0114
Indians[b]	165							0.67	0.33	0.0173
Indians[b] ND	80							0.82	0.17	∠ 0.0001
AFRICA										
Ethiopians[b]	169							0.592	0.401	0.7954
Beninese[b]	98							0.388	0.612	∠ 0.0001

*p** calculated using Pearson test with Yates corrections
[a]This study. [b]Castaño et al. 2006, [c]Rojas et al. 2005, [d]Brophy et al. 2001, [e]Holland et al. 2006.

Haplotypes and Arylesterase and Diazoxonase activities

Arylesterase and diazoxonase activities for PON1 haplotypes in Blacks and Caucasians are presented in Table 4. PON1 activities revealed no significant differences ($p>0.05$) among groups for the same haplotypes. Individuals with haplotype -108CT/55LM/192QR had the highest arylesterase activity (153.25 U/mL), whereas those with haplotype 108TT/55LM/192QR exhibited the lowest arylesterase (48.1 U/mL) and diazoxonase (2.38 U/mL) activities. The highest diazoxonase activity was associated with the -108TT/55LM/192QQ haplotype (13.56 U/mL). The most frequent haplotype in the whole population was -108CC/55LL/192QR. In Blacks, this is the second most frequent haplotype. The arylesterase activities for this latter haplotype were 116.16 U/ml and 146.30 U/mL in Blacks and Caucasians, respectively. Diazoxonase activities for the same latter haplotype were 10.09 U/mL and 11.25 U/mL, in Blacks and Caucasians, respectively.

Table 4**.** Arylesterase and Diazoxonase activities according to different PON1 genotypes.

Whole Popula-tion	**Genotype at Position**			**Black (n=40)**			**Caucasian (n=50)**		
(n= 90) n	**-108**	**55**	**192**	**n**	Arylesterase activity* mean (U/mL) ± SD	Diazoxonase activity* mean (U/mL) ± SD	**n**	Arylesterase activity* mean (U/mL) ± SD	Diazoxonase activity* mean (U/mL) ± SD
18	CC	LL	QR	6	116.16±33.75	10.09±3.97	12	146.39±3909	11.25±2.88
12	CC	LM	QR	7	119.53±26.87	9.03±2.05	5	113.40±18.49	8.55±2.03
11	CT	LL	QR	4	137.11±34.41	10.75±2.67	7	124.05±27.13	10.52±3.34
7	CT	LL	RR	5	145.97±16.27	9.44±2.05	2	137.31±1.56	8.67±1.59
7	CC	LL	RR	5	134.38 ± 40.67	9.03±2.18	2	151.18±25.30	9.5±2.85
6	CT	LL	QQ	1	*95.97	*9.67	5	136 45± 20.88	13.4±2.72
6	CT	LM	QR	2	153.25±1.74	13.39±1.08	4	130.62±26.94	9.09±2.56
5	CC	LL	QQ	3	132.09±8.90	11.86±1.83	2	127.54± 17.74	12.53±1.45
3	CT	LM	RR	3	138.07±4.96	9.76±1.25	0	--	--
2	TT	LM	QR	1	*93.83	*6.81	1	*48.10	*2.38
2	CC	MM	QR	1	*126.64	*8.79	1	*129.66	*8.67
2	CC	MM	QQ	1	*118.56	*9.01	1	ND	--
2	CC	LM	QQ	0	--	--	2	127.00±28.34	12.08±3.63
2	CT	MM	QQ	0	--	--	2	106.80±20.95	10.11±2.85
2	CT	LM	QQ	0	--	--	2	118.08±12.36	11.79±1.66
1	CC	LM	RR	1	*149.65	*11.80	0	--	--
1	TT	LM	QQ	0	--	--	1	*147.22	*13.56
1	CT	MM	QR	0	--	--	1	*138.7	*10.71

$p>0.005$ evaluated by Student t-test. * Not mean values and SD (standard deviation) were calculated.

DISCUSSION

To the best of our knowledge, this is the first study reporting the frequencies of PON1 polymorphisms at positions -108, 55, and 192 in the Panamanian population. The frequency of PON1 -108C allele in the whole Panamanian population was considerably higher than that for the T allele. The frequency of the -108C allele for Panamanians (0.66) is higher than any other population, except for African-Americans (0.850), and very similar to that for Caribbean-Hispanics (0.65) and Peruvians (0.61). This result may indicate that Panamanians, as well as Caribbean-Hispanics and Peruvians possess a relative higher plasma level of PON1, which means that these populations are less susceptible to organophosphate pesticide toxicity. The observed similarities of the C allele frequencies between Panamanians and other populations would be explain as a consequence of similar ancestral genetic contribution in the colonization period. The largest differences of this polymorphism were observed when we compared it to Thai, Caucasian American and Spanish, African American and Caribbean Hispanic populations ($p < 0.005$) (Table 3).

Regarding the PON1 55 polymorphism, the L allele had the highest frequency in the Panamanian population (0.80), similar to Mexicans (0.84) and Californians (0.82). By contrast, the frequency of this allele was significantly higher than that observed for Washington residents (Holland *et al.,* 2006). Due to the fact that L allele has been associated with higher plasma PON1 levels, we expect that the Panamanian population is partly protected against cardiovascular disease. Interestingly, when we compared the frequencies of the L allele and percentage of mortality caused by ischemic heart disease, the countries with lower L allele frequencies exhibit higher percentage of mortality caused by this disease. In this respect, United States has an L allele frequency of 0.64, having a 21 % of mortality; Panama, with a frequency of 0.80 for the same allele, exhibits 12 % of mortality, and Mexico, which the L allele frequency is 0.84, the caused of mortality attributed to the ischemic heart disease is 11% (World Health Organization. Mortality Country Fact Sheet, 2006).

As indicated in Table 3, the observed frequency for PON1 192 allele Q in Panamanians was slightly higher (0.58) than that for Caribbean-Hispanics (0.54) (Chen *et al*., 2003), Peruvians (0.54) (Cataño *et al.,* 2006), Mexicans (0.51) (Rojas-García *et al.,* 2005), Mexican mestizos

(0.52) (Gamboa *et al.,* 2006) and Chileans NS (0.57) (Acuña *et al*., 2004). Panamanians have a relative low frequency (0.419) of the R allele which is associated to high arylesterase activity. The arylesterase activity of PON1 only provides an estimate of the sensitivity to organophosphate pesticides due to the fact that the arylesterase activity is correlated with the hydrolysis of paraoxon, which is the catabolite of parathion (organophaste pesticide). According to these results, we expect that the Panamanian population is more susceptible to organophosphate compounds poisoning. When we compared the arylesterase and diazoxonase activities among Black and Caucasian Panamanians, no significant differences were observed for both enzymes activities. This result might be explained in terms of the genetic complexity of the Panamanian population, which is the result of a mixture, not only of these two races, but also with Native-Panamanian contribution.

As mentioned before, the Panamanian population is the result of a mixture of several racial groups with major contributions from Native Americans, Blacks and Spaniards. According to this fact, we expected some similarities among Panamanian racial groups and the corresponding ancestral groups. The frequencies for PON1 192 and PON1 55 alleles from Black Panamanians resemble more to that reported for other Black populations such as African-Americans, African-Brazilians and African-Benineses than the non Black populations. The Panamanian Black population is descendant of the African Black population that arrived to the isthmus as slaves in the colonization period or as workers from Antilles during Panama Canal construction.

The Panamanian Caucasian group had PON1 192 allele frequency more similar to the Spanish than Black African and American populations, including the Black Panamanian. However, the frequency for the L and C alleles is quite different from that observed in the other Caucasian groups (Cataño *et al.*, 2006). Unexpectedly, PON1 192 allele frequencies for Ngöbé were very different from that reported for Cayapa Indians, although both groups have the same Chibchan linguistic origin (Kolmand & Berminghan, 1997).

We found that the native Panamanian group Ngöbé possesses high frequencies of the T and Q alleles, which make them more susceptible to some organophosphates poisoning. Ironically, the Ngöbé are primarily employed as agricultural laborers in the highlands of Panama where the use of organophosphate pesticides is quite high. It is well known that exposure to high doses of these compounds have profound effects to the central nervous system, and recent evidence suggests that chronic low level exposure may affect neurodevelopment. This latter effect is important in pregnant women, newborns, and even in infants so that special considerations should be taken with this racial group.

The highest arylesterase activity of PON1 was observed, separately, in individuals with -108CC, 55LL and 192 RR genotypes. Similar results were obtained by Rojas-García *et al.*, (2005) in a Mexican population, in contrast to the results obtained by Brophy *et al*., (2001) in which individuals with PON1 192QQ genotype was associated with the highest arylesterase activity of PON1. Other researchers have found no relationship among these genotypes and arylesterase activity of PON1 in Caucasian, African-American, and Caribbean populations (Chen *et al*., 2003).

Arylesterase and diazoxonase activities for PON1 haplotypes in Black and Caucasian Panamanians are presented in Table 4. Arylesterase activity of PON1, as a function of different haplotypes, revealed that the highest arylesterase activity (151.18 U/mL) was observed in Panamanian Caucasian with the -108CC/55LL/192RR haplotype. This haplotype is generally associated with high arylesterase activiy (Rojas-García *et al*., 2004). However, these activity values are lower than those reported for the same haplotype in Mexicans (Rojas-García *et al*., 2004). These differences would be explained as a consequence of modifications in the protocol used to measure the arylesterase activity. In Black Panamanians, the haplotype associated with the highest arylesterase activity (149.65 U/mL) was -108CC/55LM/192RR. Although, the arylesterase activity for the -108CC/55LL/192RR haplotype in Black Panamanians was lower than the -108CT/55LM1/92QR haplotype, the obtained values may not be reliable because only two individuals in our study showed this haplotype and also variations in the activities have been reported for individuals with the same haplotype (Rojas-García *et al*., 2004).

In summary, the results presented in this study represent the only available information so far on the frequencies of the polymorphisms associated to the PON1 gene in the Panamanian population. The obtained allelic frequencies resemble to that reported for other Latino populations; however, Nögbé Bugles, an Amerindian Panamanian population, showed a low frequency for the -108CC genotype, which means that they are more susceptible to poisoning with organophosphate pesticides. Besides the contribution of our study, we recognize the needs for more extensive studies on the relationship between PON1 variability and the differences in susceptibility to pesticide poisoning and the development of vascular diseases.

ACKNOWLEDGMENTS

This study was sponsored by SENACYT-Panamá through grant No P-06-0013-A. We would like to thank to the Department of Biochemistry and Nutrition, Faculty of Medicine, University of Panama, for allowing the determination of the enzyme activities and to the Laboratory of Genetics and Molecular Biology of the University of Panama (VIP), for the determinations of the polymorphisms.

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Recibido agosto de 2009, aceptado octubre de 2009.