Genetic Variants of the Paraoxonases (PON1 and PON2) in the Chilean Population

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Abstract We estimated the frequencies of PON1 and PON2 variants (linked genes) in two hospital samples taken from the northern (San José Hospital, SJH) and eastern (Clínica Las Condes, CLC) parts of Santiago, Chile, using the polymerase chain reaction followed by restriction endonuclease digestion. The two hospital samples have different degrees of Amerindian admixture (SJH, 34.5%; CLC, 15.9%), which is reflected in the observed frequencies of the *PON1*B* allele (SJH, 43.1%; CLC, 33.7%) and the *PON2*S* allele (SJH, 86.3%; CLC, 77.6%); both allele frequencies are significantly different between samples. The frequencies of the combined PON1–PON2 genotypes **A/*B-*C/*C, *A/*B-*S/*S,* and **B/*B-*S/*S* and of the haplotypes *PON*A,C* and *PON*B,S* were significantly different between the SJH and CLC groups. None of the genotype frequencies deviated significantly from those predicted by the Hardy–Weinberg equation. No linkage disequilibrium was found between the PON1 alleles and any of the PON2 alleles in either group (all $p > 0.05$). In our samples 38.52% (SJH) and 26.25% (CLC) of chromosomes must have the haplotype *PON*B,S*, presumed to be related to the risk of coronary artery disease. Twenty-four of 193 (12.4%) SJH individuals and 7 of 122 (5.7%) CLC individuals were homozygotes for this haplotype. Finally, our data indicate ethnic-group-dependent genetic differences in the vulnerability to toxic organophosphorus.

The paraoxonase gene family contains at least three members—PON1, PON2, and PON3—all located on chromosome 7q21.3–22.1 (Mochizuki et al. 1998). The PON1 gene product is serum paraoxonase (EC 3.1.1.2), which hydrolyzes paraoxon, a commonly used insecticide, to p-nitrophenol and diethylphosphate (La Du 1992). The PON1 product circulates on a subfraction of high-density lipoproteins and appears to use phospholipids of both low- and high-density lipoprotein particles as a physiological substrate. This functional relationship might explain the reported association between common variants of the PON1 gene and phenotypes related to atherosclerosis and lipoprotein metabolism (Hegele 1999).

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In contrast, PON2 mRNA is expressed ubiquitously, and to date there are no mechanistic experiments that yield insights into its physiological role. However, there have been reports of association between variation in PON2 and some quantitative metabolic phenotypes, such as plasma lipoproteins, plasma glucose, and birth weight (Hegele 1999). At present, the function of the PON3 gene product is poorly understood (Hegele 1999).

PON1 has been extensively studied in the field of toxicology because of its ability to detoxify organophosphate insecticides and nerve gases (Humbert et al. 1993). It is genetically polymorphic; the two common isoforms differ by the substitution of the amino acid Gln → Arg at codon 192 (Davies et al. 1996). Individuals homozygous for Gln (the *PON1*A* allele) have a lower PON1 activity than individuals homozygous for Arg (the *PON1*B* allele), who have eight times more activity than individuals homozygous for Gln in hydrolyzing paraoxon. The Arg192 isoform hydrolyzes paraoxon rapidly, but it hydrolyzes diazoxon, soman, and especially sarin slowly. In contrast, the Gln192 isoform hydrolyzes paraoxon slowly, but it hydrolyzes diazoxon, soman, and sarin more rapidly (Humbert et al. 1993). Sanghera et al. (1998) described a common polymorphism at codon 311 of the PON2 gene [cys311 *(PON2*C*) to ser311 (*PON2*S*)]. They found that *PON1*B* and *PON2*S* alleles contribute synergistically to coronary heart disease risk in Asian Indians.

The frequency of the *PON1*B* allele has been found to range from 0.23 to 0.32 in different European populations (Roy et al. 1991; Motti et al. 2001). Limited research has been carried out in Asian and African populations (Roy et al. 1991; Motti et al. 2001). The frequency of *PON1*B* was found to be very low in most Asian populations: 0.14 in Chinese and 0.04 in Filipinos (Roy et al. 1991). In Chile a similar low frequency of *PON1*B* has been reported in Atacameño Indians of Asian origin (Goedde et al. 1984). This is in contrast to a very high frequency of the allele reported in the Indonesian (0.91) and Korean (0.696) populations (Roy et al. 1991). The gene frequency for *PON1*B* in African populations is 0.47 in Sudan and 0.76 in Ghana (Roy et al. 1991). There are few studies of the PON2 locus in the world. The frequencies reported for allele *PON2*S* in Dutch, Italian, Canadian, and Asian populations are 0.74, 0.65, 0.88, and 0.61, respectively (Sanghera et al. 1998; Motti et al. 2001; Boright et al. 1998; Leus et al. 1999).

In Chile allele frequencies for genetic markers are known to vary according to socioeconomic level. For example, the *ABO*A* allele of the ABO blood group and the *RHD*d* allele of the Rh system are more frequent in the higher socioeconomic level, which has more genes of European origin, whereas native Amerindian alleles are more prevalent in the lower socioeconomic stratum (Valenzuela 1988). This is explained by the origin of the Chilean population, which is a mixture of Spanish and Chilean aborigines. Since colonization (1541), aborigines and Mestizos were considered to belong to a lower social class than the Spanish, for whom the government positions, property ownership, and jobs with the highest prestige and power were reserved. Hard labor was given first to aborigines

and then to Mestizos. These factors led to a cline of mixture between the two extreme classes (aborigines and Spanish) (Valenzuela 1988).

The objective of this study is to estimate the allele frequencies of PON1 and PON2 in two urban subpopulations of Santiago, Chile, according to socioeconomic status, and to compare these frequencies with those of other populations. There are no studies for the PON genes in Chilean populations; therefore this information has practical relevance because it allows estimation of the expected frequency of individuals at risk for coronary heart disease, atherosclerosis, and other diseases in Chileans.

Materials and Methods

Blood samples, collected in tubes containing EDTA, were obtained from healthy individuals at the San José Hospital (SJH; northern Santiago), which belongs to the National Health Service and serves mainly low socioeconomic classes, and from healthy individuals at the Clínica Las Condes (CLC; eastern Santiago), a private hospital that serves principally high socioeconomic classes.

To determine the PON1 and PON2 variants, we isolated DNA fragments using routine methods and amplified them using the polymerase chain reaction (PCR). Four oligonucleotide primers were synthesized specifically to amplify the polymorphic region of PON1 (sense primer 5′-TATTGTTGCTGTGGGACCTGAG-3′ and antisense primer 5′-CACGCTAAACCCAAATACATCTC-3′) and PON2 (sense primer 5′-ACATGCATGTACGGTGGTCTTATA-3′ and antisense primer 5′-AGCAATTCATAGATTAATTGTTA-3′). The PON1 and PON2 polymorphisms were detected by digesting the PCR-amplified product (PON1, 99 pb; PON2, 262 pb) with the restriction enzymes *Alw*I and *Dde*I. DNA fragments were separated in agarose gels (3%) containing ethidium bromide, with a DNA molecular weight marker (100 pb, New England Biolabs) for comparison (Humbert et al. 1993; Sanghera et al. 1998).

Allele frequencies were calculated using allele counting, and haplotype frequencies of the polymorphisms were estimated using the maximum-likelihood method (Hartl and Clark 1988). The chi-square test was used to compare genotype distributions and allele frequencies between the studied groups and to test for Hardy–Weinberg equilibrium in the genotype distributions (Hartl and Clark 1988).

For both the SJH and CLC groups the percentage of aboriginal admixture was estimated (Bernstein 1937), using ABO and Rh (anti-D serum) systems as genetic markers and published estimates of their frequencies in the parental populations: $ABO^*O = 0.65$ and $RHD^*d = 0.41$ for the Spanish population (Campillo 1976) and $ABO^*O = 0.98$, $RHD^*d = 0.0$ for Chilean aborigines (Matson et al. 1967; Llop and Rothhammer, 1988). A linkage disequilibrium coefficient was used to estimate the degree of linkage disequilibrium between the alleles of the PON1 and PON2 genes in each group (Weir 1990). The nominal level of significance for all analyses was $p < 0.05$.

Results and Discussion

Table 1 presents the genotype, allele, and haplotype frequencies for the different variants of PON1 and PON2 and for both loci together (PON1–PON2) and the Amerindian component in the two sample hospitals based on the ABO and Rh systems. The results reveal that the degree of Amerindian admixture in the SJH group is higher than the degree in the CLC group, as expected. The *PON1*A* and *PON2*S* alleles are more frequent in both groups. The frequencies of the genotypes *PON1*A/*A*, *PON1*B/*B*, *PON2*C/*C,* and *PON2*S/*S* were significantly different between the samples. The frequencies of the *PON1*B* and *PON2*S* alleles in the SJH group were significantly higher than the frequencies observed in the CLC group. The frequencies of the combined PON1–PON2 genotypes **A/*B-*C/*C, *A/*B-*S/*S,* and **B/*B-*S/*S* and of the haplotypes *PON*A,C* and *PON*B,S* were significantly different between the SJH and CLC groups. None of the genotype frequencies deviated significantly from those predicted by Hardy–Weinberg equilibrium. There was no linkage disequilibrium between the PON1 alleles and any of the PON2 alleles in either group (all $p > 0.05$).

Our results for the PON1 locus are logical, considering that genetic markers in the Chilean population generally show allele frequencies between the ones observed in European whites and Amerindians and that the percentage of Amerindian admixture in the CLC group is lower than in the SJH group. CLC individuals presented allele frequencies for the PON1 locus nearer to those for the European populations than SJH individuals did. This demonstrates once again that the proportions of European and Amerindian genes, which contributed to the Chilean population, are different in the different socioeconomic strata.

We conclude that the frequencies of *PON1*B* in SJH and CLC individuals are due to admixture of an ancestral Spanish population with an allele frequency similar to those currently found in Europe, with an ancestral indigenous population with a frequency similar to that found in Korea (0.7). The frequency of only 0.19 for Indians of the Atacama Desert in northern Chile, reported by Goedde et al. (1984), is incompatible with our results, because the aborigines of central Chile were closely related to those of the Atacama region (Rothhammer and Llop 2003). We suggest that the method used by Goedde et al. (1984) is of limited value compared with the greater degree of discrimination possible with the genotyping method using DNA.

In our samples 38.52% and 26.25% of chromosomes must have the presumed risk-related haplotype *PON*B,S*. Twenty-four out of 193 (12.4%) SJH individuals and 7 out of 122 (5.7%) CLC individuals were homozygotes for this haplotype. This information could be applied to association studies aimed at assessing the role of PON genes and their polymorphisms in many clinical settings.

Finally, our data reveal ethnic-group-dependent genetic differences in the relative frequencies of alleles that have been demonstrated to be associated with interindividual variation in serum paraoxonase activity. This is important because

Table 1. Phenotype Distribution, Allele and Haplotype Frequencies for PON1, PON2, PON1–PON2, ABO, and Rh, and Amerindian Component in the SJH and CLC Samples

	HSJ		*CLC*	
	N	%	*N*	%
PON1				
Phenotype				
A,A	59	30.26	52	40.31
A,B	104	53.33	67	51.94
B,B	32	16.41	10	7.75
Total	195		129	
Allele				
*PON1*B*		43.08		33.72
PON2				
Phenotype				
C,C	3	1.49	9	7.20
C,S	49	24.38	38	30.40
S,S	149	74.13	78	62.40
Total	201		125	
Allele				
*PON2*S*		86.32		77.6
PON1–PON2				
Phenotype				
A,A-C,C	1	0.52	3	2.46
A,A-C,S	18	9.33	15	12.30
A,A-S,S	39	20.21	31	25.41
A,B-C,C	2	1.04	6	4.92
A,B-C,S	21	10.88	20	16.39
A,B-S,S	80	41.45	37	30.33
B,B-C,S	8	4.15	3	2.46
B,B-S,S	24	12.44	7	5.74
Total	193		122	
Haplotype				
*PON*A,S*		47.75		50.79
*PON*A,C*		8.99		15.19
*PON*B,S*		38.52		26.25
*PON*B,C*		4.74		7.77
ABO				
Phenotype				
A,B	2	1.4	2	1.31
A	50	34.97	68	44.44
B	11	7.69	14	9.15
O	80	55.94	69	45.10
Total	143		153	
Allele				
*ABO*A*		20.26		26.49
*ABO*B*		4.66		5.40
*ABO*O*		75.08		68.10

Table 1. Continued.

	HSJ		*CLC*	
	N	%	*N*	%
Rh				
Phenotype				
DD + Dd	130	94.89	137	90.73
dd	7	5.11	14	9.27
Total	137		151	
Allele				
*RHD*d*		22.60		30.45
Amerindian component		34.51		15.87

individuals with low serum paraoxonase activity are more susceptible to the toxic effects of organophosphorus than are individuals with higher activity levels.

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