Original Research

Paraoxonase activity and genetic polymorphisms in northern Han Chinese workers exposed to organophosphate pesticides

Xiaofeng Zhang[1], Hong Sui[1], Haibo Li[2], Jing Zheng[3], Fengqian Wang[1], Baixiang Li[1] and Yang Zhang[1]

[1]School of Public Health, Harbin Medical University, Harbin, 150081 China; [2]The Third Affiliated Hospital, Harbin Medical University, Harbin, 150081 China; [3]Department of Public Health Monitoring, Heilongjiang Provincial Center for Disease Control and Prevention, Harbin, 150030 China

The last two authors contributed equally to this work.

Corresponding authors: Baixiang Li. Email: lbxhyd@163.com; Yang Zhang. Email: zhangyang1962@sina.com

Abstract

Paraoxonase (PON1) is one of the major players in the detoxification of organophosphates (OPs). This study presents our investigation into the effect of OPs on serum PON1 activity and the distribution of common PON1 polymorphisms in Han Chinese workers with repeated high exposure to OP pesticides, and the factors modulating PON1 activity. In all, 400 participants, including 180 workers exposed to OP pesticides occupationally, and 220 controls were investigated. Serum PON1 and cholinesterase (ChE) activity were measured, and genotyping was done using polymerase chain reaction-restriction fragment length polymorphism. The association between PON1 activity and PON1 polymorphisms, and the influencing factors of PON1 activity, were analyzed. The results revealed that repeated OP exposures significantly decreased serum PON1 and ChE activity ($P < 0.05$), although the exposed workers did not complain of health problems. Higher L and R allele frequencies for the L55M and Q192R polymorphisms of PON1 were observed. PON1 polymorphisms (especially the Q192R polymorphism) and pesticide exposures significantly affected serum PON1 activity in the study population. Therefore, the results of this investigation indicate PON1 polymorphisms and pesticide exposures may be important risk predictors for OP poisoning in the Han Chinese population, who display very high frequencies of the M allele and R allele for PON1 polymorphisms at the positions 55 and 192, respectively.

Keywords: Organophosphates, paraoxonase; genetic polymorphisms, Chinese

***Experimental Biology and Medicine* 2014; 239: 232–239. DOI: 10.1177/1535370213513983**

Introduction

Organophosphates (OPs), the esters of phosphoric acid, are widely used as pesticides worldwide for agricultural, domestic and industrial purposes as they are cost-effective and efficient. Almost everyone has some level of background exposure to OPs, but agricultural workers and pesticide-producing workers have the highest exposure. OP poisoning is a progressively distressing phenomenon, as worldwide production and consumption of OP pesticides have doubled in developing countries, including many Asian countries.[1]

OPs are internalized via oral intake, or skin and respiratory exposure, and exert their toxicity by inhibiting acetyl cholinesterase (AChE) at nerve endings leading to accumulation of acetylcholine (ACh) and subsequent over-stimulation of muscarinic and nicotinic receptors. There are studies linking OP exposure to detrimental chronic effects on human health, including impacts on neuropsychological, endocrine and immune function, as well as the potential to cause cancers.[2,3] Some sheep dippers occupationally exposed to OPs over their working life suffer from long-term health effects including bradycardia, hypoventilation and paralysis, among others.[4] In addition, researchers have observed that there is a strong association between OP exposure and neurological symptoms.[4,5] Biomarkers of OP exposure have been developed and include measurements of AChE in erythrocytes and butyryl cholinesterase in plasma, which are collectively referred to as cholinesterases (ChE), as well as urinary dialkyl phosphates.[6,7] Nevertheless, susceptibility biomarkers of OP poisoning are not yet completely established.

The hydrolysis of OPs by serum paraoxonase (PON1) is a major determinant of OP toxicity to vertebrates, including humans. Human serum PON1 can break down OP compounds before they bind to ChE. PON1 derived its name

Copyright © 2013 by the Society for Experimental Biology and Medicine

originally from its ability to hydrolyze paraoxon (an OP compound) and is a member of the A-esterase family that is synthesized in the liver and secreted into the blood where it associates with high-density lipoprotein particles.[8,9] Studies have revealed that PON1 polymorphisms can lead to individual differences in the ability to detoxify OPs metabolized via the cytochrome P450/PON1 pathway.[10,11] The PON1 gene has two major coding region polymorphisms due to amino acid substitutions, the Methionine (M allele) to Leucine (L allele) substitution at position 55 and the Arginine (R allele) to Glutamine (Q allele) interchange at position 192, affecting the catalytic activity of PON1.[12,13]

To our knowledge, there have been many investigations into OP pesticide exposures and health symptoms. Also there are a few studies on genotypic distribution of PON1 polymorphisms in different ethnicities and regional populations including Mexican, American, Spanish and Thai populations, but information is limited with regard to the Chinese population. Therefore, the aims of this study are (1) to assess the effect of OP exposure on serum PON1 activity; (2) to determine the distribution of common PON1 gene polymorphisms (L55M and Q192R) among the northern Han Chinese population, including workers highly exposed to pesticides and (3) to study the potential factors modulating PON1 activity. We also hoped to provide evidence for a biomarker of OP poisoning that can be used to screen susceptible populations.

Material and methods

Study population

Workers in OP insecticide factories from northern China between April 2010 and June 2011 were recruited as the OP exposure population. Workers from food processing companies in the same region were recruited as the control population. All participants were Han Chinese. These workers generally worked 8 h a day for a period of at least 4 months in these industries. All individuals were given detailed information about this study, agreed to participate and signed a consent form. A questionnaire was used by trained interviewers to collect demographic characteristics, smoking habits, alcohol intake, medical history, detailed occupational history, pesticides exposure duration and residential exposure to chemicals. Exposed workers were also asked about usage of personal protective equipment and detailed pesticides exposure at work. All participants underwent a medical interview carried out by medical staff and laboratory testing (liver and kidney function tests). Finally, 180 eligible workers exposed to pesticides and 220 control workers with no evidence of known chronic diseases participated in the study. This study was reviewed and approved by the Ethics Committee of Harbin Medical University in China.

Blood collection and determination

Blood was collected via venipuncture using both vacuum tubes and heparinized vacuum tubes, and then was centrifuged for 10 min at 2500 r/min. The serum were saved at −20°C for laboratory tests, including liver and kidney function indicators (alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, glucose, total protein, albumin, total bilirubin, total cholesterol, triglyceride, lactate dehydrogenase, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, urea nitrogen, creatinine and ChE) and PON1 activity. Liver and kidney function tests were determined using Kits by biochemistry analyzer (Modular PPD, Roche, USA) at the second affiliated hospital of Harbin Medical University, China.

Determination of serum PON1 activity

Serum PON1 activities toward phenyl-acetate and paraoxon were analyzed using spectrophotometry as described previously.[14,15] Briefly, the PON1 activity toward phenyl-acetate was determined at 270 nm by adding 50 μL serum (diluted 1:10) to 1 mL Tris-HCl (0.1 M, pH 8.0) containing 5 mM phenyl-acetate (Sigma, USA) and 2 mM $CaCl_2$. The PON1 activity toward paraoxon was assessed at 25°C and 412 nm, by adding 10 μL serum to 1.2 mL glycine buffer (75 mM, pH 10.5) containing 4 mM paraoxon (O, O diethyl-o-p-nitrophenyl phosphate, Sigma, USA) and 2 mM $CaCl_2$, and then 0.1 M EDTA was added into the buffer to stop the reaction before determination. All samples were run in triplicate and were kept at 4°C before detection.

Determination of PON1 genotype

Genomic DNA of heparinized blood was extracted from peripheral blood leukocytes using the Genomic DNA Mini Preparation Kit with Spin Column (HuaShun, China) following the manufacturer's recommendations. Purified DNA was stored at –20°C for further PCR amplification. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed to determine the genotype of L55M and Q192R polymorphisms of PON1. The 183 bp fragment encompassing L to M and 181 bp fragment encompassing Q to R polymorphic site in the coding region of PON1 were amplified, respectively, using specific primers ($PON1_{55}$: F-5′-TGAAAGACTTAAACTGCCAGTCC-3′ and R-5′-TGGATCCACATCCTGCAATA-3′; $PON1_{192}$: F-5′-CTGTGG GACCTGAGCACTTT-3′ and R-5′-CCATCGGGTGAA ATGTTGAT-3′). The polymorphic region was amplified by PCR on a thermal cycler (Eppendorf 5331, German) in 20 μL reaction solution containing 100 ng of genomic DNA, 2 × Taq PCR Master mix (TianGen, China) and 0.1 μmol of each primer (Invitrogen, USA). The following PCR program was used: 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 s, annealing at 61°C ($PON1_{55}$) or 65°C ($PON1_{192}$) for 30 s and extension at 72°C for 30 s and a final extension at 72°C for 5 min. After confirmation of successful PCR amplification, each PCR product was digested overnight with NIaIII (NEB, USA) for $PON1_{55}$ or AlwI (NEB, USA) for $PON1_{192}$, according to the manufacturer's protocol and analyzed by 2.5% agarose gel electrophoresis. For quality control, genotyping was conducted without knowledge of the exposure/control status of the subjects, and a 5% random sample from each group was genotyped twice by different persons and the reproducibility was 100%.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation (SD) while qualitative values were expressed as percentages. Student's *t*-test (for continuous variables) and Chi square (χ^2) test (for categorical variables) were used to evaluate differences between exposure and control population. The Hardy-Weinberg equilibrium was evaluated using Pearson's χ^2 test. The χ^2 test was used to determine if the observed genotype frequencies deviated from Hardy-Weinberg equilibrium expectations and to evaluate the significance of the linkage disequilibrium between each polymorphism pair. ANOVA was used to test for differences in parameters between genotypes. Multiple regression analysis was used to determine the influencing factors of PON1 activities. A *P* value of less than 0.05 was considered statistically significant. All the statistical analyses were conducted with SPSS version 13.0 for Windows software.

Results

Characteristic of the study population

Detailed characteristics of the study population are shown in Table 1. The two populations have similar characteristics, except that the gender ratio is opposite between the two groups. In the population exposed to pesticides, the participants took part in the production, mixture and subpackaging of OP pesticides with personal protective equipment (gauze masks, gloves, caps and work clothes). Participants were mainly exposed to insecticides (Fenitrothion, Omethoate and Phorate, etc.) and herbicides (Glyphosate) during this investigation. In the control population, the subjects were not exposed to the pesticides and chemicals while working. It is important to note that all individuals had similar background exposure to residential chemicals aperiodically. The results of serum liver and kidney function tests did not differ between the exposure and control populations (data not shown). However, serum ChE activity in the control population was higher than that in the exposure population (Table 2).

Serum PON1 activity

The serum PON1 activities toward phenyl-acetate and paraoxon substrates in the study population are shown in Table 2. There were significant differences in PON1 activities toward these two substrates and ChE activity between the exposure and control populations. Although the serum PON1 activity toward paraoxon in exposed population and ChE activity in both populations for men was higher than that for women ($P < 0.05$), they could not provide the helpful information related to this study. There is a similar variation (3.4-fold) in serum PON1 activity toward phenyl-acetate among exposure individuals (70.94–244.03 μmol/min/mL) and control individuals (73.21–254.85 μmol/min/mL). However, there is a 16.7-fold variation in serum PON1 activity toward paraoxon among exposure individuals (13.01–217.54 nmol/min/mL) and a 2.6-fold variation among the control population (90.78–236.41 nmol/min/mL).

PON1 genotype and allele frequencies

Table 3 and Figure 1 show the genotype distribution of PON1 polymorphisms at position 55 and 192 in the study populations. The genotype frequencies of these two polymorphisms conformed to Hardy-Weinberg equilibrium expectations in the two populations (χ^2 test, $P > 0.05$). There were no differences of genotype frequencies of L55M and Q192R polymorphism of PON1 between exposure and control populations. The most common genotype of the study population at both positions was LL and RR/QR, respectively, whereas the rarest genotypes were MM and QQ homozygotes. The random combination of L and M alleles at position 55 and R and Q at position 192 is expected to give rise to nine possible combinations. The most common genotype combination of PON1 polymorphisms were 55LL/192RR and 55LL/192QR in this population, while 55MM/192XX (X = R or Q) carriers do not exist or are very rare in this population (Figure 2).

Table 1 Characteristics of the study population

		Exposure population		Control population		
Parameters		**N**	**%**	**N**	**%**	***P***
Gender	Male	85	47.2	118	53.4	0.202
	Female	95	52.8	102	46.6	
Smoking status	Smoker	57	31.7	55	25.0	0.398
	Non-smoker*	123	68.3	165	75.0	
Drinking status	<30 g/day (Non-drinking)	109	60.6	135	61.5	0.303
	≥30 g/day (Drinking)	71	39.4	85	38.5	
Age (years)		38.0 ± 8.60		38.2 ± 8.06		0.813
Pesticide exposures duration (years)		4.8 ± 3.02		NA		
Personal protection equipment		180	100%	NA		

NA: not applicable.
*Including individuals who have given up smoking over 2 years.

Table 2 Serum PON1 and ChE activity in the study population

		PON1 activity toward phenyl-acetate (μmol/min/mL)	PON1 activity toward paraoxon (nmol/min/mL)	ChE activity (U/L)
Exposure population	Average	156 ± 36.4*	121 ± 40.6*	7761 ± 1852*
	Men	161 ± 37.0	129 ± 40.9†	8436 ± 1971†
	Women	151 ± 35.5	114 ± 39.3	7158 ± 1510
Control population	Average	168 ± 41.5	158 ± 46.2	8911 ± 1656
	Men	172 ± 41.1	162 ± 42.1	9533 ± 1512†
	Women	163 ± 41.6	155 ± 49.5	8192 ± 1523

*Compared to control population, $P < 0.05$.
† Compared to women within the group, $P < 0.05$.

Table 3 Genotype and allele frequencies of PON1 polymorphisms in the study population

		Exposure population	Control population	χ^2	*P*
Frequency of $PON1_{55}$ genotype (%)	LL	88.3	87.7	5.2200	0.073
	LM	7.78	11.4		
	MM	3.89	0.91		
Allele frequency	L	0.92	0.93	0.4215	0.5162
	M	0.08	0.07		
Frequency of $PON1_{192}$ genotype (%)	RR	44.4	44.6	1.3880	0.4996
	QR	40.0	38.6		
	QQ	15.6	16.8		
Allele frequency	R	0.64	0.64	1.0559	0.3042
	Q	0.36	0.36		

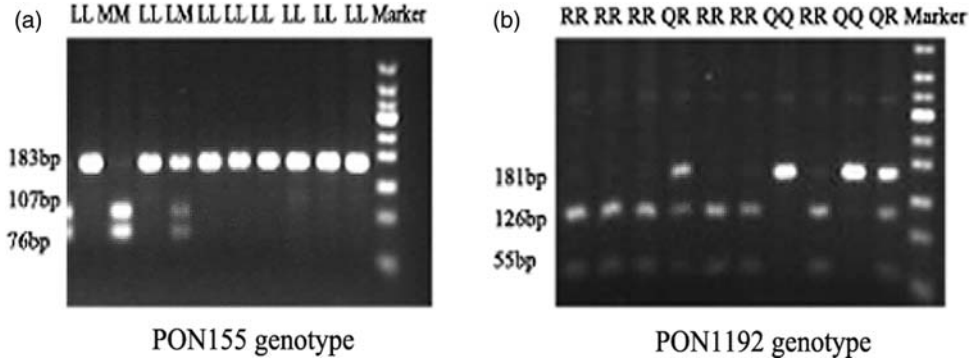

Figure 1 Genotype of PON1 polymorphisms at positions 55 and 192. $PON1_{55}$ genotype: samples with single 183 bp were scored as LL homozygote, samples digested into two fragments, 107 bp and 76 bp, as MM homozygote, and 183 bp, 107 bp and 76 bp as LM heterozygote. $PON1_{192}$ genotype: samples with single 181 bp were scored as QQ homozygote, those digested into two fragments, 126 bp and 55 bp, as RR homozygote, and 181 bp, 126 bp and 55 bp as QR heterozygote

Influence of PON1 polymorphisms on serum PON1 activity

According to the different genotypes, serum PON1 activities toward phenyl-acetate and paraoxon are presented in Figure 3. The 55LL and 192RR and the 55MM and 192QQ genotypes showed the highest and lowest PON1 activities, respectively, whereas the 55LM and 192QR heterozygotes had intermediate activity in the two populations. In the combined genotype groups (Table 4), PON1 activities toward phenyl-acetate and paraoxon substrates were significantly lower in the LL/RR and LL/QR genotypes for the pesticide exposures group compared to the control group ($P < 0.05$). Both the $PON1_{55}$ and $PON1_{192}$ polymorphisms independently affected PON1 activities in the

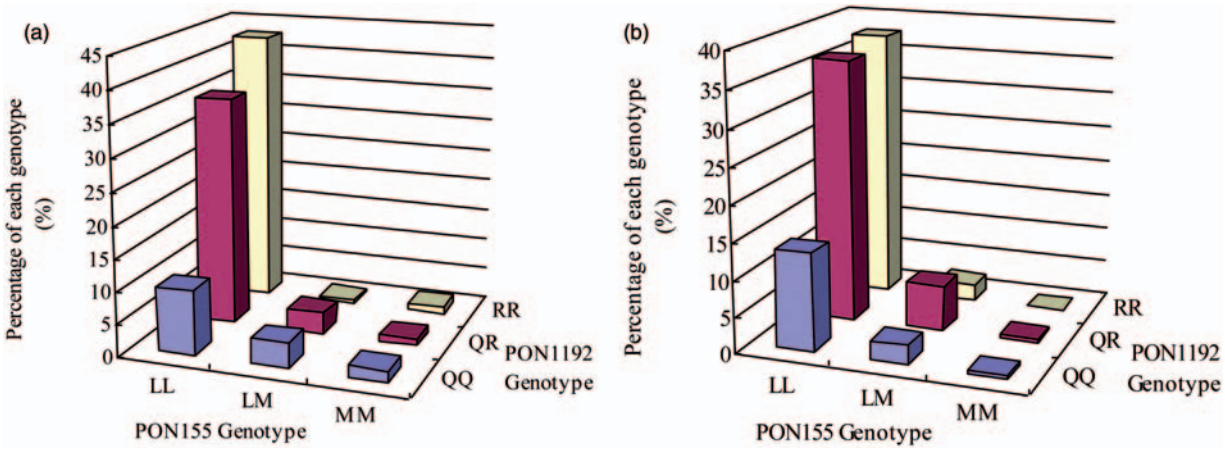

Figure 2 Genotype combination for PON1 polymorphisms in the study population. (a) Exposure population; (b) Control population. (A color version of this figure is available in the online journal.)

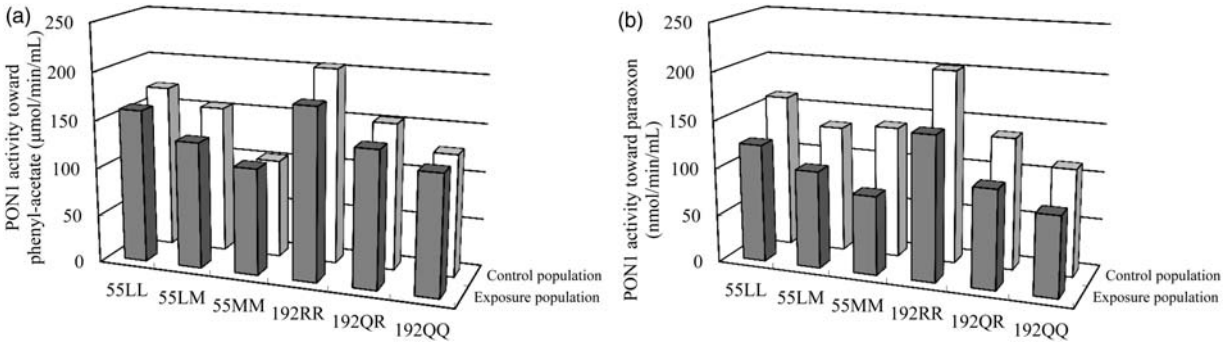

Figure 3 Effect of PON1 polymorphisms on PON1 activities toward phenyl-acetate (a) and paraoxon (b)

Table 4 PON1 activities according to $PON1_{55}$ and $PON1_{192}$ genotype

$PON1_{55/192}$ genotype	N (Exposure/ Control)	PON1 activity toward phenyl-acetate (μmol/min/mL)		PON1 activity toward paraoxon (nmol/min/mL)	
		Exposure	Control	Exposure	Control
LL/RR	77/80	179 ± 28.6*	203 ± 27.7	151 ± 29.2*	204 ± 33.0
LL/QR	64/83	142 ± 31.1*	154 ± 31.0	105 ± 31.5*	138 ± 26.9
LL/QQ	18/30	139 ± 28.3	129 ± 27.4	77.8 ± 24.6	113 ± 27.8
LM/RR	1/5	170	210 ± 15.7	171	169 ± 7.79
LM/QR	6/14	153 ± 10.4	147 ± 31.8	83.5 ± 43.8	134 ± 28.0
LM/QQ	7/6	108 ± 23.1	125 ± 32.9	107 ± 18.6	103 ± 7.05
MM/RR	2/0	150 ± 18.3	—	119 ± 1.55	—
MM/QR	2/1	112 ± 9.53	104	78.3 ± 6.48	165
MM/QQ	3/1	86.2 ± 8.21	102	61.4 ± 2.03	161

Compared to control population *$p < 0.05$.

two populations, however, the $PON1_{192}$ polymorphism had a higher contribution to PON1 activity than the $PON1_{55}$ polymorphism.

Parameters influencing serum PON1 activities

Predictors influencing serum PON1 activity, including gender, age, smoking and drinking, pesticide exposures, $PON1_{55}$ and $PON1_{192}$ polymorphisms, as well as serum ChE activity, were analyzed using multiple regression analysis with stepwise screening (Table 5). There is a linear relationship between serum PON1 activity towards phenyl-acetate and pesticide exposures, and between PON1 L55M and PON1 Q192R polymorphisms ($F = 59.24$, adjusted $R^2 = 0.4669$). Therefore, it is thought that these

Table 5 Parameters influencing serum PON1 activities toward two substrates

	phenyl-acetate (adjusted $R^2 = 0.4669$)					Paraoxon (adjusted $R^2 = 0.6002$)				
Predictor variable	**RC**	**SE**	***t***	***P***	**SPRC**	**RC**	**SE**	***t***	***P***	**SPRC**
Gender	−7.311	3.355	−2.18	0. 299	−0.092	−1.819	3.491	−0.52	0.6027	−0.019
Age	1.171	2.943	0.40	0.6909	0.015	−1.353	3.062	−0.44	0.6589	−0.014
Smoking	2.555	3.698	0.69	0.4900	0.029	0.369	3.848	0.10	0.9237	0.003
Drinking	−1.454	3.450	−0.42	0.6737	−0.017	0.058	3.590	0.02	0.9871	0.001
Pesticide exposures	13.54	3.012	4.49	<0.0001	0.170	40.63	3.045	13.35	<0.0001	0.426
$PON1_{55}$ MM	−38.60	10.15	−3.80	0.0002	−0.144	−11.47	10.56	−1.09	0.2781	−0.036
$PON1_{55}$ LM	−4.583	5.059	−0.91	0.3655	−0.034	−5.941	5.264	−1.13	0.2598	−0.037
$PON1_{192}$ QQ	−62.20	4.415	−14.09	<0.0001	−0.578	−77.36	4.594	−16.84	<0.0001	−0.601
$PON1_{192}$ QR	−44.15	3.223	−13.70	<0.0001	−0.550	−56.18	3.354	−16.75	<0.0001	−0.585
Serum ChE activity	0.024	0.016	1.53	0.1268	0.058	0.002	0.017	0.10	0.9203	0.003

RC: Regression coefficient; SE: Standard error; SPRC: Standard partial regression coefficient.

factors were significant contributors to serum PON1 activity towards phenyl-acetate in the study population. The sequence of parameters influencing PON1 activity toward phenyl-acetate was $PON1_{192}$ QQ > $PON1_{192}$ QR > $PON1_{55}$ MM > pesticide exposures > $PON1_{55}$ LM. Also, pesticide exposures and the $PON1_{192}$ polymorphisms showed significant contributions to serum PON1 activity towards paraoxon in the two populations ($F = 120.78$, adjusted $R^2 = 0.6002$). The decrement sequence of parameters influencing PON1 activity toward paraoxon was $PON1_{192}$ QQ, $PON1_{192}$ QR and pesticide exposures. Although there was no significant association of L55M with PON1 activity toward paraoxon, Q192R conferred significant risk of PON1 activities toward phenyl-acetate and paraoxon in the overall study population. So in the present study, PON1 polymorphisms and pesticide exposures are thought to be factors in modulating human serum PON1 activity.

Discussion

Risk assessment of pesticides involves the biological indicators of exposure (pesticides or their residues in biological fluids), effect (measurement of enzymes) and susceptibility biomarkers (polymorphisms of enzymes interacting with pesticides). In the present study, we used serum ChE activity, one of the biomarkers of OP exposures, to assess the exposure level of OPs in the study population. The results showed that the serum ChE activity of the workers with repeated exposures to OP pesticides was significantly lower than that of the control workers ($P < 0.05$), although the observed workers exposed to OPs did not complain of health problems. This suggests that the effect of chronic OP exposure on an individual's health may be insidious, but that OP exposure affects ChE activity earlier.

Individuals with specific defects in OP metabolism might be expected to be at greater risk of adverse health effects following exposure. A series of studies in rodents have provided important evidence to ascertain the role of PON1 in modulating OP toxicity.[16] Mice lacking PON1 activity are more susceptible to oxon and parent OP-induced toxicity, and animals that received exogenous PON1 are more resistant to the cholinergic toxicity than controls when challenged with OPs.[17,18] PON1 knockout mice, which have no detectable hydrolytic activity toward paraoxon and diazoxon, have increased sensitivity to the toxicity of chlorpyrifos and diazoxon, as well as small increased sensitivity to their parent compounds. However, they do not show any increased sensitivity to paraoxon, so PON1 activity is thought to be substrate sensitivity-dependent.[18,19] In this study, PON1 activities toward phenyl-acetate and paraoxon substrates are significantly decreased in the population exposed to OPs, compared to the controls. Although the pesticide-producing workers had personal protection equipment while working, this equipment was not effective in protecting the participants. The workers also lacked education in self-protection against insecticides. Thus, there was a decrease of serum PON1 activity due to repeated OP pesticide exposure.

There is a wide range of individual variation (10–40 fold) of serum PON1 activity in humans. In our samples, there was a 17-fold variation in PON1 activity toward paraoxon in the population. To a great extent, this variation is due to PON1 gene polymorphisms. PON1 polymorphisms are of special interest to researchers as they are genetically determined and may predispose individuals to an increased risk when exposed to OP compounds. In this investigation, we detected PON1 polymorphisms (L55M and Q192R) in the Han Chinese population and found that L and M, as well as Q and R allele frequencies were similar to those of Japanese,[20,21] Korean,[21] Malay[22,23] and Mexican populations,[24] who displayed very low frequencies of M allele and high frequencies of R allele ($M < 0.10$, $R = 0.60$). However, these results were different from those of Indian,[25] Europeans of white ancestry[26–29] and Caucasian populations ($M = 0.25$–0.40, $R = 0.25$–0.35).[13,30,31] Although Q and R allele frequencies for the PON1 Q192R polymorphism in Thai populations that were observed in two studies were inconsistent ($R = 0.29$ and 0.61, respectively), the difference was not found in L and M allele frequencies for PON1 L55M polymorphisms between the population in our study and the Thai populations.[32,33] Our results support the previous studies that revealed inter-population

differences in allele frequencies for PON1 polymorphisms[34] and also partly provide evidence that Asiatics might share a common ancestral origin because the populations from Asia demonstrate stronger genetic affinities of PON1.

In addition, we observed significant variations in serum PON1 activity depending on genetic polymorphisms of PON1. PON1 activity increased as a function of genotype in the order 192RR > 192QR > 192QQ and 55LL > 55LM > 55MM, but the Q192 R polymorphism was found to have a higher contribution to PON1 activity than the L55M polymorphism. That is to say, high levels of serum PON1 activity can increase resistance to OPs when individuals are confronting OP compounds, and there is better protection with 55LL and 192RR isoforms than 55MM and 192QQ isoforms, respectively. This finding is in agreement with other populations.[35,36] Therefore, it is thought that PON1 genetic variation at the coding region affects OP metabolism, and is regarded as a positive predictor of PON1 activity. As a result, genotyping individuals for PON1 polymorphisms (L55M and Q192R) may provide a method for the identification of individuals at higher risk for OP poisoning.

At the same time, serum PON1 activity among individuals with the same genotype differed in this investigation. There was a 5.9-fold and 4.9-fold variation of serum PON1 activity toward paraoxon among the individuals with a single genotype (55LL genotype) in the exposure and control populations, respectively. Other studies have revealed that this difference in PON1 activity is up to 13-fold among the individuals with the same genotype.[35,37] It is likely that other contributors, such as nutritional, pharmacological and environmental factors, account for the discrepancies.[38,39]

In this study, pesticide exposure significantly affected serum PON1 activity, except for PON1 genetic variations. However, there was no significant contribution of other predictors considered in this study to enzyme activity. Although ChE activity is a important parameter to reflect OP exposure, there was no significant contribution of ChE activity to PON1 activities, which is in line with the results from Ellison et al.[40] So far there are some investigations of the association between PON1 polymorphisms and risk of diseases (such as coronary heart disease, diabetes mellitus and cerebral infarction) in the Chinese population,[41–43] but there is little information on PON1 activity and the association between PON1 status and sensitivity to OP toxicity in the Chinese population who are occupationally exposed to pesticides. Our current investigation might be the first study to show an association between PON1 polymorphisms and susceptibility to OP toxicity in China.

Long-term OP exposure decreases serum PON1 activity, which is also modulated by PON1 polymorphisms. L and R genotype frequencies for PON1 coding region (L55M and Q192R) polymorphisms are higher in the northern Han Chinese population compared to Europeans, suggesting the lower sensitivity to OP toxicity in Chinese than in Europeans. Our study also supports evidence for the use of PON1 polymorphisms to monitor the sensitivity of occupational workers to OP pesticides. However, much more work is needed to support the potential usefulness of PON1 genotyping as a biomarker of susceptibility to OP poisoning. Moreover, although the results of the present study are suggestive, it is necessary to conduct further studies that take into account more factors (such as other metabolic pathways of OPs) to identify the main variables influencing serum PON1 activity. The identification of these variables is useful for those populations who are more susceptible to the adverse effects of OP pesticides.

Authors' contributions: XZ participated in the experimental design, preparation of the manuscript and performed the questionnaire investigation of study population. HS was responsible for statistical analysis. HL and FW performed the test of *PON1* gene polymorphisms. JZ participated in the determination of serum PON1 activity. This study and its design were conceived by BL and YZ, who coordinated the project, participated in the experimental design and helped to draft the manuscript. All authors have read and approved the final manuscript.

ACKNOWLEDGEMENTS

We would like to thank the people who have participated in the study. We are grateful to Stuart Brain Carter at the school of Medicine, New York University, USA, for critically reading and revising the manuscript. This study was supported by the National Nature Science Foundation of China (grant number 30901207).

REFERENCES

1. Soares WL, Porto MF. Pesticide use and economic impacts on health. *Rev Saude Publica* 2012;**46**:209–17
2. Murphy HH, Hoan NP, Matteson P, Abubakar AL. Farmers' self-surveillance of pesticide poisoning: a 12-month pilot study in North Vietnam. *Int J Occup Environ Health* 2002;**8**:201–11
3. Ross SM, McManus IC, Harrison V, Mason O. Neurobehavioral problems following low-level exposure to organophosphate pesticides: a systematic and meta-analytic review. *Crit Rev Toxicol* 2013;**43**:21–44
4. Pilkington A, Buchanan D, Jamal GA, Gillham R, Hansen S, Kidd M, Hurley JF, Soutar CA. An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers. *Occup Environ Med* 2001;**58**:702–710
5. Hernández AF, López O, Rodrigo L, Gil F. Changes in erythrocyte enzymes in humans long term exposed to pesticides: influence of several markers of individual susceptibility. *Toxicol Lett* 2005;**159**:13–21
6. Kamanyire R, Karalliedde L. Organophosphate toxicity and occupational exposure. *Occup Med* 2004;**54**:69–75
7. Sudakin DL, Stone DL. Dialkyl phosphates as biomarkers of organophosphates: the current divide between epidemiology and clinical toxicology. *Clin Toxicol* 2011;**49**:771–781
8. Cole TB, Jansen K, Park S, Li WF, Furlong CE, Costa LG. The toxicity of mixtures of specific organophosphate compounds is modulated by paraoxonase 1 status. *Adv Exp Med Biol* 2010;**660**:47–60
9. La Du BN, Billecke S, Hsu C, Haley RW, Broomfield CA. Serum paraoxonase (PON1) isozymes: The quantitative analysis of isozymes effecting individual sensitivity to environmental chemicals. *Drug Metab Disp* 2001;**29**:566–9
10. Costa LG, Giordano G, Cole TB, Marsillach J, Furlong CE. Paraoxonase 1 (PON1) as a genetic determinant of susceptibility to organophosphate toxicity. *Toxicology* 2013;**307**:115–22
11. Tsatsakis AM, Androutsopoulos VP, Zafiropoulos A, Babatsikou F, Alegakis T, Dialyna I, Tzatzarakis M, Koutis C. Associations of xenobiotic-metabolizing enzyme genotypes PON1Q192R, PON1L55M and

CYP1A1*2A MspI with pathological symptoms of a rural population in south Greece. *Xenobiotica* 2011;**41**:914–25
12. Altuner D, Suzen SH, Ates I, Koc G.V, Aral Y, Karakaya A. Are PON1 Q/R 192 and M/L 55 polymorphisms risk factors for diabetes complications in Turkish population? *Clin Biochem* 2011;**44**:372–6
13. Aydin M, Gencer M, Cetinkaya Y, Ozkok E, Ozbek Z, Kilic G, Orken C, Tireli H, Kara I. PON1 55/192 polymorphism, oxidative stress, type, prognosis and severity of stroke. *IUBMB Life* 2006;**58**:165–72
14. Richter RJ, Furlong CE. Determination of paraoxonase (PON1) status requires more than genotyping. *Pharmacogenetics* 1999;**9**:745–53
15. Tomas M, Senti M, García-Faria F, Vila J, Torrents A, Covas M, Covas M, Marrugat J. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol* 2000;**20**:2113–9
16. Li WF, Furlong CE, Costa LG. Paraoxonase protects against chlorpyrifos toxicity in mice. *Toxicol Lett* 1995;**76**:219–26
17. Foxenberg RJ, McGarrigle BP, Knaak JB, Kostyniak PJ, Olson JR. Human hepatic cytochrome p450-specific metabolism of parathion and chlorpyrifos. *Drug Metab Dispos* 2007;**35**:189–93
18. Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 1998;**394**:284–7
19. Li WF, Costa LG, Richter RJ, Hagen T, Shih DM, Tward A, Lusis AJ, Furlong CE. Catalytic efficiency determines the in vivo efficacy of PON1 for detoxifying organophosphates. *Pharmacogenetics* 2000;**10**:767–79
20. Suehiro T, Nakamura T, Inoue M, Shiinoki T, Ikeda Y, Kumon Y, Shindo M, Tanaka H, Hashimoto K. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. *Atherosclerosis* 2000;**150**:295–8
21. Ueno T, Shimazaki E, Matsumoto T, Watanabe H, Tsunemi A, Takahashi Y, Mori M, Hamano R, Fujioka T, Soma M, Matsumoto K, Kanmatsuse K. Paraoxonase1 polymorphism Leu- Met55 is associated with cerebral infarction in Japanese population. *Med Sci Monit* 2003;**9**:208–12
22. Chia SE, Mohamed Ali S, Yap PH, Gan L, Ong YB, Chia KS. Distribution of PON1 polymorphisms PON1Q192R and PON1L55M among Chinese, Malay and Indian males in Singapore and possible susceptibility to organophosphate exposure. *Neurotoxicology* 2009;**30**:214–9
23. Naidu R, Har YC, Taib NA. Genetic polymorphisms of paraoxonase 1 (PON1) gene: association between L55M or Q192R with breast cancer risk and clinico-pathological parameters. *Pathol Oncol Res* 2010;**16**:533–40
24. López-Flores I, Lacasaña M, Blanco-Muñoz J, Aguilar-Garduño C, Sanchez-Villegas P, Pérez-Méndez OA, Gamboa-Avila R. Relationship between human paraoxonase-1 activity and PON1 polymorphisms in Mexican workers exposed to organophosphate pesticides. *Toxicol Lett* 2009;**188**:84–90
25. Shin BS, Oh SY, Kim YS, Kim KW. The paraoxonase gene polymorphism in stroke patients and lipid profile. *Acta Neurol Scand* 2008;**117**:237–43
26. Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik G.P, Furlong CE. Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am J Hum Genet* 2001;**68**:1428–36
27. Hernández AF, Mackness B, Rodrigo L, López O, Pla A, Gil F, Durrington PN, Pena G, Parrón T, Serrano JL, Mackness MI. Paraoxonase activity and genetic polymorphisms in greenhouse workers with long term pesticide exposure. *Hum Exp Toxicol* 2003;**22**:565–74
28. Leviev I, James RW. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arterioscler Thromb Vasc Biol* 2000;**20**:516–21
29. Sanghera DK, Saha N, Kamboh MI. The codon 55 polymorphism in the paraoxonase1 gene is not associated with the risk of coronary heart disease in Asian Indian and Chinese. *Atherosclerosis* 1998;**136**:217–23
30. Can Demirdogen B, Turkanoglu A, Bek S, Sanisoglu Y, Demirkaya S, Vural O, Arinc E, Adali O. Paraoxonase/arylesterase ratio, PON1 192Q/R polymorphism and PON1 status are associated with increased risk of ischemic stroke. *Clin Biochem* 2008;**41**:1–9
31. Schiavon R, Turazzini M, De Fanti E, Battaglia P, Targa L, Del Colle R, Fasolin A, Silvestri M, Biasioli S, Guidi G. PON1 activity and genotype in patients with arterial ischemic stroke and in healthy individuals. *Acta Neurol Scand* 2007;**116**:6–30
32. Phuntuwate W, Suthisisang C, Koanantakul B, Mackness MI, Mackness B. Paraoxonase 1 status in the Thai population. *J Hum Genet* 2005;**50**:293–300
33. Sirivarasai J, Kaojarern S, Yoovathaworn K, Sura T. Paraoxonase (PON1) polymorphism and activity as the determinants of sensitivity to organophosphates in human subjects. *Chem Biol Interact* 2007;**168**:184–92
34. Scacchi R, Corbo RM, Rickards O, Stefano GF. New Data on the world distribution of paraosonase (PON1 Gln192→Arg) gene frequencies. *Hum Biol* 2003;**75**:365–73
35. Catano H, Cueva JL, Cardenas AM, Izaguirre V, Zavaleta A, Carranza E, Hernández AF. Distribution of paraoxonase-1 gene polymorphisms and enzyme activity in a Peruvian population. *Environ Mol Mutagen* 2006;**47**:699–706
36. Rojas-García AE, Solís-Heredia MJ, Pina-Guzmán B, Vega L, López-Carrillo L, Quintanilla-Vega B. Genetic Polymorphisms and activity of PON1 in a Mexican population. *Toxicol Appl Pharm* 2005;**205**:282–9
37. Sepahvand F, Rahimi-Moghaddam P, Shafiei M, Ghaffari SM, Rostam-Shirazi M, Mahmoudian M. Frequency of paraoxonase 192/55 polymorphism in an Iranian population. *J Toxicol Environ Health Part A* 2007;**70**:1125–9
38. Camps J, Marsillach J, Joven J. Pharmacological and lifestyle factors modulating serum paraoxonase-1 activity. *Mini Rev Med Chem* 2009;**9**:911–20
39. Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. *Biochem Pharmacol* 2005;**69**:541–50
40. Ellison CA, Crane AL, Bonner MR, Knaak JB, Browne RW, Lein PJ, Olson JR. PON1 status does not influence cholinesterase activity in Egyptian agricultural workers exposed to chlorpyrifos. *Toxicol Appl Pharmacol* 2012;**265**:308–15
41. Chen DY, Wang CY, Wen MS, Lee TH, Chu Y, Hsieh MJ, Chang SH, Lee CH, Wang JL, Chen CC, Lu LS, Lee MT, Yeh SJ, Lin FC, Hsieh IC. Paraoxonase-1 is not a major determinant of stent thrombosis in a Taiwanese population. *PloS One* 2012;**7**: e39178
42. Huang Q, Liu YH, Yang QD, Xiao B, Ge L, Zhang N, Xia J, Zhang L, Liu ZJ. Human serum paraoxonase gene polymorphisms, Q192R and L55M, are not associated with the risk of cerebral infarction in Chinese Han population. *Neurol Res* 2006;**28**:549–54
43. Wang X, Fan Z, Huang J, Su S, Yu Q, Zhao J, Hui R, Yao Z, Shen Y, Qiang B, Gu D. Extensive association analysis between polymorphisms of PON gene cluster with coronary heart disease in Chinese Han population. *Arterioscler Thromb Vasc Biol* 2003;**23**:328–34

(Received June 28, 2013, Accepted October 9, 2013)