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Paraoxonase Polymorphism and Its Effect on Male Reproductive Outcomes Among Chinese Pesticide Factory Workers

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Background *Serum paraoxonase has been associated with the metabolism of organophosphate pesticides in humans. Molecular analysis of the human paraoxonase gene (PON1) has revealed that Arg_{192} homozygotes have a greater detoxifying capability than Gln_{192} homozygotes. We examined the effects of PON1 genotypes on male reproductive outcomes and its interaction with exposure to organophosphate pesticides.*
Methods *We studied 60 Chinese pesticide-factory workers and 89 textile-factory workers who were unexposed to pesticides. The respective allele frequencies of Arg_{192} and Gln_{192} were 0.62 and 0.38. Pesticide exposure among 36 exposed subjects and 12 unexposed subjects, regardless of gender, was assessed by personal measurement of pesticide residues over an entire 8-hr shift and measurement of urinary p-nitrophenol level over a 24-hr period. We analyzed semen and hormone data collected from male subjects.*
Results *When the three PON1 genotypes were analyzed separately, a gene dose effect was not detected. We used the unexposed Arg_{192} homo/heterozygotes as the reference group, and re-analyzed the data. Exposed Arg_{192} homo/heterozygotes had significantly lower sperm count ($\chi^2 = 9.01$, $P < 0.01$) and lower percentage of sperm with normal morphology ($\chi^2 = 4.18$, $P < 0.05$) than the reference group. Both unexposed Gln_{192} homozygotes ($\chi^2 = 4.90$, $P < 0.05$) and exposed Arg_{192} homo/heterozygotes ($\chi^2 = 10.00$, $P < 0.01$) showed significantly lower sperm concentrations than the reference group. In addition, exposed Arg_{192} homo/heterozygotes had significantly higher serum LH levels ($\chi 2 = 7.94$, $P < 0.01$) than the reference group.*
Conclusions *Because of a small sample size, our findings are highly preliminary. Nevertheless, it calls for further investigation of the interaction between the PON1 genotype and organophosphate pesticide exposure on male reproductive outcomes.*
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KEY WORDS: paraoxonase; organophosphate pesticide; semen quality; reproductive hormone

INTRODUCTION

With the recent advances in molecular epidemiology, evidence of the role of gene-environment interactions on individual susceptibility to the toxic effects of environmental or occupational exposures to hazardous chemicals is accumulating. The enzyme paraoxonase is known to have two major functions in the human body: metabolism of

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lipids (particularly high density lipoproteins) and metabolism of paraoxon [Mackness et al., 1996]. Once it enters the body, parathion, a potent organophosphate pesticide, is bioactivated by the liver enzyme cytochrome-P_{450}-dependent microsomal mono-oxygenases into paraoxon, an acetylcholinesterase inhibitor that is more potent than the parent compound. Paraoxon is subsequently detoxified by other liver enzymes. In mammals, any oxon that escapes hepatic detoxification can be hydrolyzed in the blood by serum paraoxonase. The metabolic end-products are then excreted in the urine. Because of their rapid excretion after exposure to organophosphate pesticides, two types of urinary metabolites-alkyl phosphate and phenolic metabolites [Davies and Peterson, 1997] have proven to be useful indices of recent exposure.

Mathew et al. [1992] reported that in mice, methyl parathion induces a significant increase in sperm shape abnormalities. Chou and Cook [1994] demonstrated that paraoxon (a metabolite of parathion) inhibits the sperm capacitation and the fertilization of gametes in vitro. There is also evidence of clastogenicity and mutagenicity of methyl- and ethyl-parathion including sister chromatid exchanges (SCEs) and human chromosomal aberrations [van Bao et al., 1974; Chen et al., 1981]. In terms of reproductive hormone disturbance, Rattner et al. [1986] reported that parathion exposure transiently depressed plasma luteinizing hormone (LH) in quail, suggesting a cholinergic component in the regulation of LH secretion. On the whole, there may be multiple mechanisms that account for the effect of organophosphates on male reproduction: (1) increased rate of sperm abnormality and inhibition of sperm capacitation; (2) increased number of SCEs and chromosomal aberrations in sperm; and (3) disturbances in male reproductive hormone regulation. In 1976, Playfer et al. showed the presence of a genetic polymorphism with regard to plasma paroxonase activity in different ethnic groups. In 1993, Humbert et al. successfully identified the amino acid substitution at position 192 of serum paraoxonase leading to the polymorphism; arginine specifies high-level activity or more rapid clearance and is thus protective against parathion, while glutamine specifies the low-level activity variant.

On the basis of animal and human studies, Li et al. [1993] suggested that serum paraoxonase status may serve as a biomarker for insecticide susceptibility in humans. Weber hypothesized that the paraoxonase system would benefit individuals subject to repeated low-level exposure by catalyzing the detoxification process [Weber, 1995]. However, the extent to which serum paraoxonase contributes to the metabolism of organophosphates in humans is unknown [Mackness et al., 1996].

As part of an epidemiologic study of the effects of organophosphate pesticide exposure on male reproductive toxicity among Chinese pesticide factory workers, we conducted a genetic epidemiologic investigation of the interaction of the human paraoxonase *PON*1 genotype with pesticide exposure in relation to biological markers of exposure and male reproductive outcomes.

MATERIALS AND METHODS

One hundred forty-nine subjects from Anqing, China, were randomly recruited into the study. Sixty subjects were employed at a large pesticide-manufacturing plant and were occupationally exposed to one of the three organophosphate pesticides: ethyl-parathion, methyl-parathion, or methamidophos. The remaining 89 workers were employed in a nearby textile factory and were considered unexposed.

Both exposed and unexposed subjects met the following selection criteria: aged between 19–56 years, continuous work in the factory for at least three months before the study, and no congenital anomalies or acquired diseases of the external genitalia. Exposed and unexposed subjects were comparable with regard to job description, educational background, and socioeconomic status.

Collection of Data and Biological Specimens

An appointment was made for an interview, a physical examination, and biological specimen collection. For male subjects, the initial physical examination included examination of the testes by a physician blinded to pesticide exposure status. A questionnaire was administered to each subject to obtain information on general lifestyle, medical history, reproductive history, and occupational and environmental exposures to other potential reproductive toxins.

Collection of Blood and DNA Extraction

At the end of a work shift, each subject donated 10 ml of blood, which was collected into vacutainer tubes containing EDTA. Tubes were kept on ice and were spun subsequently for 10 min in a refrigerated centrifuge at $2{,}000 \times g$. The serum portion was aliquoted for hormone analysis and the buffy coat layer was separated for genetic analysis. All samples were frozen at -80°C until analyzed at the Program for Population Genetics in Boston. DNA was extracted from buffy coat samples according to the protocol, previously described by Niu et al. [1998]. The concentration of DNA was measured with SOFTmax PRO version P1.12 software (Molecular Devices, Sunnyvale, CA). Blood cells are usually separated at room temperature, and relatively inefficient separations may be obtained when the blood is chilled. Since the serum proteins (e.g., the serum hormones) are less stable at room temperature, it may be still reasonable to separate blood cells in a refrigerated centrifuge, as we need to conduct further serum hormone

analyses. We have extracted DNA from chilled blood samples in variety of human genetic studies, and the yield is typically 300 μg per 10 mL whole blood.

Genotype Analysis

*PON*1 genotypes were determined by means of polymerase chain reaction (PCR) and restriction enzyme digestion according to a published protocol [Humbert et al., 1993; Serrato and Marian, 1995]. In brief, a sense primer 5′-TATTGTTGCTGTGGGACCTGAG-3′ and an antisense primer 5′-CACGCTAAACCCAAATACATCTC-3′ which included the genomic region of interest, were used. The 10-μL PCR mixture consisted of 50 ng of genomic DNA, 10 mM Tris-HCl, 50 mM KCl (pH 8.3 at 20°C), 2 mM $MgCl_2$, 8 μmol of each primer, 400 μM of dNTPs, 0.3 U of Taq DNA polymerase (Boehringer Mannheim). PCR was carried out under the following conditions: an initial denaturation at 94°C for 3 min; 45 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min, and extension at 72°C for 1 min; and a final extension at 72°C for 7 min. After PCR amplification, the reaction products were subjected to digestion by the restriction enzyme *Alw*1 (New England Biolabs, Beverly, MA) for 3–4 hr at 37°C. The digested products were analyzed by running them in a horizontal 3.5% agarose gel, with ethidium bromide staining.

Hormone Analysis

Each male subject was also instructed to collect his first-voided urine sample at home for three consecutive days for hormone analysis. Upon receipt, each urine sample was immediately aliquoted into 5-mL tubes and stored in a freezer at −20°C until the time of analysis.

Both the serum and the urine samples were analyzed at the Program for Population Genetics in Boston. All serum samples were analyzed by commercial radioimmunoassay kits, "Coat-A-Count" (Diagnostic Product Corporation, Los Angeles, CA), for the concentrations of follicle-stimulating hormone (FSH), LH and testosterone. A noncompetitive enzyme linked immunosorbent assay (ELISA) was employed for urinary FSH and LH analyses according to the protocol described by Qiu et al. [1998]. Competitive ELISA was performed for urinary analysis of the testosterone metabolite, estrone conjugate (E1C), following a protocol described by Munro et al. [1991]. All urinary hormone measurements were indexed by the corresponding urinary creatinine levels prior to data analysis.

Semen Collection and Analysis

Of the 78 male workers, all 43 male participants were asked to abstain from ejaculation for 3–5 days before semen collection, which took place in a private room in the factory clinic at the end of a work shift. All semen analyses, including smear slide preparations, were completed within 1 hr after collection. Parameters analyzed on site according to the protocol developed by Overstreet and Brazil [1997] included liquefaction time, volume, pH, agglutination, sperm concentration, sperm count, percent motility, and progressive sperm motility score. Three semen smear slides per subject were shipped to the Institute of Toxicology and Environmental Health at the University of California, Davis, for evaluation of the percentage of sperm with normal morphology.

Exposure Assessment

A subset of exposed ($n = 36$) and unexposed ($n = 12$) subjects of both genders was randomly selected to participate in an exposure assessment, which consisted of two components. The first involved measuring pesticide residues over an entire 8-hr shift by attaching 5×5 cm gauze pieces to nine body areas, and attaching a personal pump (Sibata Scientific Technology) to the subject's shirt. The second component involved measuring the urinary metabolite *p*-nitrophenol in five urine samples collected at the end of a shift and 1, 6, 12, and 24 hr after. All specimens were analyzed at the Anqing-Meizhong Biomedical Institute according to the guidelines of the Institute of Occupational Health, Chinese Academy of Preventive Medicine [Xian, 1994a]. Acetylcholine esterase activity was measured by the ferric chloride test [Xian, 1994b] in aliquots of serum obtained for hormone analysis.

Statistical Analysis

Eight subjects did not provide blood samples. Therefore, data analysis for genotypes was restricted to 141 subjects. Frequencies of Arg_{192} and Gln_{192} were calculated, and the Hardy–Weinberg equilibrium was tested by chi-square analysis.

Of the 72 male subjects who had serum samples, 42 donated semen samples, while 44 had urine samples available. Two stages of statistical analyses of the association between pesticide exposure and reproductive outcomes were carried out among these male subjects. In the first stage, we aimed at testing the gene dosage effect, and individuals with genotypes Arg_{192}/Arg_{192}, Arg_{192}/Gln_{192} and Gln_{192}/Gln_{192} were grouped separately. The nonparametric ANOVA (i.e., Kruskal–Wallis Test) was performed using the Statistical Analysis System version 6.12 computer package (SAS Institute Inc., Cary, NC, USA) to assess mean differences in sperm count, concentration, percentage motility, and percentage of sperm with normal morphology

across the different groups. Similar analyses were performed with respect to serum FSH, LH, and testosterone levels, urinary FSH, LH, and E1C levels, and the profile of mean urinary *p*-nitrophenol level at several time points over a 24-hr period. In the second stage, we considered the Gln_{192} variant as a recessive allele, and repeated the analyses by classifying the subjects into the following four groups: (1) unexposed Arg_{192} homo/heterozygotes (the reference group); (2) exposed Arg_{192} homo/heterozygotes; (3) unexposed Gln_{192} homozygotes; and (4) exposed Gln_{192} homozygotes. To evaluate the separate and combined effects of the *PON*1 genotype and pesticide exposure, a nonparametric method was used to compare the mean of the reference group with the means of the other three groups in a pair-wise manner.

Age and cigarette smoking may potentially influence sperm markers, and we carried out the following statistical analyses to test the significance of these covariates' impacts. We created three binary variables in the regression model for the unexposed Gln_{192} homozygotes (group 1), the exposed Arg_{192} homo/heterozygotes (group 2), and the exposed Gln_{192} homozygotes (group 3), considering the unexposed Arg_{192} homo/heterozygotes as the reference group. We compared the coefficient estimates of the three groups before and after the adjustment for age and cigarette smoking. In regard to sperm concentration, the values of the coefficient estimates only changed 1.0%, 0.6%, and 2.1% after the adjustment for these covariates in groups 1, 2, and 3, respectively. Regarding other sperm parameters, controlling for age and cigarette smoking did not have significant effects on the sperm parameters either. Thus, we did not adjust for these factors in our analyses using nonparametric methods.

RESULTS

The allelic frequencies were 0.62 and 0.38 for the Arg_{192} and Gln_{192} alleles, respectively, according to the genotype distribution of Arg_{192}/Arg_{192}, Arg_{192}/Gln_{192}, and Gln_{192}/Gln_{192} shown in Table I. The frequencies observed did not deviate significantly from the Hardy–Weinberg equilibrium ($P > 0.05$).

Biological exposure markers were not substantially different among the three genotypes (Table II). However, stratification of urinary *p*-nitrophenol profiles by type of pesticide exposure and *PON*1 genotype revealed a number of interesting trends (Table III). For each genotype, the subjects exposed to parathion excreted a higher level of *p*-nitrophenol than those exposed to methamidophos. Meanwhile, given the same type of pesticides, Arg_{192} homo/

TABLE I. Frequency of *PON*1 Genotypes among 141 Chinese Factory Workers*

Genotype	N	%	Observed	Expected
Homozygous Gln_{192}	25	18	0.18	0.14
Heterozygous	57	40	0.40	0.48
Homozygous Arg_{192}	59	42	0.42	0.38
Total	141	100	–	–

*Including 72 male and 69 female workers, respectively. $\chi^2_{(1)} = 2.83$, $P = 0.09$.

TABLE II. Quantitative Exposure Assessment of 36 Chinese Pesticide Factory Workers Classified by *PON*1 Genotype*

	Mean ± SD [Range] for Indicated Genotype		
Genotype	Gln_{192}/Gln_{192}	Arg_{192}/Gln_{192}	Arg_{192}/Arg_{192}
N	11	11	13
PNP			
End of shift	0.24 ± 0.42 [0.00–1.15]	0.24 ± 0.36 [0.00–1.18]	0.20 ± 0.41 [0.00–1.29]
1 hr	0.15 ± 0.38 [0.00–1.18]	0.41 ± 0.48 [0.00–1.30]	0.41 ± 0.49 [0.00–1.71]
6 hr	0.20 ± 0.27 [0.00–0.74]	0.65 ± 0.91 [0.00–2.89]	0.39 ± 0.46 [0.00–1.28]
12 hr	0.09 ± 0.10 [0.00–0.27]	0.45 ± 0.39 [0.00–1.25]	0.43 ± 0.78 [0.00–1.87]
24 hr	0.34 ± 0.66 [0.00–1.96]	0.56 ± 0.60 [0.00–1.51]	0.15 ± 0.19 [0.00–0.48]
Personal pump**			
Ethylparathion	0.02 ± 0.00	0.02 ± 0.01 [0.00–0.03]	0.06 ± 0.09 [0.00–0.16]
Methamidophos	0.05 ± 0.07 [0–0.20]	0.01 ± 0.01 [0.00–0.03]	0.07 ± 0.06 [0.00–0.18]
Plasma AChE level	2.88 ± 0.71 [1.86–4.30]	2.88 ± 0.62 [2.08–4.17]	2.67 ± 0.87 [1.54–4.74]

*Including 18 male and 17 female workers; One female subject did not have a blood sample taken for genotype analysis.

**Each subject was exposed to either ethylparathion or methamidophos (mg/m^3).

PNP = urinary *p*-nitrophenol (mg/L), AChE = plasma acetylcholinesterase enzyme activity (μmol at 37°C).

TABLE III. Mean Amount of Urinary *p*-Nitrophenol Excreted at Different Time Points Over a Period of 24 Hr Among 32 Exposed Pesticide Factory Workers* of Both Genders Stratified by Type of Pesticide and *PON*1 Genotype**

	Ethylparathion			Methamidophos		
Genotype	Arg_{192}/Arg_{192}	Arg_{192}/Gln_{192}	Gln_{192}/Gln_{192}	Arg_{192}/Arg_{192}	Arg_{192}/Gln_{192}	Gln_{192}/Gln_{192}
N	3	5	3	8	6	7
PNP						
End of Shift	0.693	0.426	0.696	0.017	0.077	0.006
1 hr	0.833	0.392	0.506	0.247	0.420	0.000
6 hr	0.520	0.918	0.393	0.290	0.305	0.120
12 hr	0.573	0.466	0.110	0.365	0.430	0.093
24 hr	0.160	0.543	0.653	0.150	0.593	0.154

*Values may not reflect the total number of subjects because of missing data.
**Each subject was exposed to either ethylparathion or methamidophos (mg/m^3).
PNP = urinary *p*-nitrophenol (mg/L).

TABLE IV. General Characteristics of Male Chinese Factory Workers by *PON*1 Genotype and Organophosphate Pesticide Exposure Status[a]

	Unexposed			Exposed		
Genotype	Arg_{192}/Arg_{192}	Arg_{192}/Gln_{192}	Gln_{192}/Gln_{192}	Arg_{192}/Arg_{192}	Arg_{192}/Gln_{192}	Gln_{192}/Gln_{192}
N	16	17	4	17	6	9
Age (years)	34 ± 8	29 ± 8	34 ± 8	30 ± 11	32 ± 11	36 ± 12
Income (Yuan/month)[b]	380 ± 138	351 ± 53	325 ± 65	358 ± 39	366 ± 50	414 ± 58
Marriage duration (years)	11 ± 8	8 ± 6	8 ± 6	10 ± 10	16 ± 7	15 ± 9
Number of pregnancies fathered	2 ± 1	2 ± 1	2 ± 1	3 ± 2	4 ± 1	4 ± 1
Current						
Cigarette smoker						
Yes	10 (62)	11 (65)	4 (100)	9 (53)	6 (100)	5 (63)
No	6 (38)	6 (35)	–	8 (47)	–	3 (37)
Current						
Alcohol drinker						
Yes	3 (19)	6 (35)	–	1 (6)	1 (17)	2 (22)
No	13 (81)	11 (65)	4 (100)	16 (94)	5 (83)	7 (78)

[a]Excluding subjects who refused to have blood collected (n = 6): age, 35 ± 9 years; income, 278 ± 52 Yuan/month; length of marriage, 9 ± 6 years; number of pregnancies fathered 2 ± 1.
[b]1 Yuan = 0.82 USD $.
Values are expressed as mean ± SD for age, income, marriage duration and number of pregnancies and as number (percentage) for smoking and drinking status. In some cases, values may not reflect the total number of subjects because of missing data.

heterozygotes appeared to excrete higher levels of *p*-nitrophenol than the Gln_{192} homozygotes. When we considered the 24-hr urinary profile of each group, parathion-exposed Arg_{192} homo/heterozygotes excreted a larger total amount of *p*-nitrophenol but cleared *p*-nitrophenol more slowly than the parathion-exposed Gln_{192} homozygotes.

Table IV shows the general characteristics of male subjects. The marriage duration for the exposed Arg_{192}/Gln_{192} and Gln_{192}/Gln_{192} subjects was, on average, 4–8 years longer than for the other subgroups. This may account for the observation that the pesticide-exposed Arg_{192}/Gln_{192} and Gln_{192}/Gln_{192} subjects appeared to father more pregnancies.

TABLE V. Selected Semen Parameters Among 42 Male Chinese Factory Workers by *PON*1 Genotype and Organophosphate Pesticide Exposure Status

	Unexposed*			Exposed		
Genotype	Arg_{192}/Arg_{192}	Arg_{192}/Gln_{192}	Gln_{192}/Gln_{192}	Arg_{192}/Arg_{192}	Arg_{192}/Gln_{192}	Gln_{192}/Gln_{192}
N	10	8	4	7	6	7
Abstinence period (days)	7 ± 6 [1–18]	9 ± 9 [3–30]	5 ± 4 [2–10]	5 ± 4 [1–13]	6 ± 5 [2–15]	5 ± 3 [1–10]
Sperm count[a] ($\times 10^6$)	259 ± 174 [61–557]	228 ± 169 [27–493]	145 ± 55 [97–211]	108 ± 74 [24–196]	63 ± 40 [18–124]	160 ± 99 [70–325]
Sperm concentration[b] ($\times 10^6$/mL)	77 ± 33 [38–147]	94 ± 56 [17–159]	38 ± 4 [31–41]	37 ± 20 [14–68]	37 ± 21 [8–63]	53 ± 25 [17–85]
Percentage motility[c] (%)	56 ± 14 [36–82]	55 ± 16 [32–79]	62 ± 8 [52–70]	48 ± 19 [25–72]	45 ± 14 [25–62]	48 ± 14 [31–72]
Percentage with normal morphology[c] (%)	61 ± 5 [54–67]	63 ± 5 [58–70]	56 ± 19 [29–68]	57 ± 8 [50–69]	57 ± 5 [48–63]	58 ± 12 [40–74]

*One unexposed subject did not have a blood sample taken for genotyping.
[a]Significant ($P < 0.01$) by nonparametric test comparing exposed Arg_{192} homo/heterozygotes with the reference group.
[b]Significant ($P < 0.05$) by nonparametric test comparing unexposed Gln_{192} homozygotes; significant ($P < 0.01$) by nonparametric test comparing exposed Arg_{192} homo/heterozygotes with the reference group.
[c]Significant ($P < 0.05$) by nonparametric test comparing exposed Arg_{192} homo/heterozygotes with the reference group.
Values are expressed as mean ± SD [Range]. The reference group refers to the unexposed Arg_{192} homo/heterozygotes.

TABLE VI. Reproductive Hormone Levels Among Male Chinese Factory Workers by *PON*1 Genotype and Organophosphate Pesticide Exposure Status

	Unexposed			Exposed		
Genotype	Arg_{192}/Arg_{192}	Arg_{192}/Gln_{192}	Gln_{192}/Gln_{192}	Arg_{192}/Arg_{192}	Arg_{192}/Gln_{192}	Gln_{192}/Gln_{192}
N	16	17	5	19	6	9
Serum FSH (mIU/mL)	15.3 ± 3.9 [7.7–21.1]	15.2 ± 8.8 [5.4–36.3]	13.7 ± 3.8 [8.5–17.6]	14.8 ± 5.7 [6.8–31.0]	19.2 ± 9.4 [7.6–33.9]	14.9 ± 4.8 [10.0–23.6]
Serum LH[a] (pM)	20.5 ± 4.1 [13.2–26.9]	21.7 ± 7.3 [10.5–37.8]	24.6 ± 6.1 [20.2–33.2]	27.9 ± 12.0 [10.8–52.8]	38.0 ± 16.3 [22.6–60.3]	28.0 ± 13.1 [12.7–60.2]
Serum testosterone (ng/dL)	126.1 ±52.1 [43.9–269.1]	138.0 ± 78.9 [63.2–388.2]	125.8 ± 64.0 [48.3–217.6]	108.3 ± 35.2 [30.9–167.3]	115.6 ± 63.0 [24.4–210.9]	105.2 ± 53.7 [33.3–218.7]
N	10	5	3	13	4	9
Urinary[b] FSH (ng/mL)	3.5 ± 0.9 [2.3–5.2]	3.1 ± 2.7 [0.0–7.2]	2.5 ± 1.0 [1.5–3.5]	2.5 ± 1.3 [0.0–4.3]	3.5 ± 1.6 [1.6–5.2]	2.3 ± 1.2 [2.8–17.6]
Urinary[b] LH (pM)	4.2 ± 2.2 [2.0–8.8]	7.8 ± 6.7 [2.5–18.7]	4.7 ± 1.8 [2.9–6.5]	3.7 ± 1.6 [1.3–6.2]	13.1 ±18.0 [2.7–40.0]	6.2 ± 4.8 [2.8–17.6]
Urinary[b] E1C (pg/mL)	62.5 ± 147.8 [1.5–479]	7.8 ± 4.4 [4.3–15.2]	24.3 ± 13.2 [9.3–34.2]	25.3 ± 18.8 [6.7–77.6]	23.2 ± 34.5 [2.8–74.8]	22.2 ± 23.9 [0.5–77.5]

[a]Significant ($P < 0.01$) by nonparametric test comparing exposed Arg_{192} homo/heterozygotes with the reference group.
[b]First-day urine sample only.
FSH = follicle stimulating hormone, LH = luteinizing hormone, E1C = estrone conjugate. Values are expressed as mean ± SD [Range]. The reference group refers to the unexposed Arg_{192} homo/heterozygotes.

Associations between *PON*1 genotype, pesticide exposure and reproductive health outcomes are presented in Tables V (sperm parameters) and VI (hormone parameters). In both the exposed group and the unexposed group, no gene-dose effect of the *PON*1 genotype was observed with respect to sperm and hormone parameters (Tables V and VI). We combined Arg_{192}/Arg_{192} and Arg_{192}/Gln_{192} as one group (thus assuming the Gln_{192} allele to be recessive), and re-analyzed the data. Compared to the unexposed Arg_{192} homo/heterozygotes (the reference group), exposed Arg_{192} homo/heterozygotes had a significant decrease in both sperm count ($\chi^2 = 9.01$, $P < 0.01$) and percentage of sperm with normal morphology ($\chi^2 = 4.18$, $P < 0.05$) (Table V). Both unexposed Gln_{192} homozygotes ($\chi^2 = 4.90$, $P < 0.05$) and exposed Arg_{192} homo/heterozygotes ($\chi^2 = 10.00$, $P < 0.01$) showed significant decreases in sperm concentrations compared to the reference group. However, within the exposed group, the Gln_{192} homozygotes appeared to have a significantly higher sperm count ($\chi^2 = 4.08$, $P < 0.05$) than the Arg_{192} homo/heterozygotes (Table V). In terms of serum and urinary hormone levels, exposed Arg_{192} homo/heterozygotes seemed to have a significant increase in serum LH levels ($\chi^2 = 7.94$, $P < 0.01$) compared to the reference group (Table VI).

DISCUSSION

The major findings of our study were: (1) parathion exposure was significantly associated with a decrease in sperm count and sperm concentration; (2) parathion exposure was significantly associated with an increase in serum LH levels; (3) *PON*1 Arg192Gln polymorphism had a notable influence on the association between parathion exposure and sperm parameters.

The frequency of the Gln_{192} variant differed remarkably across various ethnic groups: 0.69 in Caucasians [Seratto and Marian, 1995], 0.73 in Saudi Arabians [Nogueira et al., 1993], 0.50 in Hungarians [Benkmann et al., 1993], and 0.34 in Japanese [Yamasaki et al., 1996]. The Gln_{192} variant frequency in this Chinese population (0.38) thus appeared similar to that reported in Japanese populations.

Playfer et al. [1976] suggested that the low-activity variant, Gln_{192}, reflected a recessive trait. Therefore, the Arg_{192}/Gln_{192} heterozygotes would have higher paraoxonase activity than the Gln_{192} homozygotes. Li et al. [1993] demonstrated that, indeed, the enzyme activity of the low-activity allele group was substantially different from heterozygotes and homozygotes for the high-activity allele. In other studies [Furlong et al., 1988, 1989], a few heterozygotes exhibited enzyme activity at a lower level than that of the low-activity homozygotes, but when given another substrate for enzyme paraoxonase, chloropyrifos, enzyme activity for these subjects clearly resembled the high-activity homozygotes.

Urinary *p*-nitrophenol excretion may serve as a biomarker of parathion exposure [Denga et al., 1995; Davies and Peterson, 1997] and has been used as the basis of paraoxonase phenotypic determination [Furlong et al., 1988]. From the PNP excretion profile of our subjects (Table III), it was apparent that the Gln_{192} homozygotes excreted less PNP at a given point of time and a lower total amount of PNP, compared to the heterozygotes and the Arg_{192} homozygotes, regardless of direct parathion exposure. Though the sample size was extremely small (especially for the parathion-exposed Gln_{192} homozygotes ($n = 3$)), the results seem to imply that paraoxonase activity for the heterozygotes was higher than that of the Gln_{192} homozygotes.

The hypothesis of the study was that lower sperm counts would be observed in pesticide-exposed Gln_{192} homozygotes. By contrast, the opposite trends were indicated for sperm parameters in the pesticide-exposed group (Table V). There are several possible explanations for the discrepancies between the hypothesis and our findings. (1) Because this occupational health study utilized a cross-sectional study design, Gln_{192} homozygous workers who are exposed to pesticides may have dropped out of this industry early in their employment due to other illnesses. Such a healthy worker effect may underestimate (or even bias) the exposure-response relation between *PON*1 genotype and sperm parameters. (2) The sample sizes of both exposed and unexposed groups were limited and thus these observations were highly preliminary. (3) Other mutations of the *PON*1 gene, such as the Met54Leu (this same position is designated as ''55'' in this study) polymorphism, appeared to be of central importance to paraoxonase function, by virtue of its significant association with serum PON1 concentrations [Garin et al., 1997]; therefore, the Met54Leu polymorphism may influence the results between the *PON*1 Arg192Gln polymorphism and sperm parameters.

Parathion has been found to depress plasma LH levels in quail [Rattner et al., 1982] via its anticholinergic mechanism and to induce abnormal sperm morphology in mice [Mathew et al., 1992]. Our findings suggest that LH regulation might be sensitive to parathion exposure in humans, and further investigations are clearly needed to corroborate our findings.

The major limitation of our study was the small sample size. The detection of gene-environment interaction requires far larger sample sizes than a study of main effects, especially in this case, given a low prevalence of Gln_{192} homozygotes. In this study, selection bias could be ruled out with regard to genotype but not with regard to exposure and reproductive outcomes. We did not find a higher participation rate among exposed or more fertile subjects. Though

laboratory technicians were blinded to the exposure status of subjects, nondifferential misclassification of exposure, outcome, and genotype may have occurred as a result of random measurement errors. Additionally, significant excretion of *p*-nitrophenol by methamidophos-exposed workers reflected some mixed exposure to ethyl- and methyl-parathion. Nevertheless, the allegedly unexposed workers were indeed virtually free of pesticide exposure because they did not excrete any urinary *p*-nitrophenol.

In addition to *PON*1 Arg192Gln genotype, the activity of serum paraoxonase has been associated with other factors, such as age [Playfer et al., 1977], gender [Williams et al., 1993], and cigarette smoke exposure [Nishio and Watanabe, 1997]. Unfortunately, due to a small sample size, our study had insufficient power to evaluate the effects of age, gender, or smoking.

This study attempted to investigate the relationship among *PON*1 Arg192Gln polymorphism, organophosphate pesticide exposure, and male reproductive outcomes in humans. Despite limited statistical power, our results reflect that *PON*1 genotype may modify an association between environmental exposures and reproductive health effects. Further studies with a larger sample size will be able to characterize more accurately the gene-environment interaction with pesticide and other risk factors and draw firmer conclusions. Nevertheless, this study yielded notable preliminary information relevant to the application of biomarkers – whether of exposure, susceptibility, or male reproductive outcomes – in an epidemiological study.

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