Journal of Occupational and Environmental Medicine
Volume 45 • Number 2 • February 2003

ORIGINAL ARTICLES

CME Article #2

Association Between Human Paraoxonase Gene Polymorphism and Chronic Symptoms in Pesticide-Exposed Workers

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David Christiani's research was supported by a grant from the National Institutes of Health.

Learning Objectives

- Explain the role of paraoxonase (*PON*) in organophosphate metabolism and the implications of polymorphism in the human *PON* gene for farm workers exposed to pesticides.
- Identify demographic and clinical correlates of chronic toxicity in farm workers exposed to pesticides when applying them to crops.
- Relate the occurrence of chronic pesticide toxicity to the *PON* genotypes associated with "fast" and "slow" enzymatic hydrolysis of toxic pesticide metabolites.

Abstract

Pesticides, such as parathion, are metabolized by cytochrome p-450 system to paraoxon, which is a potent cholinesterase inhibitor. Paraoxonase (PON) **catalyzes the hydrolysis of these toxic metabolites and protects against pesticide toxicity. A glutamine/arginine (Gln/Arg) polymorphism at amino acid position 192 of *PON* has been described. The Arg/Arg genotype is associated with higher serum paraoxonase activity compared to Gln/Gln. The Arg/Gln genotype is associated with intermediate serum *PON* activity. The potential association between *PON* genotype and symptoms of chronic pesticide toxicity was examined among 100 farm workers. As part of a cross-sectional study of pesticide toxicity among mixed-race farm workers in the Western Cape, South Africa, 100 farm workers were genotyped for polymorphism of the paraoxonase gene at amino acid position 192. Subjects with two or more of the following symptoms were considered to have evidence of chronic toxicity: abdominal pain, nausea, rhinorrhea, dizziness, headache, somnolence, fatigue, gait disturbance, limb numbness, paresthesias, limb pain, or limb weakness. In multivariable logistic regression analysis, the independent predictors of chronic toxicity were previous history of head trauma resulting in loss of consciousness (OR 2.8, 95% CI = 1.7–6.7), having worked as a pesticide applicator (OR 5.4, 95% CI = 3.2–8.9), and having one of the two "slow metabolism" (Gln/Gln or Gln/Arg) genotypes (OR 2.9, 95% CI = 1.7–6.9). Furthermore, the prevalence of chronic toxicity increased in a stepwise fashion from 15% among pesticide nonapplicators with a "fast metabolism" (Arg/Arg) genotype, to 42.9% among pesticide nonapplicators with "slow metabolism" (Gln/Gln or Gln/Arg) genotypes, to 58.8% among pesticide applicators with**

"fast metabolism" genotype, and 75.0% among pesticide applicators with "slow metabolism" genotypes ($P = 0.001$). Age, number of years on the job, smoking history, alcohol history, education level, plasma or red blood cell cholinesterase level, or previous history of acute organophosphate poisoning were not statistically significant predictors of chronic toxicity. The *PON* genotype is an important determinant of a farmworker's susceptibility to chronic pesticide poisoning.

Introduction

At least three million pesticide poisonings occur each year and result in over 200,000 deaths throughout the world. [1] Many more toxicities occur from lower level chronic exposure to pesticides. [2] [3] Pesticides such as parathion are metabolized by cytochrome p-450 system to paraoxon, which is a potent cholinesterase inhibitor. [4] Acute pesticide poisoning causes inhibition of acetyl cholinesterase and increases the level of acetylcholine in the synapse. Clinical manifestations of acute pesticide poisoning may include bradycardia, tachycardia, hemodynamic instability, arrhythmia, seizure, ataxia, respiratory depression, obtundation, fasciculation, paralysis, tremor, numbness, hypersalivation, tearing, bronchorrhea, cough, wheezing, nausea, vomiting, diarrhea, abdominal pain, blurred vision, and miosis. [5] Clinical manifestations of chronic pesticide toxicity may differ significantly from that of acute poisoning and include abdominal pain, nausea, vomiting, rhinorrhea, dizziness, headache, fatigue, irritability, restlessness, depression, anxiety, somnolence, sleep disturbance, gait disturbance, limb pain, limb numbness, paresthesias, and limb weakness. [5] [6] [7]

Human serum paraoxonase (*PON*) catalyzes the hydrolysis of organophosphates and their metabolites. [8] In rats, infusion of *PON* protects against organophosphate toxicity. [9] The LD_{50} (lethal dose 50, or dose which will kill 50% of the subjects) for organophosphate poisoning for given species varies with its *PON* activity. [10] In humans, the *PON* activity varies 10- to 40-fold among individuals. The human *PON* gene is located close to the cystic fibrosis gene on the long arm of chromosome 7. [11] A glutamine/arginine (Gln/Arg) polymorphism at amino acid position 192 of *PON* has been described in humans. The Arg/Arg, Gln/Arg, and Gln/Gln genotypes are associated with high, intermediate, and low serum paraoxonase activities, respectively. [12] Therefore, we postulated that among farm workers exposed to organophosphates, those with slower *PON* activity would have a higher risk of pesticide toxicity compared to those with faster *PON* activity.

Methods

Subjects were derived from a cross-sectional epidemiologic survey of deciduous fruit farm workers in Western Cape, South Africa in 1983 and have been described previously. [7] The survey included 347 subjects which represents 68% of the eligible farm workers from 73 participating farms in the region (65% of eligible farms). One nonapplicator from the same farm was selected for every two applicators of pesticides. The nonapplicator and applicators were matched for age (within 2 years) and educational status (within 1 year). Nonapplicator versus applicator status was determined at the time of the survey. The study was conducted in accordance with the World Medical Association's Declaration of Helsinki [13] and received approval from the Ethics Committee of the University of Cape Town Medical School. Workers were interviewed regarding demographic information, symptoms, habits (pack-years of smoking, alcohol use, and problem drinking as measured by the CAGE questionnaire [14]), pesticide applicator history, and medical history, including self-reported head injuries. The symptoms had to be present within 3 months of the survey. In addition, two "dummy" symptoms not related to chronic pesticide poisoning (chest pain and earache) were included in the interview to test the internal validity of comparisons based on symptom reporting. Subjects were excluded if there was a history of vitamin deficiency, encephalopathy of known origin, psychotropic medication use, or previous injury of the lower limbs interfering with peripheral sensation. Only one subject was excluded from the survey (due to prior injury of the limb). Blood samples were analyzed for γ-glutamyl transferase, serum albumin (as marker of chronic undernutrition), and plasma and erythrocyte cholinesterase (as markers of acute organophosphate exposure and toxicity, respectively).

The following symptoms were considered to be compatible with chronic toxicity: chronic abdominal pain, nausea, rhinorrhea, dizziness, headache, somnolence, fatigue, gait disturbance, limb numbness, paresthesias, limb pain, or limb weakness. Initially, a neurologic symptom score was constructed based on a point for each positive symptom. Because there were very few subjects whose scores were greater than 4, subjects were categorized as having toxic symptoms if the score was 2 or greater; those with scores of 0 to 1 were categorized as not having toxic symptoms. A cut-off score of 2 was chosen because it divided the population in half.

Of the 347 farm workers in the survey, 100 subjects selected by convenience had

blood samples drawn toward the end of the study to investigate genetic susceptibility to chronic pesticide toxicity. The techniques used for DNA extraction and genotyping were conventional and as described previously. [12] [15] [16] In brief, a standard polymerase chain reaction technique was used to amplify a 99 bp segment of genomic DNA containing the polymorphic region of the *PON* gene. Polymerase chain reaction product was digested with AlwI restriction endonuclease, resolved on 3.5% TBE-agarose gel, and visualized with ethidium bromide. Samples that revealed the presence of a single band (99 bp) were categorized as Arg/Arg genotype, two bands (65 and 34 bps) were categorized as Gln/Gln genotype, and three bands (99, 65, 34 bps) were categorized as Gln/Arg genotype. The genotype determination was done blindly.

The two-tailed *t* test for unpaired data, Wilcoxon rank sum test, and Fisher exact test were used to compare the means of continuous normal variables, continuous nonnormal variables, and categorical variables, respectively. The Mantel-Haenszel test for trend was used to compare pesticide exposure status-genotype categories and frequency of toxic symptoms. Variables found to have significant or near significant ($P < 0.10$) association with toxic symptoms in bivariate analysis were included in a multivariable logistic regression model. All statistical analyses were performed on SAS software (SAS Institute Inc, Cary, NC).

Results

Of the 100 subjects in this study, 50 reported toxic symptoms. The baseline characteristics of farm workers with and without symptoms of chronic pesticide toxicity are summarized in Table 1. The proportion of subjects with a prior history of head injury resulting in loss of consciousness was significantly higher for those with toxic symptoms than those without toxic symptoms (56 versus 32%, respectively, $P = 0.02$). The proportion of subjects answering "yes" to two or more of alcohol abuse questions (positive CAGE) was also higher among those with toxic symptoms but the difference did not reach statistical significance (74 versus 56%, $P = 0.06$). There were no significant differences between the two groups in regard to age, years as farm workers, smoking history, serum albumin and γ-glutamyl transferase levels, plasma and red blood cell cholinesterase levels, education level, or current alcohol use. History of acute pesticide poisoning did not differ statistically between the two groups. As expected, the neurologic symptom score was higher for those with toxic symptoms.

Table 1. Baseline Characteristics of Farm Workers With and Without Symptoms of Chronic Toxicity

Variable	≤1 Toxic Symptoms	≥2 Toxic Symptoms
Age (years)*	32.2 (8.2)	32.8 (7.2)
Time as farm worker (years)*	14.8 (9.9)	15.7 (7.6)
Smoking (pack-years)*	8.7 (6.3)	11.0 (16.0)
Serum albumin (mg/dl)*	46.8 (3.1)	46.0 (2.6)
Serum GGT (U/L)*	20.5 (10.9)	20.2 (15.7)
Cholinesterase (plasma, U/L)*	6745 (1407)	6308 (1382)
Cholinesterase (RBC, U/L)*	36.3 (4.1)	36.8 (4.4)
7 or more years of school (%)	42.0	44.0
Current alcohol use (%)	76.0	84.0
Positive CAGE (%)#	56.0	74.0+
History of head injury (%)	32.0	56.0++
Neurologic symptom score	0.4 (0.5)	4.1 (2.3)+++
History of acute pesticide poisoning (%)	2.0	8.0

* Mean values (S.D.) unless otherwise indicated

Answers yes to two or more alcohol abuse questions.

+ $P = .06$;

++ $P = 0.02$;

+++ $P = .0001$

Of the subjects in the study, 49 were pesticide applicators. As expected, the proportion of subjects with toxic symptoms was significantly higher among applicators than non-applicators of pesticides (63.3% versus 37.3%; OR = 2.9 [1.3–6.5], $P = 0.009$).

Of the 100 subjects in the study, 82 underwent genotype analysis. The frequency of Gln/Gln, Gln/Arg, and Arg/Arg genotypes were 11, 45, and 43%, respectively. These frequencies differ substantially from that of the Caucasian population. [17] Subjects with the Arg/Arg genotype was categorized as "fast" genotype ($n = 37$). Because of the small number of subjects with the Gln/Gln genotype, those with the Gln/Gln or the Gln/Arg genotypes were combined and categorized as "slow" genotype ($n = 45$; 60%). A significantly higher proportion of subjects with the slow genotype reported two or more symptoms of chronic toxicity compared to those with fast *PON* activity (60 versus 35.1%; OR = 2.8 [1.1–6.7]). However, there was no association between *PON* genotype and the prevalence of non-specific "dummy" symptoms.

The samples from remaining 18 subjects did not contain adequate blood specimens for DNA analysis, hence had no genotype information available. However, subjects with inadequate blood specimens for genotype determination did not differ significantly from those with adequate specimens. (Table 2). Specifically, there were no differences in the history of head trauma proportion, which had been pesticide applicators, or neurologic symptom score.

Table 2. Characteristics of Farm Workers with Known vs. Unknown Genotype

	Genotype Known (N = 82)	**Genotype Unknown** (N = 18)
Age (years)*	32.7 (7.7)	31.6 (8.0)
Time as farm worker (years)*	15.3 (8.5)	14.8 (10.3)
Smoking (pack-years)*	9.5 (12.1)	11.5 (12.1)
Serum albumin (mg/dl)*	46.4 (2.8)	46.3 (3.1)
Serum GGT (U/L)*	21.4 (14.2)	15.4 (7.2)

	Genotype Known	Genotype Unknown
Cholinesterase (plasma, U/L)*	1 (1275)	6960 (1874)
Cholinesterase (RBC, U/L)*	36.8 (4.4)	35.5 (3.2)
7 or more years of school (%)		61 1
Current alcohol use (%)	79.3	83.3
	43.9	
	2.2 (2.5)	2.3 (2.2)
	50.0	44.4

Mean values (S.D.) unless otherwise indicated.
Answers yes to two or more alcohol abuse questions
+ p = 0.06; ++ p = 0.02; +++ p = 0.0001

Figure 1illustrates the interaction among genotype, pesticide exposure status, and chronic pesticide toxicity. The proportion of subjects with toxic symptoms increased in a stepwise fashion from nonapplicator-fast genotype, nonapplicator-slow genotype, applicator-fast genotype, and applicator-slow genotype categories (15, 42.9, 58.8, and 75%, respectively, Mantel-Haenszel Trend Test, $P = 0.001$).

Fig Exposure status, genotype, and toxic symptoms

In multivariable logistic regression analysis (Table 3), the independent predictors of chronic pesticide toxicity were previous history of head injury, slow genotype (Gln/Gln or Gln/Arg), and history of having worked as a pesticide applicator.

Table 3. Independent Predictors of Chronic Toxicity (2 or more symptoms) in

a Multivariable Logistic Regression Model

	Coefficient B	OR (95% CI)
Applicator	1.63	5.1 (3.2–8.9)
Slow genotype (Gln/Gln or Gln/Arg)	1.07	2.9 (1.7–4.9)
History of head injury	1.03	2.8 (1.7–4.7)

Model: In (p / (1-p)) = B_0 + B_1 (applicator) + B_2 (genotype) + B_3 (Head Injury) + E

Discussion

Although *PON* activity remains stable over time within a given individual, [18] it varies 10- to 40-fold between individuals. [19] [20] [21] This variation is due to genetic polymorphism at amino acid position 192 of *PON*. The Arg/Arg, Gln/Arg, and Gln/Gln genotypes are associated with high, intermediate, and slow serum *PON* activities, respectively. [12] In this study of 100 South African farm workers, symptoms consistent with chronic organophosphate toxicity was significantly more likely among subjects with the slow (Gln/Gln or Gln/Arg) *PON* genotypes compared to those with the fast (Arg/Arg) *PON* genotype (OR 2.8 [1.1–6.7], P = 0.02). This association between slow *PON* genotypes and chronic toxicity is biologically consistent with previous animal and human data demonstrating that *PON* has an important role in the metabolism of organophosphates. [8] [9] [10] [22] .

Furthermore, nonapplicators of pesticides with fast *PON* genotype had the lowest prevalence of chronic toxicity whereas applicators of pesticides with slow *PON* genotype had the highest prevalence of chronic toxicity. In fact, the proportion of subjects with toxic symptoms increased in a stepwise fashion from nonapplicator-slow genotype, nonapplicator-slow genotype, applicator-fast genotype, to applicator-slow genotype subjects (15, 42, 58.8, and 75%, respectively, Mantel-Haenszel Trend Test, P = 0.001). This observation further strengthens the

association between slow *PON* genotypes and chronic organophosphate toxicity.

In multivariable logistic regression analysis, previous history of head trauma resulting in loss of consciousness, history of having worked as a pesticide applicator, and slow *PON* genotype were independent predictors of chronic toxicity. History of head trauma is probably not associated with pesticide toxicity, per se. Rather, prior history of head trauma is associated with neurologic symptoms that overlap with symptoms of chronic organophosphate toxicity.

Based on this study, it is not clear why nonapplicators had toxic symptoms. Nonapplicators with the slow *PON* genotypes reported a higher prevalence of symptoms than those with the fast *PON* genotype. This observation is consistent with the hypothesis that even the non-applicators were exposed to pesticides. Because nonapplicator versus applicator status was determined at the time of the survey, some of nonapplicators may have worked as an applicator in the past. In addition, some of the non-applicators may have been performing tasks that still expose them to pesticides through indirect means. Such pesticide exposure among the non-applicators would lead to exposure misclassification but the direction of such bias would have been toward the null hypothesis.

It is possible that applicators of pesticides simply reported more symptoms than non-applicators because of recall or other biases. However, it is unlikely that such bias played a role in the association between the *PON* genotype and toxic symptoms since the subjects were not aware of their genotype at the time of the interview. Furthermore, the *PON* genotype was determined without knowledge of subject's baseline characteristics or symptom profile. Moreover, comparison of dummy symptom reporting showed no difference between the *PON* genotype groups, making reporting bias unlikely.

Of the 100 subjects in the study, genotype could not be determined for 18 subjects because there were no detectable levels of DNA in their blood samples. This most likely represents an error in collection, storage, or transport of the specimens. However, there were no statistically significant differences between subjects with known and unknown genotypes in terms of demographic characteristics, symptom profile, history of head injury, or pesticide applicator history (Table 2). Thus, it is unlikely that the findings would have been altered if the genotype information had been available for those 18 subjects.

Conclusion

In conclusion, the *PON* genotype appears to be an important determinant of susceptibility to chronic organophosphate poisoning. Whether the *PON* genotype influences the susceptibility to acute organophosphate poisoning should be investigated in future studies.

Acknowledgment

The authors acknowledge the technical assistance of Ms. Li Su, Ms. Hanlie van Heerden, and Ms. Aneleh Midgeley. The farmers and farm workers in the South African deciduous fruit industry are acknowledged for their support and cooperation.

Supported in part by NIH Center Grant ES00002 and by the grants from the International Development Research Council (Canada) and the South African Medical Research Council.

References

1. World Health Organization. Public Health Impact of Pesticides Used in Agriculture. Geneva, Switzerland: WHO; 1990.

2. Innes DF, Fuller BH, Berger GMB. Low serum cholinesterase levels in rural workers exposed to organophosphate pesticide sprays. S Afr Med J. 1990; 78: 581–583. Abstract

3. Brown SK, Ames RG, Mengle DC. Occupational illness from cholinesterase-inhibiting pesticides among agricultural applicators in California 1982–1985. Arch Environ Health. 1989; 44: 35–39. Abstract

4. Gage JC. A cholinesterase inhibitor derived from O,O-diethyl O-p-nitrophenyl thiophosphate in vivo. Biochem J. 1954; 54: 426–430.

5. Bardin PG, van Eeden SF, Moolman JA, et al. Organophosphate and carbamate poisoning. Arch Intern Med. 1994; 154: 1433–1441. Abstract

6. Perold JG, Bezuidenhout DJJ. Chronic organophosphate poisoning. S Afr Med J. 1980; 57: 7–9. Abstract

7. London L, Nell V, Thompson ML, et al. Effects of long-term organophosphate exposures on neurological symptoms, vibration sense, and tremor among South African farm workers. Scand J Work Environ Health. 1998; 24: 18–29. Abstract

8. Aldridge WN. An enzyme hydrolysing diethyl p-nitrophenyl phosphate (E 600) and its identity with the A-esterase of mammalian sera. Biochem J. 1954; 53: 117–124.

9. Costa, LG. Serum paraoxonase and its influence on paraoxon and chlorpyrifos oxon toxicity in rats. Toxicol Appl Pharmacol. 1990; 103: 66–76. Abstract

10. Costa LG. Species differences in serum paraoxonase correlate with sensitivity to paraoxon toxicity. In: Costa LG (ed.), Toxicology of Pesticides: Experimental, Clinical, and Regulatory Aspects. Berlin: Springer-Verlag; 1987; 263–266.

11. Tsui LC, Buchwald M, Barker D, et al. Cystic fibrosis locus defined by a genetically linked polymorphic DNA marker. Science. 1985; 230: 1054–1057. Abstract

12. Humbert R, Adler DA, Disteche CM, et al. The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet. 1993; 3: 73–76. Abstract

13. World Medical Association. The Declaration of Helsinki. World Med J. 1982; 29: 86–88.

14. Mayfield DG, McLeod G, Hall P. The CAGE questionnaire: validation of new alcoholism screening instrument. Am J Psychiatry. 1974; 131: 1121–1123. Citation

15. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16: 1175.

16. Serrato M, Marian AJ. Variant of human paraoxonase/arylesterase (HUM*PON*A) gene is a risk factor for coronary artery disease. J Clin Invest. 1995; 96: 3005–3008. Abstract

17. La Du BN. Human serum paraoxonase/arylesterase. In: Kalow(ed). Pharmacogenetics of

Drug Metabolism. New York: Pergamon; 1992; 51–91.

18. Geldmacher-von Mallinckrodt M. Genetisch determinierter polymorphismus der menschlichen serum-paraoxonase. Humangenetik. 1973; 17: 331–335. Citation

19. Zech R, Zurcher K. Organophosphate splitting enzymes in different mammals. Comp Biochem Physiol. 1974; 48B: 427–433.

20. Furlong CE, Richter RJ, Seidel SJ, et al. Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon. Am J Hum Genet. 1988; 43: 230–238. Abstract

21. Playfer JR, Eze LC, Bullen MF, et al. Genetic polymorphism and interethnic variability of plasma paraoxonase activity. J Med Genet. 1976; 13: 337–342. Abstract

22. Li WF, Furlong CE, Costa LG. Paraoxonase protects against chlorpyrifos toxicity in mice. Toxicol Lett. 1995; 76: 197–216. Abstract

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