# Pesticides Exposure and Genetic Polymorphism of Paraoxonase in the Susceptibility of Parkinson's Disease

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**Abstract-**

*Purpose:* The manifestation of Parkinson's disease (PD) is characterized by bradykinesia, resting tremor, and rigidity. The etiology of PD remains unknown. Recently several studies suggest that some environmental and genetic factors may be related to the cause of PD. Genetic variation in xenobiotic metabolizing enzymes involved in the disposition of pesticides, such as paraoxonase I (PON 1), may increase the risk of PD. We investigated the association between PON1 polymorphism, pesticides exposure and risk of Parkinson's disease in Taiwanese population.

*Methods:* We enrolled 162 controls and 125 patients with idiopathic PD. Histories of exposures to environmental factors and other information were collected with a questionnaire filled out during a face-to-face interview with the subject. The data included years of farming, drinking water sources, occupational exposures to pesticides, duration and the initial age of the pesticides exposure. Buccal mucosa cells are collected from each subject and PON1 polymorphism at codon 54 (L and M alleles) is studied with PCR-based restriction fragment length polymorphism (RFLP) analysis.

*Results:* There is significant association between the risk of PD and exposure to pesticides (OR=1.72, 95% CI=1.07-2.75). On the otherhand, no significant differences are found in PON1 genotype or allelic distribution between PD and control groups. We further investigated participants who had reported exposure to pesticides and found that the frequency distribution of PON1 genotypes did not differ significantly between patients and controls.

*Conclusion:* The present survey reveals the close relationship between exposure to pesticides and Parkinson's disease. There are no significant differences in the distribution of PON1 genotypes between cases and controls.

**Key Words:** Parkinson's disease, Pesticide, PON1 polymorphism

## INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders among the elderly. PD is characterized by bradykinesia, resting tremor, rigidity, gait disorder and postural reflex impairment. The cardi-

nal symptoms in PD result from the degeneration of midbrain dopaminergic nuclei, most markedly pars compacta of substantia nigra. The cause of PD is unknown. Various metabolic defects in PD have been reported, implying that PD patients have less effective detoxification systemsand raising the possibility that PD may be related to an increased susceptibility to endogenous or exogenous toxin. Among the toxin, MPTP (1-methy-4-phenyl-1,2,3,6-tetra-hydro-pyridine) has been most emphasized. The discovery of neurotoxin MPTP as a cause of PD-like abnormalities has encouraged the search for comparable environmental factors, such as rural residence, use of well water, farming, and contact with pesticides. The chemical resemblance between MPTP and some pesticides, the increased use of these pesticides in rural areas, and the possibilities of contamination of well water by the pesticides suggest the existence of MPTP-like environmental toxins[1].

Genetic variation in xenobiotic metabolizing enzymes involved in the disposition of pesticides may increase the risk of PD[2]. Pesticides, such as organophosphates parathion, have been widespreadly used in Taiwan. Parathion is converted to its active species, paraoxon, via cytochrome P450 of liver. Hydrolysis of paraoxon is catalyzed by serum paraoxonase I (PON1)[3]. PON1 also hydrolyzes other organophosphates such as chlorpyrifos, diazinon, sarin, and soman. PON1 has two polymorphisms at codon 191 and codon 54. The first polymorphism consists of an A ≥ G base pair transition that results in a change of amino acid 191 from glutamine (A allele) to arginine (B allele). The second polymorphism consists of an T ≥ A base pair transition that results in a change of amino acid 54 from leucine (L allele) to methionine (M allele). The A allele and M allele are associated with slow hydrolysis, and the B allele and L allele with fast hydrolysis, of paraoxon. Thus, genotyping of PON1 polymorphisms may provide a method for identification of patients with high risk of organophosphates poisoning[4].

In Caucasian studies[5-7], PON1 Gln191Arg polymorphism is not related to susceptibility of PD. However, polymorphism of codon 54 is associated with marked modulation of serum concentrations of the enzyme independently of the polymorphism at codon 191. The investigation of the association between Parkinson's disease and polymorphism of codon 54 may therefore of importantance[8]. Furthermore, genetic susceptibility to Parkinson's disease appears to be different in different races. In Taiwan, exposures to pesticides potentially could play a more important role in the development of PD. However, the interactions between pesticide exposure, genetic polymorphism of PON1 Met54Leu, and susceptibility to PD in Taiwan have never been explored. We therefore investigated the foregoing interactionsin a Taiwanese population.

## METHODS

One hundred and twenty five patients with idiopathic Parkinson's disease enrolled from Dalin Tzu Chi General Hospital between July 2002 and June 2004 are included in this study. Disease onset is defined as the time at which the first symptom of typical Parkinson's disease began. All patients are evaluated by neurologists at the neurological department of Dalin Tzu Chi General Hospital. The diagnostic criteria for Parkinson's disease are the presence of at least three of the four cardinal signs (tremor at rest, bradykinesia, rigidity, and asymmetric onset) and presence of sustained L-dopa response. Patients with atypical features suggesting multiple system atrophy, progressive supranuclear gaze palsy or secondary causes of parkinsonism (including neuroleptic drug, infection, tumor previous cerebrovascular accident or known toxins) were excluded. The control group consists of 162 unrelated individuals matched with the patients on age and sex. The control subjects were recruited from the outpatient clinic with the diagnoses of back pain or cervical spondylosis. Information was collected with a questionnaire filled out during a face-to-face interview with the subjects and their family members, if available. Age at the onset of PD was defined as the age at which the first symptom of PD became evident according to the patient's recollection. Data included years of farming, drinking water sources, and occupational exposures to pesticides. A positive exposure was defined as an occupational or residential contact with a

given factor for at least 12 months prior to the onset of Parkinson's disease. If the patients indicated that they had been exposed to pesticides, they would be asked to define the duration and the initial age of the exposure.

Buccal mucosa cells are collected from each Parkinson's disease patients and control subjects. The brushed cells are dried at room temperature and stored for genomic DNA extraction. According to a previously reported method[9], the genomic DNA was amplified by PCR with the primers 5' TCTGGCAGAAACTGG-CTCTGAAGCC 'and 5' CTTAAACTGCCAGTCCTA-GAAAACG 3'. The PCR samples were initially denatured for 5 min at 95° followed by 30 cycles of 92° for 1 min, 52° for 45 s, and 72° for 45 s, with a final extension at 72° for 5 min. The PCR products were digested with Nco I endonuclease at 36° overnight. The T→A transition corresponding Leu54Met amino acid substitution of the PON1 gene creates a cleavage site for restriction enzyme of Nco I and produces two bands 25 and 105 base pairs in length after digestion. Finally, the digestion products were separated by 3.5% agarose gel electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light (Fig.).

Each exposure variable was analyzed separately for its strength of association with PD. Logistic regression was used to estimate the odds ratio (OR), 95% CI, and p value of various risk factors. p<0.05 was considered statistically significant. The distribution of the genotypes and alleles frequencies in the PD patients was compared with that in the control subjects.

## RESULTS

A total of 69 women and 56 men with PD were studied. Their mean age was 69.06 years (range: 51 to 86 years). Mean age at the onset of PD was 64.94 (range: 44 to 84 years). Mean Hoehn and Yahr stage of Parkinson's disease patients was 2.4 (range: 1 to 5). There were 90 women and 72 men in the control group. The mean age of the control subjects was 68.0 years (range: 54 to 84 years). The risk of PD was not increased for those which had ever used well water. There is significant association between the risk of Parkinson's disease and exposure to pesticides (OR=1.72, 95% CI=1.07-2.75). We examined the relationship between PD and the duration of exposure to pesticides, and found that the relative risk of PD was 2.14 with a 35-year or longer exposure to pesticides (Table 1).

The genotypes are shown in Table 2. The genotype frequencies display Hardy-Weinberg equilibrium. We found the following genotype distribution of the Leu54Met polymorphism in the Taiwanese population: LL 93.2%, LM 6.2% and MM 0.6%. This pattern is quite different from that in Caucasian populations (49.3%, 38.6% and 12.1%)[9] but is more similar to Mainland China population (92.2%, 7.7% and 0.6%)[10].
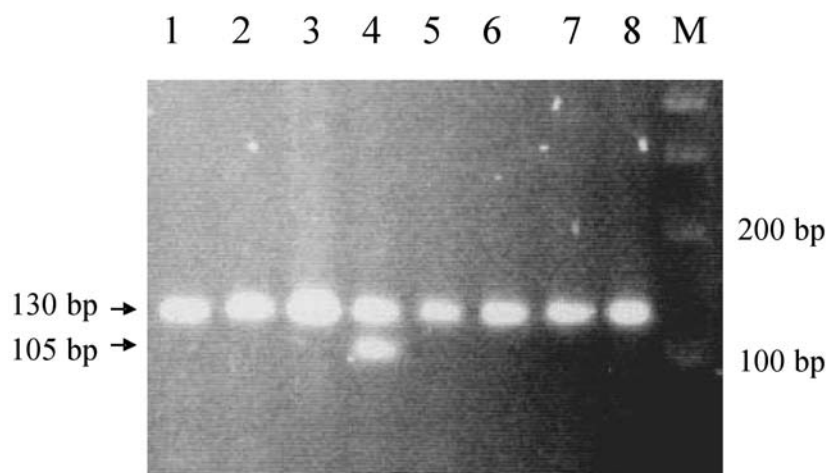


Figure. PCR-RFLP analysis for the mutation of the *Paraoxonase* gene. PCR products were digested with *Nco* I endonuclease prior to 3.5% agarose gel electrophoresis. M: DNA marker ladder; lanes 4: M/L heterozygote, the other DNA samples were all L/L homozygotes.

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**Table 1.** *Analysis for the association between PD and pesticide exposure*

|  | Controls n=162 (%) | Patients n=125 (%) | OR | 95% CI | P value |
|---|---|---|---|---|---|
| Pesticide use | 69 (42.6) | 70 (56.0) | 1.72 | 1.07-2.75 | 0.025 |
| Exposure time (years) |  |  |  |  |  |
| 0 | 93 (57.4) | 55 (44.0) | 1.00 |  |  |
| ≤ 35 (1-35) | 31 (19.1) | 22 (17.6) | 1.20 | 0.63-2.28 | 0.644 |
| ≥ 36 (36-50) | 38 (23.5) | 48 (38.4) | 2.14 | 1.23-3.71 | 0.005 |

**Table 2.** *The distribution of PON1 genotypes and allelic frequencies*

| PON1 genotype | Subjects with pesticide exposure | | Subjects without pesticide exposure | | All subjects | |
|---|---|---|---|---|---|---|
|  | Controls (n=69) n (%) | Patients (n=70) n (%) | Controls (n=93) n (%) | Patients (n=55) n (%) | Controls (n=162) n (%) | Patients (n=125) n (%) |
| LL | 64 (92.8) | 66 (94.3) | 87 (93.5) | 54 (98.2) | 151 (93.2) | 120 (96.0) |
| LM | 5 ( 7.2) | 4 ( 5.7) | 5 ( 5.4) | 0 ( 0.0) | 10 ( 6.2) | 4 ( 3.2) |
| MM | 0 ( 0.0) | 0 ( 0.0) | 1 ( 1.1) | 1 ( 1.8) | 1 ( 0.6) | 1 ( 0.8) |
| M allele | 5 ( 3.6) | 4 ( 2.9) | 7 ( 3.8) | 2 ( 1.8) | 12 ( 3.7) | 6 ( 2.4) |

There is no difference between the patients and the control subjects groups in the genotype of PON1 (p=0.504). Furthermore, the subjects who reported or did not report exposure to pesticides were analyzed and the distribution of the PON1 genotypes did not differ significantly. The M allele was not significantly increased in PD patients compared with the controls (p=0.374). Even among the subjects who reported or did not report exposure to pesticides, the M allele frequency of the PON1 genotypes did not differ significantly between patients and controls (p=0.718 versus p=0.346).

## DISCUSSION

There are only limited reports on the risk of PD with pesticide exposure. A significant association between PD and pesticide use but not between PD and other rural factors was reported in Germany[11]. Reports from Hong Kong showed a 3.6-fold increased relative risk of PD[12] in the subjects who had previously used herbicides/pesticides. Our results concurred with previous observations and showed that occupational exposure to pesticides was significantly associated with PD.

Paraoxonase is an important enzyme for the detoxification of environmental pollution and may therefore prevent organophosphate poisoning[13]. It hydrolyzes many substrates, including aromatic carboxylic acid, nerve gases such as sarin, and paraoxon, a metabolic product of the widely used pesticides. Paraoxon is a potent inhibitor of the cholinesterases that break down the neurotransmitter acetylcholine. Thus, paraoxonase in blood might help to prevent paraoxon from reaching the nervous system, where the pesticide would cause acetylcholine to accumulate at the cholinergic synaptic junction and therefore excessively stimulate the neurons. The experiments on rats and mice have shown that intravenously injected PON1 provides protection against paraoxon toxicity. PON1 knockout mice displayed much greater sensitivity to chlorpyrifos oxon toxicity than wild mice[14].

Serum levels of paraoxonase activity vary widely among individuals, which may partly account for differences in susceptibility to organophosphate poisoning[15]. The molecular basis for this difference has been ascribed to the presence of DNA polymorphisms in the PON1 gene at amino acid positions 54 and 191[4,7]. Two poly-

morphisms of PON1 are in linkage disequlibrium, giving rise to leucine at position 54 and arginine at position 191. The Leu54Met polymorphism was associated with the concentration and activity of paraoxonase and was independent of the polymorphism at position 191. The M allele was associated with lower concentrations of the enzyme and MM homozygotes had the lowest level of paraoxonase activity, irrespective of the presence of glutamine or arginine at position 191[7]. Hence, environmental toxin metabolism might be more closely related to Leu54Met polymorphism than to Gln191Arg polymorphism. It was the reason that we specifically investigated the relationship between Leu54Met polymorphism and the risk of Parkinson's disease.

The frequency of the Met 54 allele of PON1 is significantly increased in patients with PD in a study based on Russian population[9]. The relative risk of PD in the Met 54 allele carriers has been estimated to be 2.3-fold higher than in that in the homozygotes ofthe L allele. They suggested that the Met 54 allele may be considered as an independent risk factor for PD. We find no evidence in our data to support the findings in the Russian study. Similar negative findings were also reported in China[10]. It could be explained by ethnic differences between Western and Eastern populations. It is also possible that some patients with a genetic predisposition may have to contact with some "appropriate" environmental toxins before phenotypic expression of PD. We analyzed the subjects who reported or did not report exposure to pesticides, and still could not find that the distribution of the PON1 genotypes differed significantly between patients and controls (p=0.379 versus p=0.508). There may be more than one susceptible gene contributing to PD, orinteractions of PON1 with other genes may play an important role in the susceptibility of PD. Therefore, complex gene-environmental and gene-gene interactions should be investigated in the future.

In conclusion, the present study revealed the close relationship between long exposure to pesticides and the occurrence of Parkinson's disease. However, there is no significant difference in the distribution of PON1 genotypes between PD patients and controls.

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