

SHORT REPORT

## Genetic and environmental risk factors for Parkinson's disease in a Chinese population

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**Abstract**

**An epidemiological study of the environmental and genetic factors as well as the possible interplay between them was conducted among 215 patients with Parkinson's disease and 313 controls in a Chinese population in Hong Kong. In univariate analysis, a regular tea drinking habit was found to be a protective factor, which had not been reported before. Smoking (a protective factor), family history, duration of pesticide exposure (in years) in farming and pesticide exposure during farming in women (both risk factors) have been reported previously. In multivariate analysis, current smoking reached borderline significance at the 5% level and the variables, years exposed to pesticides and family history were significant at the 10% level. By contrast with the common occurrence of polymorphism of the CYP2D6 gene (a gene involved with xenobiotic metabolism) in white people, it is very rare in China and is not thought to be a significant factor contributing to Parkinson's disease in Chinese people.**

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Epidemiological studies suggest that environmental factors may be important for the development of Parkinson's disease. The most consistent although not universal finding has been smoking and its inverse relation to Parkinson's disease.<sup>1-4</sup> Results of studies into other risk factors have been less consistent. Nevertheless, some large studies<sup>2 5 6</sup> have identified pesticide or herbicide exposure as putative risk factors. More conflicting are the results on, for example, rural living, farming, and drinking well water.<sup>2 5-8</sup> Methodological differences, sample sizes, and regional differences in farming practices may explain possible differences.

Genetic factors may also be important. Asymptomatic monozygotic twins can show signs of Parkinson's disease some years after the appearance of the disease in their identical siblings.<sup>9</sup> Recently, a potential mechanism for the genetic susceptibility to Parkinson's disease has been postulated. White patients with a

metabolic defect in the cytochrome P-450 CYP2D6-debrisoquine hydroxylase gene with the poor metaboliser phenotype had a 2.54-fold increased risk of Parkinson's disease than controls.<sup>10</sup> Cytochrome P-450 CYP2D6 polymorphism is an autosomal recessive trait associated with impaired debrisoquine metabolism in 5%-10% of white people. It is known that this gene is also responsible for the metabolism of antipsychotic drugs such as haloperidol and the detoxification of certain environmental toxins. Furthermore, it is important in the metabolism of MPTP,<sup>11</sup> which is known to cause dopaminergic neuronal damage and parkinsonism in humans and experimental animals.

We hypothesise that the mutant CYP2D6 gene may interact with environmental toxins to cause Parkinson's disease. We therefore undertook a case-control study which included putative environmental factors and the presence of the mutant CYP2D6 gene in a Chinese population in Hong Kong.

**Methods**

A total of 215 patients with Parkinson's disease were recruited largely from two major hospitals in Hong Kong (Prince of Wales Hospital and United Christian Hospital). All patients were examined by a specialist geriatrician or neurologist. Inclusion criteria for Parkinson's disease were according to those of Marangonore *et al.*<sup>12</sup> The exclusion criteria were all other causes for parkinsonism.

Three hundred and thirteen controls were recruited from the same sources. In the selection of controls, 10 year age group, sex and locality (hospital catchment area) were taken into consideration to make the control group as comparable with the Parkinson's disease group as possible (table 1). All controls were examined by a geriatrician or neurologist and those with signs of Parkinson's disease or parkinsonism were excluded.

**EPIDEMIOLOGICAL STUDY**

A standard questionnaire focusing on possible risk factors for Parkinson's disease was completed by all participants by personal interview. To minimise bias, the participants were blinded to the specific hypothesis being investigated. Several doctors and nurses were used

Table 1 All subjects combined

	Cases n=215		Controls n=313	
	n	(%)	n	(%)
Age group:				
<60	29	(13.5)	51	(16.3)
60-69	72	(33.5)	100	(31.9)
70-79	72	(33.5)	107	(34.1)
80+	42	(19.5)	55	(17.6)
Sex:				
Female	101	(47.0)	142	(45.4)
Male	114	(53.0)	171	(54.6)
Hospital:				
UCH	97	(45.1)	125	(39.9)
PWH	118	(54.9)	188	(60.1)

as interviewers. However, the interviews were conducted strictly according to the standard questionnaire (in Chinese), and particular care was taken to instruct the interviewers not to prompt the interviewees in the answering of questions.

The information obtained included age of onset of Parkinson's disease, place of residence (urban *v* rural), duration of residence in urban or rural environment, past occupation in farming and duration, pesticide exposure during farming, consumption of raw vegetables, use of vitamin supplements or cod liver oil, frequency of consumption of fruits and leafy green vegetables (as an indication of antioxidant intake), smoking habits, tea drinking habits, consumption of raw vegetables, and family history of Parkinson's disease.

#### LABORATORY METHODS

Venous blood was obtained from all participants. Genomic DNA was extracted from the whole blood by the salting out method.<sup>13</sup> Two allele specific polymerase chain reaction (PCR)

assays were carried out using primers as described to detect a G-to-A substitution at the junction of intron 3/exon 4 and a base pair deletion in exon 5.<sup>10</sup> The mutant alleles were deleted by restriction analysis with endonucleases *Bst*NI and *Hpa*II respectively. These two PCR assays can identify up to 90% of those with the CYP2D6 gene polymorphism.

#### STATISTICAL ANALYSIS

The statistical package SPSS was used. Univariate logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals<sup>14</sup> as well as the p values of various risk factors. The same process was repeated again by stratifying sex to study the difference in the risk factors between men and women with Parkinson's disease. Multivariate logistic regression was used to analyse all the risk factors, with the same statistical package.

## Results

### EPIDEMIOLOGICAL SURVEY

In the univariate analysis there were significant inverse relations between Parkinson's disease in this population with current smoking and tea drinking. A significant positive relation with the presence of a family history of the disease was also found (table 2). People who drank infrequently (<1 cup a day) were found to have an OR of 1.51 (95% CI 1.06-2.15; p=0.02) compared with regular drinkers ( $\geq$  1 cup a day) for having Parkinson's disease. Current smokers were found to be less likely to have Parkinson's disease than controls (OR=0.437; 95% CI 0.228-0.839; p=0.013) whereas a positive family history had a 5.21-fold increased risk of Parkinson's disease (95% CI 1.07-25.3; p=0.04).

Table 2 All subjects combined

Factors	Cases n=215		Controls n=313		Univariate results		Multivariate results	
	n	(%)	n	(%)	OR (95% CI)	p Value	Adjusted OR (95% CI)	p Value
Smoking history:								
(1) Current	14	(10.61)	41	(21.35)	0.437 (0.228-0.839)	0.013	0.514 (0.262-1.01)	0.054
Non-smoker	118	(89.39)	151	(78.65)	1			
(2) Ex-smoker	83	(41.29)	121	(44.49)	0.878 (0.607-1.27)	0.489	0.907 (0.618-1.33)	0.621
Non-smoker	118	(58.71)	151	(55.51)	1			
Number of years exposed to pesticide:					1.05 (1.01-1.09)	0.018	1.05 (0.992-1.11)	0.090
Tea (cups per day)								
<1	102	(47.44)	117	(37.38)	1.51 (1.06-2.15)	0.021	1.35 (0.931-1.95)	0.114
$\geq$ 1	113	(52.56)	196	(62.62)	1			
Family history:								
History	7	(3.26)	2	(0.64)	5.21 (1.07-25.3)	0.041	4.04 (0.802-20.4)	0.091
No history	208	(96.74)	311	(99.36)	1			
Pesticide exposure in farming:								
Pesticide	19	(8.84)	16	(5.11)	1.80 (0.903-3.58)	0.095	0.754 (0.257-2.22)	0.608
No pesticide	196	(91.16)	297	(94.89)	1			
Percentage of stay in rural area					1.01 (0.999-1.01)	0.122	1.00 (0.995-1.01)	0.408
Vitamin or cod liver oil supplement (times/week):								
<1	184	(85.58)	280	(89.46)	0.700 (0.414-1.18)	0.182	0.685 (0.396-1.19)	0.178
$\geq$ 1	31	(14.42)	33	(10.54)	1			
Consume raw vegetables (times/week):								
<1	210	(97.67)	309	(98.72)	0.544 (0.144-2.05)	0.368	0.577 (0.149-2.23)	0.426
$\geq$ 1	5	(2.33)	4	(1.28)	1			
Fruit consumption (times per week):								
<1	11	(5.12)	19	(6.07)	0.834 (0.389-1.79)	0.642	0.850 (0.383-1.89)	0.689
$\geq$ 1	204	(94.88)	294	(93.93)	1			
Vegetable consumption (times/week):								
<1	2	(0.93)	2	(0.64)	1.46 (0.204-10.4)	0.706	1.58 (0.215-11.6)	0.655
$\geq$ 1	213	(99.07)	311	(99.36)	1			
Drinking well water:								
	134	(62.33)	193	(61.66)	1.03 (0.719-1.47)	0.877	1.04 (0.701-1.54)	0.850
	81	(37.67)	120	(38.34)	1			
Farming:								
	84	(39.07)	124	(39.62)	0.977 (0.685-1.39)	0.900	0.919 (0.592-1.43)	0.709
	131	(60.93)	189	(60.38)	1			

Duration of pesticide exposure (in years) during farming was found to have a marginally increased risk of Parkinson's disease (OR=1.05; 95% CI 1.01–1.09;  $p=0.018$ ). Exposure to pesticides during farming was not significantly related to Parkinson's disease for the patient group as a whole (OR=1.80; 95% CI 0.903–3.58;  $p=0.095$ ), but when the group was analysed according to sex, this factor was significantly related in women (OR=6.84; 95% CI 1.90–24.7;  $p=0.003$ ) but not in men (OR=0.68; 95% CI 0.25–1.83;  $p=0.44$ ). However, the numbers of patients and controls exposed to pesticides were few in both groups (patients: women 13, men six; controls: women three, men 13).

Rural residence, farming, drinking well water, and consumption of fruits and leafy green vegetables were found not to have a significant relation with Parkinson's disease in this population. Infrequent consumption (<once a week) of raw vegetables (OR=0.54; 95% CI 0.14–2.05;  $p=0.37$ ), and infrequent (<1 time a week) vitamins and cod liver oil supplements (OR=0.70; 95% CI 0.41–1.18;  $p=0.18$ ) showed a trend towards a lower risk of Parkinson's disease, but in neither case was this significant.

When the process (univariate analysis) was repeated by stratifying the sex, the differences found between the sexes were exposure to pesticides and duration of exposure to pesticides, both risk factors in women (OR=6.84; 95% CI 1.90–24.7;  $p=0.003$  and OR=1.07; 95% CI 1.004–1.14;  $p=0.039$  respectively). By contrast, current smoking was found to be a protective factor in men (OR=0.441; 95% CI 0.204–0.955;  $p=0.038$ )

If all subjects were analysed together (table 2), no variable was found to be significant at the 5% level in the multivariate analysis but the variables that were significant in the univariate analysis remain top of the list in terms of degree of significance. The variable current smoking reached borderline significance at the 5% level and the variables, years exposed to pesticide and family history, were significant at the 10% level. It should be stressed that any significant effect detected is independent of all other variables concerned.

#### GENETICS

Two polymorphic sites of the CYP2D6 gene were analysed in 437 subjects—a total of 874 alleles. Only three mutant alleles were found, all of which were heterozygous, in three subjects at the intron 3 site for a G-to-A transition at G1934. Two subjects were controls and one was a patient. No polymorphism was detected at the exon 5 site in this study.

#### Discussion

This case-control study investigated the role of environmental and genetic factors and the possible interplay between them, in promoting susceptibility to Parkinson's disease in a Chinese population in Hong Kong. In common with other case-control studies, selection bias of both cases and controls cannot be completely excluded. However, the two major hos-

pitals that participated in this study are the only hospitals that serve patients living within their respective catchment areas. Furthermore, a large number of patients with Parkinson's disease and controls were recruited from the two hospitals to minimise bias, and the controls were selected with similar characteristics in age, sex, and locality compared with the subjects with Parkinson's disease.

the finding in this study of an increased risk of acquiring Parkinson's disease in those with a positive family history of the condition accords with other studies<sup>15,16</sup> as does the finding of a decreased risk associated with cigarette smoking.<sup>2-4</sup> However, the decreased risk associated with tea drinking found in the current study has not been reported previously, although the fact that the lower limit of the 95% CI of the OR only just excluded unity requires that the result be interpreted with some caution. Further studies would be helpful to clarify the issue. If tea is protective against the acquisition of Parkinson's disease, it may be because of its antioxidant qualities, or because it contains tannins which form tight complexes with iron, thus inhibiting iron absorption. Increased iron deposition has been found in the substantia nigra of patients with Parkinson's disease, raising the possibility that iron is involved in the pathogenesis of the disease<sup>17</sup>

By contrast with a previous case-control study conducted in Hong Kong,<sup>18</sup> we found no association between prior rural dwelling or farming, and the development of Parkinson's disease. Urbanisation in Hong Kong in recent years may explain the differing results of the two studies.

Pesticides have been reported in some studies as a risk factor for the development of Parkinson's disease.<sup>2-5</sup> We found that duration of pesticide exposure (in years) is associated with a marginal increase in risk. Our study also found that pesticide exposure in women was significantly associated with Parkinson's disease, but this was not the case in men. The reason for this difference is unclear. It is possible that susceptibility to pesticide exposure as a determinant of Parkinson's disease risk is sex related, as is the development of cirrhosis from chronic exposure to alcohol. However, because of the low number exposed overall, it is necessary to be cautious in interpretation (the low exposure reflects the farming habits in Hong Kong over the past decades).

Increased vitamin, cod liver oil supplements, and ingestion of raw vegetables all showed a trend towards increasing the risk of Parkinson's disease in the current study, but without achieving significance. The use of vitamin or cod liver oil supplements among patients with Parkinson's disease was further analysed. The mean duration of use was short (0.59 years) compared with the mean duration of Parkinson's disease (5.4 years) indicating that the relation is probably not causal.

Cytochrome P-450 CYP2D6 debrisoquine hydroxylase gene polymorphism was found to be extremely rare in both patients and controls in the current study, and thus it seems very unlikely that this gene has a significant role in

determining risk for Parkinson's disease in the Chinese population. This finding is contrary to Smith's study in white people,<sup>10</sup> but is in agreement with a study of Chinese subjects in Taiwan.<sup>19</sup> Epidemiological studies from China<sup>20, 21</sup> have shown that the prevalence of Parkinson's disease is much lower than in white people.<sup>22</sup> The low frequency of this genetic polymorphism in the Chinese population may provide one possible explanation for the low prevalence of Parkinson's disease in this racial group.

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