ORIGINAL ARTICLE

Gene-environment interactions in parkinsonism and Parkinson's disease: the Geoparkinson study

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The full results of all the genetic and gene-environment interaction analyses are available online at the *OEM* website (http://oem.bmj.com/supplemental).

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Objectives: To investigate associations of Parkinson's disease (PD) and parkinsonian syndromes with polymorphic genes that influence metabolism of either foreign chemical substances or dopamine and to seek evidence of gene-environment interaction effects that modify risk.
Methods: A case-control study of 959 prevalent cases of parkinsonism (767 with PD) and 1989 controls across five European centres. Occupational hygienists estimated the average annual intensity of exposure to solvents, pesticides and metals, (iron, copper, manganese), blind to disease status. *CYP2D6*, *PON1*, *GSTM1*, *GSTT1*, *GSTM3*, *GSTP1*, *NQO1*, *CYP1B1*, *MAO-A*, *MAO-B*, *SOD 2*, *EPHX*, *DAT1*, *DRD2* and *NAT2* were genotyped. Results were analysed using multiple logistic regression adjusting for key confounders.
Results: There was a modest but significant association between *MAO-A* polymorphism in males and disease risk (G vs T, OR 1.30, 95% CI 1.02 to 1.66, adjusted). The majority of gene-environment analyses did not show significant interaction effects. There were possible interaction effects between *GSTM1 null* genotype and solvent exposure (which were stronger when limited to PD cases only).
Conclusions: Many small studies have reported associations between genetic polymorphisms and PD. Fewer have examined gene-environment interactions. This large study was sufficiently powered to examine these aspects. *GSTM1 null* subjects heavily exposed to solvents appear to be at increased risk of PD. There was insufficient evidence that the other gene-environment combinations investigated modified disease risk, suggesting they contribute little to the burden of PD.

The causes of Parkinson's disease (PD) are unclear although both genetic and environmental factors are believed to be important. Several occupational exposures have been studied as possible risk factors for PD including pesticides, organic solvents and metals including iron, copper and manganese. A positive family history is a risk factor,[1] but whether this reflects shared heredity or environment is uncertain. Polymorphic genes coding for enzymes involved in the metabolism of foreign chemicals (xenobiotics) may modify disease risk in exposed individuals. Similarly, polymorphic genes coding for enzymes involved in the transport or metabolism of dopamine may alter disease risk. The interested reader is referred to the additional electronic text regarding candidate genes examined as risk factors in this study. Briefly, we selected genes coding for enzymes that metabolise foreign chemicals, transport dopamine or metabolise dopamine and have polymorphisms that occur relatively frequently in the European population. The following genes were studied: *CYP2D6, CYP1B1, PON1, GSTM1, GSTT1, GSTM3, GSTP1, NQO1, MAO-A, MAO-B, SOD 2, EPHX, DAT1, DRD2* and *NAT2*.

Many association studies investigating candidate genetic polymorphisms and occupational exposures have been relatively small and have not used high quality exposure estimates. The interaction between occupational exposures and genetic factors may be important in leading to disease in susceptible individuals. Therefore this large multicentre study of environmental exposures, genes and gene-environment interactions as risk modifiers for Parkinson's disease was undertaken.

METHODS

The overall study aim was to (i) explore environmental risk factors (organic solvents, pesticides, iron, copper or manganese) for Parkinson's disease and parkinsonism; (ii) to determine if polymorphisms in a number of genes that influence metabolism of chemicals can modify the risk of PD and parkinsonism; and (iii) to establish whether there was any evidence of gene-environment interactions between these polymorphic genes and occupational exposure to solvents, pesticides, iron, copper or manganese.

The research was a multicentre case-control study, prevalent cases of parkinsonism and controls being recruited in the five participating countries (Scotland, Italy, Sweden, Romania and Malta). In an accompanying paper the study design is more fully described and the exposure assessment methods have previously been reported.[2] The findings regarding gene-tobacco smoke interactions as risk modifiers for PD will be reported separately.

Each centre aimed to recruit 200 cases and 400 age and sex balanced controls. Cases were classified as having Parkinson's disease or parkinsonism using the United Kingdom Parkinson's Disease Society Brain Bank (UK PDS Brain Bank) clinical

Abbreviations: AAI, average annual intensity; bp, base pair; CE, cumulative exposure; HWE, Hardy-Weinberg equilibrium; JEM, job exposure matrix; OEL, occupational exposure limit; OR, odds ratios; PCR, polymerase chain reaction; PD, Parkinson's disease; RFLP, restriction fragment length polymorphism

diagnostic criteria. Individuals with vascular or drug induced parkinsonism were excluded from the study, as were those with dementia. No information on race or ethnicity was collected from study participants. However, individuals with non-Maltese parents were excluded from the Maltese arm of the study. The relevant ethics committees approved the study and all subjects gave written informed consent.

Power calculations

The power to detect a significant association with a disease is dependent on the proportion of subjects with a potential risk factor (or combination of risk factors). For a single risk factor (exposed/unexposed or polymorphism present/absent), it was calculated that a two group continuity corrected χ^2 test with a 0.05 two sided significance level would have 89% power to detect the difference between a case group proportion of 0.57 and a control group proportion of 0.50 with sample sizes of 800 cases and 1600 controls, respectively. If a risk factor were much more or less common then it would be possible to detect much smaller differences in proportions between the case and control groups as statistically significant. For example, if a polymorphism were present in 15% of controls then a difference of plus or minus 5% in the percentage within the cases would be detected with more than 80% power. If the polymorphism, or a combination of polymorphisms, were present in 5% of the controls then a difference of plus or minus 3% within the cases would be detectable with 80% power. Provided the number of the subjects with a particular combination of characteristics is not extremely small then the sample size would have sufficient power to detect interactions between exposure and genetic factors at the 5% significance level. With this sample size, the width of the 95% confidence interval for the log odds ratios presented by De Palma *et al* for an interaction between solvent exposure, CYP2D6 status and Parkinson's disease would be reduced by a factor of 0.35.[3] Then the odds ratios would be 1.15 (0.94 to 1.40) for solvent exposure, 0.69 (0.48 to 1.00) for the presence of two variant alleles at the CYP2D6 locus and 14.47 (5.94 to 35.09) for their interaction. Then the odds ratio for solvent exposure would be very close to indicating statistical significance and the interaction result would be much more significant than reported in that paper.

Questionnaire

A questionnaire in English was developed, piloted and subsequently translated into Italian, Swedish, Romanian and Maltese. Trained interviewers administered the questionnaire. Occupational history was obtained together with information as to the duration and likely intensity of occupational and hobby exposure to solvents, pesticides, iron, copper and manganese. Private water supply use such as a well, river or spring was recorded by location and duration. Smoking (''ever consumed a tobacco product,'' defined as smoking one cigarette per day or two cigars or two pipes per week or use of snuff/chewable tobacco for a period greater than 6 months) and alcohol histories were obtained. History of having been knocked unconscious (defined as any loss of consciousness) and of Parkinson's disease in first and second degree relatives was recorded. Interviewers administered a core questionnaire to all subjects with a series of questions that triggered exposure specific questionnaires where relevant.

Exposure estimation

An occupational hygienist produced a job exposure matrix (JEM) for commonly reported occupations, categorising exposures as zero, low, medium or high with reference to the then UK occupational exposure limit (OEL) for mixed solvents, a typical pesticide employed in the task or for iron, copper or manganese in air. The resulting exposure estimate was then modified using subjective exposure estimation techniques.[2] The hygienist employed judgment as to the most likely agents for the described task where the specific agent was not recalled. Exposure intensity for each job was combined with data on exposure duration (number of hours per day, days per year and years exposed) to calculate a job cumulative exposure. This was expressed in OEL years where 1 OEL year was equivalent to working at the then UK OEL for 8 hours per day for 240 days per year. Job cumulative exposure values were summed to provide a lifetime cumulative exposure (CE) to that chemical group. A second measure produced was the average annual intensity (AAI) of exposure derived from dividing the lifetime CE by the number of years of exposure to that material. An indirect validation of this exposure assessment methodology has been reported for solvent exposures.[2]

Genetic methods

DNA was extracted from whole blood samples or from buccal samples taken from each subject enrolled in the five participating countries. Most of the genes were selected for analysis on the basis that they have polymorphisms that occur relatively frequently in the European population and are involved in the metabolism of foreign chemicals. The following genes were studied: *CYP2D6, CYP1B1, PON1, GSTM1, GSTT1, GSTM3, GSTP1, NQO1, MAO-A, MAO-B, SOD 2, EPHX, DAT1, DRD2* and *NAT2*. All Maltese samples were analysed in Malta, whereas the other three laboratories in Scotland, Italy and Sweden analysed specific genes for the remaining countries. In Scotland *CYP2D6, PON1, DAT1* and *NAT2* were analysed; in Italy *CYP1B1, GSTM1, GSTT1, GSTM3, GSTP1* and *NQO1* were analysed; and in Sweden *MAO-A, MAO-B, SOD 2, EPHX* and *DRD2* were analysed. One per cent of all samples were sent to the laboratories a second time as blind duplicates for quality assurance purposes.

CYP2D6 genotyping was carried out using published methodology to identify *CYP2D6*4*, the major ''poor metaboliser'' allele in people of white race.[4] Single nucleotide polymorphisms in codons 55 (Leu55Met) and 192 (Gly192Arg) of the *PON1* gene were genotyped by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (PCR-RFLP) analysis.[5 6]

GSTM1 and *GSTT1* polymorphisms (homozygous large gene deletions) were characterised by a multiplex PCR method.[7] We characterised *GSTM3, GSTP1, NQO1* and *CYP1B1* polymorphisms using real time fluorescence PCR methods on a LightCycler Instrument (Roche Diagnostics GmbH, Mannheim, Germany). *GSTP1* exon 5 (Ile105Val), *NQO1* (Pro187Ser) and *CYP1B1* (Leu432Val) polymorphisms were identified applying previously described protocols.[8–10] New methods were developed to characterise the genetic polymorphisms of *GSTM3*[11] and *GSTP1* exon 6. The primer and hybridisation probe's sequences used to characterise the *GSTP1* exon 6 polymorphism are shown in table 1. The *GSTP1* exon 6 fluorescein labelled probe hybridises to a 20 bp sequence of exon 6 of the *GSTP1* gene (nt 487-468 of the X08095 GeneBank sequence), which contains the base transition and is specific for the variant allele. Therefore, the wild type allele (Ala 114) will display a lower melting temperature, compared to that of the variant allele (Val 114). For the Maltese samples the same PCR products from the *GSTM3* and the *NQO1* genes were analysed by dideoxy sequencing and by restriction enyzme digestion using Hinf I, respectively. The *GSTP1* polymorphisms[12] and *CYP1B1*[13] were analysed using previously published methods.

Genotyping for MAO-A polymorphism was carried out for the Fnu4HI site[14] that lies in the coding region of the gene (exon 8) at position 941 and was detected by DHPLC (WAVE) assay. Pyrosequencing[15] was carried out for MAO-B, intron

Table 1 Oligonucleotide primers, probes and restriction endonuclease employed in genotyping (unless otherwise listed in the text)

Genes	Molecular biology methods	
	PCR-RFLP	
	Oligonucleotide primers	Restriction endonuclease
DAT1	D9RU 5′-CATCATCTACCCGGAAGCCA-3′ D9RL 5′-CAGGGTGAGCAGCATGATGA-3′	DdeI
	Real time PCR	
	Oligonucleotide primers	Hybridisation probes
GSTP1 exon 6	Forward primer 5′-AAGCAGAGGAGAATCTGGGACTC-3′ (Reverse primer) 5′-GGCCAGATGCTCACCTGGTC-3′	5′-LC Red 640*- CCAGACACCAC-CATGTATCATCCTACTCTCp-3′ 5′-GTCATCCTTGCCCACCTCCT-FL†3′
	Pyrosequencing	
	Pyrosequencing primers	Sequencing primers
MAO-B	5′-GTCCTTTAGGGAGCAGATTAG-3′ 5′-biotin-CAGACTCTGGTTCTGACTGC-3′	5′-GATTAGAAGAAAGATGGT-3′
SOD2	5′-biotin-CAGCCCAGCCTGCGTAGACG-3′ 5′-CTGGAGCCCAGATACCCCAA-3′	5′-CCCAGATACCCCAAA-3′
	PCR and dideoxy sequencing primers	
EPHX3	5′-GGAAACTGCCTTGCCACTCC-3′ 5′-CTAGCTCTAAAGATGGACAGA-3′	
	DHPLC (WAVE)	
MAO-A exon 8	5′-TTGCTTGTGTGTGTTTTAGTGC-3′ 5′-GACAGACCAAGATTTCTAATCC-3′	
NAT2	5′-GGATTCATGCAGTAGAAATAC-3′ 5′ATAACGTGAGGGTAGAGAGG-3′	

*LightCycler Red 640; †fluorescein.

13–36 A/G (table 1). Primers were developed (table 1) for sequencing the C/T polymorphism (Val16Ala) in the *SOD2* gene using pyrosequencing.[15] For the Maltese samples these polymorphisms were analysed by dideoxy sequencing. Two amino acid polymorphisms in the *EPHX1* gene (*EPHX1* exon 3 and *EPHX1* exon 4) were determined by a PCR-RFLP assay[16 17] taking care to test for a synonymous SNP in the primer site.[18] The *EPHX1* Tyr113His substitution in exon 3 was characterised in the Maltese samples by dideoxy sequencing (table 1).

A PCR-RFLP approach identified the A1215>G polymorphism in exon 9 of the *DAT1* gene using the primers shown in table 1. The resulting 83 base pair (bp) PCR product was digested with DdeI, producing fragments of 40 bp and 43 bp for the wild type allele. Two polymorphisms in the *DRD2* gene, *DRD2* TaqI-A and *DRD2* TaqI-B, were detected by PCR-RFLP, using TaqI as restriction enzyme.[19 20]

The *NAT2* status was determined using the WAVE DNA fragment analysis system. Using this method, two polymorphisms (C282T and T341C) were identified allowing identification of the most common slow acetylator alleles found in people of white race.[21] The same polymorphisms were characterised in the Maltese samples by sequencing of the whole *NAT2* gene.

Statistical methods

Statistical analyses were undertaken relating genetic factors to disease state: cases of parkinsonism versus controls. None of the analyses assumed cases were matched with specific controls. Gene distributions in controls in the five countries overall were tested to confirm they fitted the Hardy-Weinberg equilibrium (HWE). If the overall p value was less than $p = 0.001$ then each country was tested individually and any country where $p<0.001$ was excluded from analyses for that polymorphism. *GSTM1* and *GSTT1* were not tested for HWE, as the test used does not distinguish between heterozygotes and homozygotes.

For each polymorphism odds ratios (OR) were calculated for each category compared to a prespecified reference category. Where there were fewer than 5% of subjects in one category then categories were combined and the factor was treated as binary. One genetic factor with seven categories (GSTP1 haplotype) was collapsed into three categories before statistical analysis. Since *MAO-A* and *MAO-B* are X linked, only females could belong to the heterozygous category; therefore these factors were considered separately for males and females. Depending on the number of categories a χ^2 test or χ^2 test for trend was conducted to assess the relation between the genetic factor and disease state.

Multiple logistic regression was then used to obtain estimates of ORs for genetic factors adjusting for the following prespecified covariates: age, sex, country, ever used tobacco, ever been knocked unconscious and first degree family history of Parkinson's disease. Subjects with missing information for any of these covariates were excluded from the adjusted analyses. Genetic factors with two categories were tested to establish whether the coefficient of the factor representing the log OR was significantly different from zero. For risk factors with three ordered categories a trend test was conducted using logistic regression assuming equidistance between the three categories.

All of the above analyses were then repeated restricting cases to those with a diagnosis of PD. No adjustment was made for multiple significance testing. Occupational exposures were arbitrarily split into low and high exposure categories taking high exposure as being an average AAI of >20% of the current UK OEL value for mixed solvents, iron or manganese. For pesticides and copper, where exposures were generally lower, the cut-off for high exposure was taken as an average annual intensity of 2% of the relevant OEL.[2]

Prespecified gene-environment interactions were evaluated by first calculating a 2×4 table showing the numbers of cases and controls in each combination of genetic and occupational exposure with respect to the reference category—that is, the combination of the reference genotype and no exposure. From the 2×4 table various ORs may be presented including those for having one or both of the two factors with respect to this reference.[22] In each case an OR representing the deviation from the multiplicative model of interaction was calculated.

When factors with three or more levels were involved, appropriate extensions to the 2×4 table were produced. In addition to the gene-environment interactions some gene-gene interactions were assessed using identical methods. Two three

Table 2 Analysis of genetic factors by case-control status (all cases versus controls) after adjustment*

Genetic polymorphisms	Reference group	Studied groups	OR (95% CI)	p Value**
CYP2D6	*1*1	*1*4	0.90 (0.75 to 1.07)	0.16**
		*4*4	0.83 (0.55 to 1.26)	
PON1 L55M	M/M	L/M	0.92 (0.77 to 1.09)	0.31**
		L/L	0.90 (0.69 to 1.18)	
PON1 Q192R	Q/Q	Q/R	0.99 (0.83 to 1.17)	0.67**
		R/R	1.40 (0.83 to 1.57)	
GSTM1	Positive	Null	1.12 (0.95 to 1.31)	0.18
GSTT1	Positive	Null	0.93 (0.75 to 1.56)	0.52
GSTM3	AA	AB	1.01 (0.84 to 1.20)	0.54**
		BB	0.71 (0.42 to 1.22)	
GSTP1 haplotype	AA	AB/AC/AD	0.98 (0.82 to 1.16)	0.74
		BB/BC/CC	1.10 (0.83 to 1.47)	
NQO1	*1*1	*1*2	0.95 (0.80 to 1.13)	0.41**
		*2*2	0.86 (0.55 to 1.34)	
CYP1B1	*1*1	*1*3	0.95 (0.79 to 1.13)	0.76**
		*3*3	0.98 (0.77 to 1.24)	
MAO-A (males)	T	G	1.30 (1.02 to 1.66)	0.04
MAO-A (females)	TT	TG	1.06 (0.82 to 1.36)	0.55**
		GG	1.26 (0.72 to 1.76)	
MAO-B (males)	A	G	1.09 (0.87 to 1.36)	0.45
MAO-B (females)	AA	AG	1.14 (0.86 to 1.51)	0.49**
		GG	1.11 (0.79 to 1.56)	
SOD2 Val_9Ala	AA	AV	1.00 (0.82 to 1.22)	0.95**
		VV	0.99 (0.79 to 1.25)	
EPHX3 Y113H†	YY	YH	0.89 (0.71 to 1.13)	0.28**
		HH	0.86 (0.61 to 1.21)	
EPHX4 H139R	HH	HR	0.95 (0.80 to 1.13)	0.72**
		RR	1.37 (0.89 to 2.10)	
DAT1	A/A	A/G	0.94 (0.80 to 1.12)	0.93**
		G/G	1.13 (0.82 to 1.55)	
DRD2A†††	CC	CT	0.93 (0.74 to 1.16)	0.26**
		TT	1.42 (1.00 to 2.03)	
DRD2B†	B2B2	B2B1	0.95 (0.77 to 1.67)	0.97**
		B1B1	1.27 (0.69 to 2.34)	
NAT2	Fast/fast	Fast/slow	1.04 (0.87 to 1.24)	0.97**
		Slow/slow	0.95 (0.71 to 1.26)	

*Logistic regression adjusting for age, sex, country, ever used tobacco containing product, ever knocked unconscious and first degree family history of Parkinson's disease. *MAO-A* and *MAO-B* are not adjusted for sex but are presented for males and females separately.
**For three category factors p value for trend derived from logistic regression coding genetic factors as 0, 1 and 2 variable.
†Malta excluded from analysis involving *DRD2B*.
††Sweden and Italy excluded from analysis involving *EPHX3*.
†††Sweden and Malta excluded from analysis involving *DRD2A*.

way gene-gene-environment interactions were also assessed in a similar way. Multiple logistic regression was again undertaken to adjust the analysis of interactions for confounding factors. For interactions between two binary factors, a multiplicative interaction term was included in the model described above and a test carried out to determine whether the coefficient of the interaction term was significantly different from zero.

For factors with three categories, two dummy variables were created and interactions between all combinations of these variables were included in the model. It was then tested simultaneously whether any of these interactions were significantly different from zero using a single likelihood ratio test.

Gene-environment interaction analyses were undertaken for *CYP2D6*, *GSTM1*, *GSTT1*, *GSTM3*, *GSTP1* haplotype, *NQO1*, *CYP1B1*, *SOD2*, *NAT2* and solvents. Similar analyses were undertaken for pesticide exposures and the following genes—*CYP2D6*, *PON1*, *GSTM1*, *GSTT1*, *GSTM3*, *GSTP1* haplotype, *NQO1*, *CYP1B1* and *NAT2*. For iron exposure the genes studied were *GSTM1*, *CYP1B1*, *SOD2* and *NAT2*. Analyses for gene-copper interactions were carried out for *SOD2* whereas gene-manganese interactions were carried out for *CYP2D6*, *NQO1*, *CYP1B1* and *SOD2*.

Gene-gene interaction analyses were undertaken for CYP2D6 and GSTM1, PON1 and GSTM1, GSTT1 and GSTM1, GSTM3 and GSTM1, GSTP1 haplotype and GSTM1, NQO1 and GSTM1, NAT2 and GSTM1, CYP2D6 and GSTT1, GSTP1 haplotype and GSTT1, MAO-B and NQO1, MAO-B and DRD2A, and MAO-B and DRD2B. All the MAO-A and MAO-B analyses were carried out for males and female separately. Gene-gene-environment interactions were examined for GSTT1 and GSTP1 haplotype and pesticides and also for GSTT1 and GSTP1 haplotype and solvents.

RESULTS

In all, 959 cases of parkinsonism (of whom 767 met the UK PDS Brain Bank criteria for PD) and 1989 age and sex balanced controls were recruited. For brevity, only the results of the analysis of genetic factors including all cases (after adjustment) are presented in table 2 and the all cases gene-environment interaction analysis (after adjustment) in table 3. The full results of all the genetic and gene-environment interaction analyses are available online at the *OEM* website (http://oem.bmj.com/supplemental, tables E-2 and E-3.)

The following polymorphisms did not fit the HWE: *EPHX3* (Sweden, Italy), *DRD2A* (Sweden, Malta) and *DRD2B* (Malta)

Table 3 Analysis of gene-environment interactions (all cases versus controls) after adjustment*

Genetic polymorphisms	Candidate genotype	Exposure†	Multiplicative interaction	
		Low vs high	Odds ratio (95% CI)	p Value
CYP2D6	*4*4	Solvents	1.65 (0.37 to 7.36)	0.51
CYP1B1	*3*3	Solvents	1.15 (0.49 to 2.69)	0.75
GSTM1	Null	Solvents	1.76 (0.91 to 3.41)	0.09
GSTT1	*Null*	Solvents	0.81 (0.35 to 1.89)	0.63
GSTM3	AB/BB	Solvents	0.93 (0.45 to 1.91)	0.84
GSTP1 haplotype	AB, AC, AD, BB, BC,CC	Solvents	1.53 (0.79 to 2.96)	0.21
NQO1	*1*2/*2*2	Solvents	0.54 (0.27 to 1.11)	0.09
SOD2 Val_9Ala	AV	Solvents	1.74 (0.72 to 4.23)	0.46**
	VV	Solvents	1.60 (0.60 to 4.24)	
NAT2	FS/SS	Solvents	2.31 (0.61 to 8.77)	0.22
CYP2D6	*4*4	Pesticides	1.61 (0.40 to 6.46)	0.50
PON1 L55M	LM/LL	Pesticides	4.43 (0.88 to 22.31)	0.07
PON1 Q192R	QR/RR	Pesticides	1.56 (0.70 to 3.44)	0.27
GSTM1	Null	Pesticides	0.65 (0.29 to 1.43)	0.28
GSTT1	*Null*	Pesticides	1.29 (0.42 to 3.94)	0.65
GSTM3	AB/BB	Pesticides	1.41 (0.60 to 3.30)	0.43
GSTP1 haplotype	AC/AD/BC/CC	Pesticides	2.47 (0.63 to 9.73)	0.20
NQO1	*1*2/*2*2	Pesticides	1.20 (0.54 to 2.66)	0.66
CYP1B1	*3*3	Pesticides	0.57 (0.18 to 1.91)	0.37
NAT2	FS/SS	Pesticides	1.70 (0.48 to 6.07)	0.42
GSTM1	Null	Iron	0.94 (0.48 to 1.83)	0.85
CYP1B1	*3*3	Iron	1.53 (0.61 to 3.83)	0.37
SOD2 Val_9Ala	AV	Iron	0.67 (0.29 to 1.55)	0.64**
	VV	Iron	0.75 (0.31 to 1.84)	
NAT2	FS/SS	Iron	1.45 (0.39 to 5.40)	0.58
SOD2 Val_9Ala	AV	Copper	0.78 (0.30 to 2.04)	0.66**
	VV	Copper	1.20 (0.40 to 3.55)	
CYP2D6	*4*4	Manganese	1.27 (0.28 to 5.80)	0.76
NQO1	*1*2/*2*2	Manganese	1.09 (0.53 to 2.22)	0.82
CYP1B1	*3*3	Manganese	1.75 (0.68 to 4.52)	0.25
SOD2 Val_9Ala	AV	Manganese	0.75 (0.31 to 1.82)	0.60**
	VV	Manganese	1.13 (0.44 to 2.92).	

*Logistic regression adjusting for age, sex, country, ever used tobacco containing product, ever knocked unconscious and first degree family history of Parkinson's disease. *MAO-A* and *MAO-B* are not adjusted for sex but are presented for males and females separately.
**For three category factors p value for trend derived from logistic regression coding genetic factors as 0, 1 and 2 variable.
†Cut-offs for low/high exposure: solvents/iron/manganese: 20% of an OEL (AAI); pesticides/copper: 2% of an OEL (AAI).

and analyses of these polymorphisms were restricted to those countries in the HWE.

For the majority of genetic polymorphisms there was no evidence of any association with the odds of being a case. For the unadjusted all cases analyses (data not shown) there was an increased odds ratio for disease for the *MAO-A 8 G* genotype among males only, OR 1.30, 95% CI 1.03 to 1.64, p = 0.03 unadjusted. This was not statistically significant when considering only PD cases: *MAO-A 8 G*, OR 1.25, 95% CI 0.97 to 1.61, p = 0.09 unadjusted. There were no significant results when restricting analyses to PD cases unadjusted.

These analyses were repeated after adjustment for age, sex, country, ever used a tobacco containing product, ever been knocked unconscious and first degree family history of Parkinson's disease. The adjusted (all cases) analyses (table 2) were broadly similar to the unadjusted results. Only the *MAO A 8* (males) analysis was significant at the 5% level (OR 1.30, 95% CI 1.02 to 1.66, p = 0.04 adjusted). For the PD only (adjusted) analysis, (data not shown), there were no statistically significant results.

The gene-environment interactions analysis for all cases (parkinsonism and PD) yielded little evidence of interaction effects between environmental and genetic factors; none of the analyses conducted being significant at the 5% level. However, a number of interactions may be worthy of further study. There were possible interaction effects between *GSTM1* genotype and solvent exposure, the multiplicative interaction (table 3) giving an OR 1.76 (95% CI 0.91 to 3.41, p = 0.09 adjusted); *NQO1* and solvent exposure (OR 0.54, 95% CI 0.27 to 1.11, p = 0.09 adjusted); and *PON55* and pesticides (OR 4.43, 95% CI 0.88 to 22.31, p = 0.07 adjusted). None of the gene-gene interactions (table 4) gave a significant result. Neither of the three way interactions studied (*GSTT1/GSTP1* haplotype/solvents and *GSTT1/GSTP1* haplotype/pesticides) showed any evidence of an effect.

The adjusted analyses excluding those cases without a diagnosis of PD provided a similar pattern of results: see *OEM* website (http://oem.bmj.com/supplemental, table E-3). The previously noted multiplicative interaction between *GSTM1* genotype and solvent exposure was now statistically significant with an increased OR (OR 2.34, 95% CI 1.08 to 4.62, p = 0.03 adjusted). For *NQO1* the previously noted marginally non-significant association between *NQO1 *1*2/*2*2* and solvent exposure was no longer evident (OR 0.68, 95% CI 0.32 to 1.44, p = 0.31 adjusted). For *PON55* and pesticides there was no evidence of an interaction (OR 0.55, 95% CI 0.24 to 1.30, p = 0.18 adjusted). *GSTP1* haplotype and pesticides gave a non-significant multiplicative interaction (OR 3.01, 0.75–12.02, p = 0.12). No statistically significant gene-gene interaction was identified.

DISCUSSION

There was no convincing evidence that any of the genes studied modified risk and only limited evidence that gene-environment interactions (*GSTM1* genotype and solvents) may alter the risk of Parkinson's disease. None of the significant p values were less than p = 0.01. The genetic analyses identified only one statistically significant association with Parkinson's disease. The association between *MAO-A 8 G* or *GG* genotype (reference rs6323 on NCBI SNP database) and parkinsonism that was observed was of modest size.

Table 4 Analysis of gene-gene interactions (all cases versus controls) after adjustment*

Gene-gene interactions		Multiplicative interaction	
		Odds ratio (95% CI)	p Value
CYP2D6	GSTM1	0.68 (0.30 to 1.56)	0.37
PON55	*GSTM1*	0.80 (0.48 to 1.34)	0.40
PON192	*GSTM1*	0.88 (0.64 to 1.22)	0.45
GSTT1	*GSTM1*	1.17 (0.76 to 1.80)	0.47
GSTM3	*GSTM1*	0.75 (0.52 to 1.09)	0.13
GSTP1 haplotype	GSTM1	0.88 (0.62 to 1.24)	
AB/AC/AD vs AA	Null vs pos	1.23 (0.69 to 2.19)	0.48**
BB/BC/CC vs AA	Null vs pos		
NQO1	*GSTM1*	0.96 (0.70 to 1.35)	0.82
NAT2	*GSTM1*	1.17 (0.67 to 2.04)	0.58
CYP2D6	*GSTT1*	1.21 (0.36 to 4.10)	0.76
GSTP1 haplotype	GSTT1	1.11 (0.70 to 1.74)	
AB/AC/AD vs AA	Null vs pos	1.38 (0.63 to 3.01)	0.70**
BB/BC/CC vs AA	Null vs pos		
MAO-B (males only)	NQO1	0.98 (0.62 to 1.57)	0.95
MAO-B (females only)	NQO1	0.93 (0.52 to 1.67)	
AG vs AA		0.83 (0.40 to 1.69)	0.87**
GG vs AA			
MAO-B (males only)	*DRD2A**	1.51 (0.55 to 4.15)	0.43
MAO-B (females only)	DRD2A‡‡	0.71 (0.23 to 2.21)	
AG vs AA		0.99 (0.26 to 3.74)	0.79**
GG vs AA			
MAO-B (males only)	*DRD2B*‡	0.65 (0.37 to 1.15)	0.14
MAO-B (females only)	DRD2B‡	1.01 (0.50 to 1.99)	
AG vs AA		0.58 (0.24 to 1.39)	0.37**
GG vs AA			

*Logistic regression adjusting for age, sex, country, ever used tobacco containing product, ever knocked unconscious and first degree family history of Parkinson's disease.
**For three category factors p value for trend derived from logistic regression assuming equidistance between genetic categories.
‡Excludes Malta.
‡‡Excludes Sweden and Malta.

The gene-environment interactions also gave few significant results. Allowing for the number of analyses carried out these may simply be chance associations; even if no interaction effects exist 5% of the tests performed should still be statistically significant by chance. Nonetheless there is limited evidence to support the previously observed association of *GSTM1 null* genotype, solvent exposure and neurological disease.[23] The multiplicative interaction is greater than one, suggesting that a *GSTM1 null* individual having high solvent exposure would have greater risk of disease than would be expected with only one of these factors. This interaction effect is only statistically significant for the PD only subgroup. *NQO1* homozygous mutants show poorer benzene metabolism than the wild type[24] and the reduced odds ratios for *NQO1* heterozygotes or homozygous mutants, with high solvent exposure, is therefore unexpected. The non-significantly reduced OR for *PON55 MM* genotype subjects with high pesticide exposure should be interpreted with caution as this is based on a very small number of exposed subjects.

Polymorphism in the *GSTP1* gene has previously been linked to an increased risk of Parkinson's disease in pesticide exposed individuals.[25] There was a non-significantly elevated OR that would support this association in this (larger) study where detailed exposure estimates were generated. In particular, the risk was associated with the less common *GSTP1* polymorphism, affecting exon 6, which modifies the substrate binding site of the enzyme.

A number of polymorphisms were not in HWE for some countries. There are a number of possible explanations for a gene not being in HWE, including recent mutation, mating preference linked to a polymorphism, natural selection or sampling error. It is not possible to identify a specific reason why a polymorphism is not in HWE in a specific population. It is possible that one of the conditions for HWE was not met in that population but equally it may be the result of chance, as a substantial number of polymorphisms were evaluated.

The Geoparkinson study is one of the largest case-control studies to have examined genetic, environmental and occupational risk factors for Parkinson's disease. A number of enzymes were examined (on a candidate gene basis) as potential modifiers of chemical toxicity. Published diagnostic criteria for PD were employed and subjects were recruited using a common protocol across five countries. Detailed estimates for a range of occupational exposures were produced using an indirectly validated methodology.

The literature includes many small studies describing positive associations between a range of genetic polymorphisms and Parkinson's disease.[26] Several studies report associations

Main messages

- There was a modest but significant association between *MAO-A* polymorphism in males and risk of Parkinson's disease
- Only one of the 31 gene-environment analyses showed a significant interaction effect.
- There were possible interaction effects between *GSTM1 null* genotype and solvent exposure

Implications

- There was little evidence that the genetic polymorphisms studied modified risk of Parkinson's disease.

between a number of these polymorphisms, chemical exposures and PD. It seems likely that some of these are false positive associations, occurring by chance. Publication bias ensures more of these false positive studies are published than negative studies. This large multicentre study was sufficiently powered to test these associations. One possible explanation for these largely negative findings is that the occupational and hobby exposures identified were generally of low level. An alternative explanation is that other genetic factors are more important in modifying disease risk. Some gene-environment interactions may only be revealed with substantial exposure. These results suggest that even if the polymorphisms studied interact with heavy exposures to modify disease status they do not make a major contribution to the prevalence of Parkinson's disease.

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ECHO

Causes of eosinophilic bronchitis grow

Please visit the *Occupational and Environmental Medicine* website [www.occenvmed.com] for a link to the full text of this article.

Workers with asthma-like symptoms should have their sputum checked for eosinophils as well as having other, standard, respiratory tests, say doctors who have recently reported two new causes of eosinophilic bronchitis in the workplace.

Their assertion rests on two cases associated with workplace exposure to methylene diphenyl isocyanate in a foundry worker and wheat flour in a male baker. The 44 year old foundry worker, who had been at the foundry for eight years, had developed a non-productive chronic cough in the past six months, whereas the 41 year old baker had acquired the same symptoms in the previous two years, after 10 years' exposure to flour.

The proportion of sputum eosinophils in each man was directly related to periods of exposure, non-exposure, and bronchial challenge with isocyanate (35% after a work shift; 0% not exposed; 60% after challenge) or flour (40%; 0%; 54%), respectively, and it mirrored the respiratory symptoms as they varied with exposure. FEV_1 values did not change during the 24 hour observation period after challenge. Both workers presented with non-productive cough with no wheeze or dyspnoea, which was directly related to occupational exposure in their respective workplaces. Standard blood and lung function tests gave normal results on initial testing.

So far, eosinophilic bronchitis has two other proven occupational causes: natural rubber latex and acrylates. The condition presents as a chronic cough with increased proportion of sputum eosinophils (>3% of non-squamous epithelial cells) but no variable airflow obstruction or airway hyper-responsiveness.

▲ Di Stefano F, *et al. Thorax* 2007;**62**:368–370.