Acknowledgements

We are grateful to all of those who have contributed to the Lothian Stroke Register. MRM acknowledges the support of the Brain and Spine Foundation.

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Paraoxonase 1 promoter and coding region polymorphisms in Parkinson's disease

Parkinson's disease (PD) is thought to be caused by a combination of genetic and environmental factors. Epidemiological studies have found associations of PD with pesticide exposure, or suspected pathways of pesticide exposure, such as rural residence and well water consumption. Many organophosphorous insecticides (for example, chlorpyrifos and diazinon) are bioactivated to potent cholinesterase inhibitors by the cytochromes P450, and the resulting toxic oxon forms are hydrolysed by paraoxonase (PON1). Genetic variants of detoxifying enzymes or pesticide metabolising enzymes, such as paraoxonase, may confer a predisposition to PD and thus are considered candidate genes for association studies.

Multiple polymorphisms have been identified in the *PON1* gene. The coding region contains two common polymorphisms, at amino acid codons 55 and 192. The glutamine (Q) to arginine (R) substitution at codon 192 causes substrate dependent differences in the kinetics of hydrolysis: compared with the PON1 192Q isoform, PON1 192R has higher activity towards paraoxon and chlorpyrifos oxon, but lower activity towards diazoxon, soman, and sarin. The leucine (L) to methionine (M) substitution at codon 55 does not affect the catalytic efficiency of substrate hydrolysis by the enzyme, but the *PON1* 55M allele is correlated with decreased mRNA and protein levels, because of linkage disequilibrium with a single nucleotide polymorphism (SNP) at position -108 of the promoter region of the gene. Five SNPs have been identified in the promoter region, at positions -108, -126, -162, -832, and -909. The -108 SNP has been shown to have the greatest effect on arylesterase activity, accounting for about 22% of the total variance, followed by the polymorphisms at positions 192, 55, and -162, which account for about 5.7 %, 4.1%, and 1.1% of the total variance in arylesterase activity, respectively. Cell culture studies indicate an approximately twofold change in *PON1* gene transcription attributable to the -108C/T and -162G/A SNPs, with the -108C and -162A providing more efficient transcription.

A significant association of the 192R allele with PD was found in a population of patients of Japanese ethnicity with comparatively low mean age of onset.[1] In contrast, no difference in genotypes distributions was found between PD cases and controls for the *PON1* Q192R polymorphism in an Australian study[2] nor in another study on subjects of Russian ethnicity.[3] In a more recent study by Akhmedova *et al*[4] on the same Russian population, the M55 allele was found to be associated with PD. No associations for the amino acid codon 55 and 192 polymorphisms were found in a study from China.[5]

In this study, we examined associations of two promoter (G-162A and C-108T) and two coding region (M55L and Q192R) polymorphisms in *PON1* with PD. Newly diagnosed idiopathic PD patients (n=150; 91 men and 59 women), aged 37 to 88 years, were identified by neurology and general medical practice clinics of the group health cooperative (GHC) from the Puget Sound area in western Washington State. Inclusion criteria for the cases were the presence of at least two of the four cardinal signs of PD: bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment. Exclusion criteria included the use of certain drugs during the 12 months preceding symptom onset, history of multiple cerebrovascular events, or another explanation for parkinsonism symptoms.

Controls (n=244; 158 men and 86 women), aged 44 to 84 years, were identified from GHC enrollees without past histories of PD or other neurodegenerative disorders. Controls were matched to cases by birth decade, sex, and year of enrolment in GHC. All subjects were of non-Hispanic white ethnicity. Study subjects were volunteers who were informed of the purpose of the study. Study forms and procedures were approved by the Institutional Review Board committees on Human Subjects Research at the University of Washington and GHC Center for Health Studies.

A PCR/dye terminator cycle sequencing based assay was used to detect the -162 G/A and -108 C/T genetic variants within the paraoxonase 1 (*PON1*) gene. TaqMan Detection System based assays were developed to identify the *PON1* 55 T/A and *PON1* 192 A/G variants. Odds ratios and χ^2 tests were calculated using of SPSS software for Windows; $\alpha=0.05$ was taken as the level of significance. Logistic regression models were used to calculate adjusted odds ratios and to test for statistical significance of interactions. Haplotypes were inferred using EH software.

Among controls, we observed the following allelic frequencies: -162 A = 0.23, G = 0.77; -108 T = 0.46, C = 0.54; 55M = 0.35, L = 0.65; 192R = 0.30, Q = 0.70. As shown in table 1, there were no significant differences in the genotype distributions of cases and controls for any of the four *PON1* polymorphisms. The distribution of three marker haplotypes was not significantly different between cases and controls ($\chi^2_{(7)}=4.78$, p=0.69). There also was no difference in the distribution of four marker (including -162) haplotypes between cases and controls ($\chi^2_{(15)}=9.98$, p=0.82).

We also tested for interactions between *PON1* genotypes and age (<60, =60), sex, and smoking. No conclusive evidence of interaction between any *PON1* genotype and either smoking or sex was found. However, we did detect an interaction between *PON1* 192 genotype and age. Interestingly, among cases, *PON1* 192 QQ genotype frequency increased with age ($\chi^2_{(4)}$ for genotype distribution $=3.9\times10^{-5}$), whereas among controls, 192 QQ genotype frequency decreased with age ($\chi^2_{(4)}$ for genotype distribution =0.09). Mean age at diagnosis, however, did not differ appreciably between *PON1* 192 genotypes (mean (SD) age for Q/Q cases =68.9 (9.1), Q/R cases =65.7 (10.4), R/R cases =66.5 (8.3)).

In contrast with the reports of Kondo and Yamamato[1] and Akhmedova *et al*,[4] our results do not indicate that PD is associated with specific *PON1* genotypes. In addition to the

Table 1 *PON1* –162, –108, 55, and 192 genotype frequencies in cases and controls

SNP	Genotype			χ^2 Genotype distribution	OR* (95% CI)	OR* (95% CI)
–162	GG	GA	AA		GA *v* GG	AA *v* GG
Cases (%)	79 (52.7)	56 (37.3)	15 (10)	2.68	1.28	1.72
Controls (%)	145 (60.2)	80 (33.2)	16 (6.6)	p=0.26	(0.82 to 1.98)	(0.80 to 3.68)
–108	CC	CT	TT		CT *v* CC	TT *v* CC
Cases (%)	43 (31.6)	67 (49.3)	26 (19.1)	1.34	1.09	0.75
Controls (%)	71 (31.1)	102 (44.7)	55 (24.1)	p=0.51	(0.67 to 1.78)	(0.41 to 1.38)
55	LL	LM	MM		LM *v* LL	MM *v* LL
Cases (%)	60 (40.0)	70 (46.7)	20 (13.3)	0.57	1.19	0.99
Controls (%)	105 (43.2)	104 (42.8)	34 (14)	p=0.75	(0.77 to 1.86)	(0.52 to 1.89)
192	QQ	QR	RR		QR *v* QQ	RR *v* QQ
Cases (%)	81 (54.0)	57 (38.0)	12 (8.0)	0.76	0.89	0.71
Controls (%)	121 (50.0)	97 (40.1)	24 (9.9)	p=0.68	(0.58 to 1.37)	(0.33 to 1.51)

*OR, odds ratio, adjusted for age (<60, ⩾60) and sex; CI, confidence intervals.

coding region polymorphisms investigated previously, we assessed the role of two promoter mutations but found no evidence of association. These findings suggest that *PON1* genotypes may not be predictive of PD, although there remains the possibility of interactions with pesticide exposures. Considerably larger studies will be required to investigate such interactions.

Acknowledgements

The authors would like to thank the PD patients and control subjects that participated in this study, Ms Janet Petersen for administering the questionnaire to study subjects, and the neurologists of GHC and UW for referral of PD cases, in particular Drs Robert Gotshall, Ann Hunt, Erik Kraus, Richard Mesher, Steve Pugh, Bruce R Ransom, Ali Samii, Timothy Scearc, Kurt Seiffert, Ken Uchino, Thurman Wheeler.

Funding: this research was supported by National Institute for Environmental Health Sciences Grants ES-04696, 10750, 07033, 09601, 07032 and Environmental Protection Agency Grant 826886–02.

Competing interests: none declared.

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Multifocal myoclonus secondary to tranexamic acid

Myoclonus is characterised by sudden and brief involuntary movements. We describe a patient who developed myoclonus and altered mental status following tranexamic acid overdose. The patient was a 61 year old man on chronic ambulatory peritoneal dialysis for adult polycystic kidney disease and had been prescribed lisinopril and metoprolol for long standing hypertension. He presented to hospital because of bloody effluent from his peritoneal catheter. Examination did not reveal any neurological abnormalities. Haemoglobin level was 6.2 g/dl, calcium 2.28 mmol/l, urea 20.3 mmol/l, and creatinine 1109 μmol/l, which was similar to the values taken one month before in the outpatient clinic (urea 20.2 mmol/l and creatinine 1112 μmol/l). Blood and peritoneal fluid were taken for microbiological analyses and were unrevealing.

He was transfused two units of packed cells and started on oral tranexamic acid 500 mg four times daily in order to reduce the bleeding. Six days later he became dull and developed spontaneous, arrhythmic, and multifocal myoclonus. On repeat renal function testing, the urea and creatinine levels were 20.6 mmol/l and 1190 μmol/l, respectively. An ECG and urgent brain computed tomography were unremarkable but an electroencephalogram (EEG) showed intermittent spike waves over both parasagittal regions. No other cause of epilepsy was found. Anticonvulsants were not started as it was felt that the involuntary movements were not disabling and likely to be a transient adverse drug effect. Four days after tranexamic acid had been stopped, the patient had regained his premorbid mental state and the myoclonus had ceased. Normal posterior dominant alpha activity was recorded on a repeat EEG, without further epileptiform discharges. Six months after this event the patient remains free from seizures.

Comment

Drug induced myoclonus usually occurs with encephalopathy and is often a diagnosis of exclusion. In addition, other neurological signs such as ataxia, coma, generalised seizures, and headache may be present with certain agents. Implicated drugs include antibiotics, anaesthetics, calcium channel blocking drugs, antidepressants, and antiepileptic drugs. Tranexamic acid (TAMCA; 4-(aminomethyl)cyclohexanecarboxylic acid) is commonly used in the treatment of disorders that predispose to bleeding. It is a synthetic lysine analogue that has strong antifibrinolytic activity. Plasminogen binds to fibrin to form plasmin, which in turn degrades fibrin into fibrin degradation products. TAMCA blocks the lysine binding site on plasminogen and prevents interaction with fibrin. Clinical trials have shown that TAMCA reduces blood loss in patients with primary menorrhagia, and in those undergoing cardiopulmonary bypass, prostatectomy, hip replacement, and liver transplantation.

TAMCA is generally well tolerated. Side effects are mainly gastrointestinal, such as nausea, vomiting, diarrhoea, and abdominal pain. There are, however, experimental studies of neurotoxicty.[1–4] Direct application of TAMCA to the cortex of cats produces spike-wave bursts on EEG similar in appearance to that found in feline generalised epilepsy.[4] The ability to elicit epileptic activity depended on the concentration of the drug and the area of cortex exposed. Intravenous injection causes intracranial and systemic hypertension as well as epileptiform discharges on the EEG.[3] Drugs may induce seizures by modulating neurotransmission, as application of excitatory transmitters such as kainic acid and inhibitory receptor blockers such as penicillin have been shown to evoke seizures. By applying TAMCA to the spinal cord of rats, Furtmüller was able to demonstrate a dose dependent hyperexcitibility.[5] This is blocked by the addition of muscimol, a γ-aminobutyric acid (GABA) receptor agonist, which indicates that tranexamic acid induces convulsions by blocking inhibitory GABAergic neurotransmission.

Clinically, an increase in cerebral ischaemia has resulted when TAMCA has been given for the treatment of subarachnoid haemorrhage.[3] TAMCA induced seizures have been reported in a man who was inadvertently given a 50 mg intrathecal dose during spinal anaesthesia and developed status epilepticus.[6] The patient required thiopentone infusion as the seizures were not controlled with phenytoin and midazolam; the clinical course was complicated by multiorgan dysfunction and critical illness polyneuropathy. Our patient with end stage renal failure was given an accidental overdose of oral TAMCA which resulted in myoclonus that resolved after the drug was stopped. As excretion of this drug depends on renal function, dosage adjustment is required in patients with renal failure; the efficacy of haemodialysis in removing the drug has not been studied. This case suggests that TAMCA overdose should be considered as a cause of drug induced myoclonus.

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Immunohistochemical study of caveolin-3 in idiopathic hyperCKaemia

With the increasing concern about malignant hyperthermia and with the inclusion of creatine kinase determination in the automated blood chemistry profile, performed as part of health screening, the number of subjects with raised serum creatine kinase (hyperCKaemia) without clinical signs of neuromuscular disease is continuously increasing. In 1980 Rowland *et al* coined the term "idiopathic hyperCKaemia" to describe patients with