A REVIEW OF XENOBIOTIC METABOLISM ENZYMES IN PARKINSON'S DISEASE AND MOTOR NEURON DISEASE

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SUMMARY

The role of xenobiotic metabolising enzymes (XMEs) in disease aetiology has been under investigation by numerous researchers around the world for the last two decades. The association of a number of defects in both phase I and phase II reactions with Parkinson's disease (PD) and motor neuron disease (MND) have been extensively studied. This review of the work of the group based initially at the University of Birmingham into the functional genomics of XMEs and neurodegenerative diseases has indicated that:

1. Sub-groups of patients with PD and MND can be identified with problems in xenobiotic metabolism by *in vivo* or *in vitro* methods.
2. 38-39% of the patients with MND/PD have a defect in the *S*-oxidation of the mucoactive drug, carbocysteine, by an unknown cytosolic oxidase(s). The odds risk ratio for the association of this defect with these diseases was calculated to be 10.21 for MND and 10.50 for PD.
3. Patients with PD appear to have an altered substrate specificity for monoamine oxidase B substrates in an *in vitro* platelet assay.

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4. Patients with MND have an increased capacity to *S*-methylate aliphatic sulphydryl compounds in an *in vivo* challenge as well as an *in vitro* erythrocyte thiol methyltransferase assay.

The results of over a decade of investigations into both PD and MND indicate that these are diseases with mutifactorial origins that encompass both genetic predisposition and environmental insult.

KEY WORDS

xenobiotic metabolising enzymes, pharmacogenetics, Parkinson's disease, motor neuron disease

INTRODUCTION

The idea of genetic variation and environmental cause of diseases has been actively investigated over the last 30 years /1/ and to date a number of biomarkers have been proposed: CYP2D6 and CYP2C9 with Parkinsom's disease (PD) /2,3/; NAT2 and familiar PD /4/; ApoE and Alzheimer's disease (AD) /5/; and superoxide dismutase and familial motor neuron disease (MND) /6/. To date a considerable body of evidence indicates that the late onset neurodegenerative diseases, PD, AD and MND, may be caused by slow chemical poisoning over much of an individual's lifetime /7,8/. The environmental trigger may be a chronic (lifetime exposure) or an acute insult. The acute poisoning is given support by the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) story and its association with PD /9/. Here bioactivation of the xenobiotic, MPTP, by monoamine oxidase B results in the production of a mitochondrial complex I inhibitor (4-phenyl-pyridium cation, MPP^+) which causes dopaminergic neuronal cell death /8/. In MND, acute pyrethrum poisoning and reduced sulphation of a pyrethrum metabolite have also been associated with the disease /10/. Both AD and PD have a large subclinical component which suggests that the disease process consists of steadily accumulating damage compatible with the finding at the clinical level that these diseases occur in the young adult but increase markedly in incidence with advancing age. The brain has several defence mechanisms against chemical damage. These include intracellular antioxidants and glutathione to protect against the effects of reactive oxygen species

(ROS). In addition, the glial cells are now believed to play a role in the protection of neurons. The identification of both neuronal and glial cytochrome P450 (CYP) isozymes and the presence of conjugating enzymes gives the brain both a detoxification as well as a toxification capacity.

The area of research into genetic variation of environmental compound biotransformation was ignited by the initial observation of Barbeau *et al.* /6/ that the intermediate metabolism (IM) phenotype of CYP2D6 was associated with PD. Today the genie of "pharmacogenetics" and its role as a susceptibility factor in neurological disease is well and truly 'out of the bottle' /11-19/. We review here the functional genomic activity of several XMEs, in particular those associated with sulphur metabolism, in patients with PD and MND.

Control individuals, hospital in-patients age-matched with the PD and MND patients, were selected as previously reported /11/. These patients had nondegenerative neurological diseases and were not taking medication. Typical diagnoses in this group included lumbar disc disease, benign intracranial hypertension, multiple sclerosis and those in whom no organic disease was discovered. Other in-patients studied had no neurological disease but had diverse general medical illness and were chosen because they were receiving no medication.

Patients with PD were selected as previously reported /12/. Patients with clinically definitive PD were recruited from the practice of a single neurologist. None was taking or had ever taken L-dopa-containing preparations or any other medication. No patients with atypical features, such as ocular motility disorders, pyramidal or cerebellar signs, or autonomic failure, were included. Patients who had one other family member with PD or patients with juvenile PD (age of onset less than 30 years) were also excluded. All patients exhibited at least two of the three cardinal features of the disease: tremor, rigidity and bradykinesia. Any patient failing to respond to L-dopa therapy following the pharmacogenetic investigations was excluded from the results presented.

Patients with MND were selected as previously reported /11/. Patients with clinically definitive MND were recruited from the practice of a single neurologist. All had typical features of MND that developed in adult life. No patients with atypical features, such as a long and non-progressive course, age of onset under 30 years, or sensory findings clinically or on electrophysiological testing, were

included. Also excluded were patients in whom it was impossible to tell whether the diagnosis was MND or spondylosis and those in whom the reflexes, motor or sensory conduction times (as assessed electrophysiologically), or protein level of the cerebral spinal fluid suggested a neuropathy. No patients taking medication were included. All patients had evidence of denervation with wasting and fasciculation either in their limbs or in the bulbar musculature. Patients were studied during the hospital admission, usually their first, in which a definite diagnosis of MND was made, and hence none had been suffering from the effects of severe immobility or had bulbar difficulties of the severity that might interfere with nutrition.

IN VIVO INVESTIGATION

The number of individuals and the XMEs investigated can be seen in Table 1a while the results of these investigations are presented in Table 2. There was no significant difference between the control, PD and MND patients in terms of the phase I reactions catalysed by CYP2D6 and FMO3 (see Tables for abbreviations). The non-enzymic reaction of D-penicillamine disulphide formation was also not significantly different between the controls and the patients with neurodegenerative disease (Table 2). The phase II reactions catalysed by UGT1A6, SULT1A1/2/3 and CCNAT activity also showed no significant differences between the patients with PD and MND and the controls.

However, the PD and MND patients did differ significantly from the controls in terms of the phase I reaction catalysed by carbocysteine *S*-oxidase and the phase II reactions carried out by NAT2 and TMT (Table 2). The metabolism of the L-cysteine analogue, carbocysteine, in terms of the production of urinary *S*-oxide metabolites was severely reduced in patients with PD and MND. The number of individuals phenotyped as extensive metabolisers (EM) and poor metabolisers (PM) were significantly different in PD (EM 32%; PM 39%) and MND (EM 32%; PM 38%) compared to the controls (EM 57%; PM 7%) ($p < 0.001$, χ^2 test with Yates' correction). There was however no significant difference in the proportion of individuals phenotyped as IM (controls 36%, PD 29%, MND 30%; $p > 0.05$, χ^2 test with Yates' correction). The metabolism of L-cysteine analogues was also perturbed with respect to the phase II reaction, *S*-methylation. When the

metabolism of D-penicillamine was investigated, both PD and MND patients had significantly higher concentrations of urinary *S*-methyl D-penicillamine (PD 14%, MND 16%) compared to the controls (8%) ($p < 0.01$ and < 0.001, Mann-Whitney U-test). Another phase II defect was found in arylamine metabolism when sulphadimidine metabolism was investigated. The PD population was found to have a significantly higher proportion of individuals with the EM phenotype for NAT2 (67%) compared to the controls (45%) ($p < 0.01$, χ^2 test). The MND population showed a significant decrease in the number of individuals with the EM phenotype for NAT2 (26%) compared to the controls ($p < 0.005$, χ^2 test). When the results for the *S*-oxidation of carbocysteine were analysed by the odds risk ratios (see Table 4), it was found that the PM phenotype for carbocysteine *S*-oxidase results in a 10.21 odds risk ratio for developing MND and a 10.5 odds risk ratio for developing PD. The odds risk ratio for developing either PD or MND is approximately 1.5 for the IM phenotype.

IN VITRO INVESTIGATION

The results from the *in vivo* functional genomic investigation were extended further since it is possible to assay TMT activity in erythrocytes and SULT1A1/2/3 activity in platelets (Table 1b). In addition, MAO-B activity was also assayed since MAO is involved in MPTP bioactivation.

The results of the *in vitro* functional genomic investigation can be seen in Table 3. The results of the TMT investigation indicate that the patients with MND had significantly elevated TMT activity using 2-mercaptoethanol (2-ME) and D-penicillamine as substrates (2405 and 967 pmol/mg/min; $p < 0.01$, Mann-Whitney U-test) compared to the controls (950 and 605 pmol/mg/min). The patients with PD, however, showed no significant difference with either substrate (319 and 721 pmol/mg/min; $p > 0.05$, Mann-Whitney U-test) compared to the controls. When platelet SULT1A1/2 activity was investigated using 4-nitrophenol and paracetamol as substrates, no significant differences was found between the controls and patients with PD and MND (Table 3). A similar pattern of results was seen for platelet SULT1A3 using dopamine and paracetamol as substrates (Table 3). The *in vitro* activity of platelet MAO-B was investigated using benzylamine, phenylethylamine, dopamine and MPTP as substrates. There was no

TABLE 1

Number of controls and patients with Parkinson's disease (PD) and motor neuron disease (MND) in the *in vivo* and *in vitro* functional genomic investigations

a: Functional genomics investigation of xenobiotic metabolism in vivo

Metabolic biotransformation	Enzyme	PD (n)	MND (n)	Controls (n)
***S*-Carboxymethyl-L-cysteine *S*-oxidation**	unknown	175	105	201
Debrisoquine 4-hydroxylation	CYP2D6	124	66	87
Rantidine *N*-oxidation	FMO3	25	16	30
Ranitidine *S*-oxidation	FMO3	25	16	30
Paracetamol glucuronidation	UGT1A6	70	70	70
Paracetamol sulphation	SULT1A1/2/3	70	70	70
Sulphadimidine *N*-acetylation	NAT2	28	19	40
D-Penicillamine *S*-methylation	TMT	34	34	22
D-Penicillamine *N*-acetylation	CCNAT	34	34	22

CYP2D6 Cytochrome P450 isoenzyme 2D6;
NA12 Acetyl CoA:arylamine *N*-acetyltransferase isoenzyme 2;
FMO3 Flavin monooxygenase isoenzyme 3;
UGT1A6 Uridine disphosphate glycosyltransferase isoenzyme 1A6;
SULT1A1/2/3 Sulphotransferase isoenzymes 1A1, 1A2 and 1A3;
TMT Thiol methyltransferase;
CCNT Acetyl CoA:cysteine conjugate *N*-acetyltransferase(s).

b: Functional genomics investigation of xenobiotic metabolism *in vitro*

Enzyme		PD (n)	MND (n)	Control (n)
MAO-B	benzylamine	27	17	29
	phenylethylamine	17	20	20
	dopamine	20	20	27
	MPTP	27	17	29
TMT	2-mercaptoethanol	40	53	53
	D-penicillamine	22	33	22
SULT1A1/2	4-nitrophenol	20	20	20
	paracetamol	20	20	20
SULT1A3	dopamine	20	20	20
	paracetamol	20	20	20

TMT Erythrocyte thiol methyltransferase;
SULT Platelet sulphotransferase;
MAO Platelet monoamine oxidase;
MPTP 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

TABLE 2

Results of the *in vivo* functional genomic investigations

Metabolic biotransformation	Enzyme	PD	MND	Controls
Carbocysteine *S*-oxidation	oxidase ?	**32%$^+$** (EM)	**32%$^+$** (EM)	57% (EM)
		29% (IM)	30% (IM)	36% (IM)
		39%$^+$ (PM)	**38%$^+$** (PM)	7% (PM)
Debrisoquine 4-hydroxylation	CYP2D6	56% (EM)	55% (EM)	43% (EM)
		40% (IM)	36% (IM)	48% (IM)
		4% (PM)	9% (PM)	9% (PM)
Ranitidine *N*-oxidation	FMO3	13%	17%	12%
		(±6%)	(±4%)	(±8%)
Ranitidine S-oxidation	FMO3	7%	4%	9%
		(±6%)	(±4%)	(±14%)
Paracetamol *O*-glucuronidation	UGT1A6	6%	6%	6%
		(±5%)	(±8%)	(±6%)
Paracetamol *O*-sulphation	SULT1A1/2/3	4%	5%	11%
		(±4%)	(±4%)	(±4%)

Sulphadimidine *N*-acetylation	NAT2	**67%* (EM)**	**26%** (EM)**	45% (EM)
		33% (PM)	74% (PM)	55% (PM)
D-Penicillamine *S*-methylation	TMT	**14%$^{++}$**	**16%$^{++}$**	8%
		(0-51%)	(0-63%)	(0.1-31%)
D-Penicillamine *N*-acetylation	CCNAT	10%	7%	6%
		(0-50%)	(0-47%)	(0-20%)
D-Penicillamine disulphide formation	non-enzymic	4%	5%	7%
		(0-37%)	(0-31%)	(0-11%)

CYP2D6 Cytochrome P450 isoenzyme 2D6;
NAT2 Acetyl CoA:arylamine *N*-acetyltransferase isoenzyme 2;
FMO3 Flavin monooxygenase isoenzyme 3;
UGT1A6 Uridine disphosphate glycosyltransferase isoenzyme 1A6;
SULT1A1/2/3 Sulphotransferase isoenzymes 1A1, 1A2 and 1A3;
TMT Thiol methyltransferase;
CCNAT Acetyl CoA:cysteine conjugate *N*-acetyltransferase(s);
D-Penicillamine β,β-Dimethyl-D-cysteine.

+ $p < 0.0001$, χ^2 test with Yates' correction; ++ $p < 0.01$, Mann-Whitney U-test; +++ $p < 0.001$, Mann-Whitney U-test;
* $p < 0.010$, χ^2 test; ** $p < 0.005$, χ^2 test.

% = mean (± SD) for FMO3, UGT1A6, SULT1A1/2/3; median (range) for TMT, CCNAT non-enzymic;
% of population with the various phenotypes for oxidase?, CYP2D6, NAT2.

TABLE 3

Results of the *in vitro* functional genomic investigations

Enzyme	Substrate	PD	MND	Control
MAO-B**	benzylamine	**220**$^{++}$	167	160
	(20 μM)	(± 17)	(± 19)	(± 13)
MAO-B**	phenylethylamine	**6.5**$^{+++}$	5.0	5.1
	(20 μM)	(± 1.1)	(± 0.2)	(± 0.2)
MAO-B**	dopamine	**230**$^{++++}$	451	476
	(50 μM)	(± 29)	(± 98)	(± 107)
MAO-B**	MPTP	45	39	39
	(500 μM)	(± 7)	(± 7)	(± 5)
TMT#	2-ME	319	**2405**$^{+}$	950
	(30 μM)	(± 100)	(± 750)	(± 320)
TMT#	D-penicillamine	721	**967**$^{+}$	605
	(50 mM)	(286-1711)	(242-2578)	(130-2217)
SULT1A1/2*	4-nitrophenol	440	425	430
	(4 μM)	(± 55)	(± 45)	(± 54)

	paracetamol	650	655	645	
	(16 mM)	(± 35)	(± 29)	(± 30)	
SULT1A3*	dopamine	855	880	865	
	(60 μM)	(± 45)	(± 50)	(± 42)	
	paracetamol	650	655	645	
	(16 mM)	(± 35)	(± 29)	(± 30)	

Values are means (± SD)

TMT Erythrocyte thiol methyltransferase;
2-ME 2-Mercaptoethanol;
SULT Platelet sulphotransferase;
MAO Platelet monoamine oxidase;
MPTP 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

\# pmol product formed $h^{-1}.mg^{-1}$ erythrocyte membrane protein;
* pmol product formed $h^{-1}.mg^{-1}$ platelet protein;
** pmol product formed $min^{-1}.mg^{-1}$ platelet protein.

\+ $p < 0.010$, Mann-Whitney U-test;
++ $p < 0.050$, Student's t-test;
+++ $p < 0.025$, Student's t-test;
++++ $p < 0.005$, Student's t-test.

TABLE 4

Carbocysteine *S*-oxidation as a susceptibility factor for Parkinson's disease and motor neuron disease

Population	n	<1.6%	1.6-14.3%	>14.3%	Odds risk ratio (95% CI)	
					<1.6%	1.6-14.3%
Controls (SCM)[@]	200	3%	33%	64%		
Controls	201	7%	36%	57%		
PD	175	39%*	29%	32%[+]	10.50 (5.20-21.29)	1.51 (0.9-2.52)
MND	105	38%*	30%	32%[+]	10.21 (4.52-20.28)	1.45 (0.8-2.82)

@ SCM population (Mitchell *et al.* /19/).
* $p < 0.001$, χ^2 test with Yates' correction.
+ $p < 0.05$, χ^2 test with Yates' correction.
% = % of population with the various phenotypes.

significant difference between the controls and patients with MND using these substrates (Table 3), but the patients with PD showed an interesting pattern of results. Using benzylamine and phenylethylamine as substrates, the patients with PD showed significantly elevated MAO-B activity (220 and 6.6 pmol/mg/h) compared to the controls (160 and 5.1 pmol/mg/h) ($p < 0.05$ and < 0.025, Student's t-test). However, when dopamine was used as the substrate the patients with PD showed significantly reduced MAO-B activity compared to the controls (230 versus 476 pmol/mg/h; $p < 0.005$, Student's t-test). These results were further compounded by the observation that there was no significant difference in platelet MAO-B activity between patients with PD and controls using MPTP as substrate (45 versus 39 pmol/mg/h; $p > 0.05$, Student's t-test).

DISCUSSION

All the patients reported in this investigation were newly diagnosed and thus in the early stages of the disease process. No patient was taking any medication (this was an exclusion criterion). Thus the effects of disease progression on the biotransformation pathways studied was not undertaken since previous investigations have been compromised by concurrent patient medication /20/.

The results of the *in vivo* functional genomics investigation presented in Table 2 showed some surprising results. The *S*-oxidation of the mucoactive drug, *S*-carboxymethyl-L-cysteine is polymorphic in man /21/ and the inability to produce *S*-oxide metabolites has been associated with neurodegenerative diseases /11,12,18/. The results from Tables 2 and 4 show very significant differences in the number of patients with PD and MND with EM and PM phenotypes. The population distribution for urinary recovery of *S*-oxide metabolites is not skewed or shifted but reversed in the patients with the two neurodegenerative diseases compared to the controls (Table 4). The two control populations reported show the number of individuals phenotyped as PMs as 3-7%, but in the patients with MND and PD this rose to 38-39%. The number of hemizygotes (IM) in the control, PD and MND populations was the same, while the wild-type phenotype was dramatically under-represented in the neurodegenerative disease groups.

The *CYP2D6*4* allele has been reported to be associated with PD /22,23/. Over-representation of this allele in the PD population would result in increased numbers of PD patients being classified as EMs (wild-type and hemizygous genotypes) or IMs (hemizygous genotype), depending on which antimodes are used to determine the phenotype /24/. The results here are in broad agreement with the literature /25/ which suggests that CYP2D6 polymorphism is not associated with PD or MND. The *N*- and *S*-oxidation of ranitidine by FMO3 were not significantly different in the control, PD and MND populations (Table 2), thus indicating that the FMO3 protein appears to be functioning normally in this small cohort of patients.

The phase II reaction of *N*-acetylation of the sulphonamide drug, sulphadimidine, showed clear differences between patients with PD and MND and the controls. The control population showed a split between EM and PM phenotypes for NAT2 of 45% and 55%. The patients with PD, however, showed an overrepresentation of the EM phenotype (67%), and the MND population an under-representation of the EM phenotype (26%). Previous results from genotyping of NAT2 in PD have indicated that the PM genotype was associated with familial PD /4/. However, a larger study using genotyping analysis in 1,131 individuals found no association between patients with PD and the wild-type/hemizygous genotype, but reported a significant decrease in the number of wild-type/hemizygous genotype for patients with MND /26/. The odds risk ratio was 2.33, but on statistical analysis this was found not to be significant. The sulphation (SULT1A1/2/3) and glucurondation (UGT1A6) of paracetamol were not significantly different in the control, PD and MND populations (Table 2). Similar results for urinary paracetamol sulphate recovery in PD have been reported /27/. The results from the paracetamol investigation were a surprise since previous results reported from our group showed significantly reduced urinary paracetamol sulphate recovery in patients with PD and MND /15/. The recovery of the paracetamol sulphate metabolite was normally distributed in the control, PD and MND populations. Patients with PD and MND showed a trend of lower urinary paracetamol sulphate recovery compared to the controls but this failed to reach significance.

To date, the enzyme(s) that carry out the *S*-oxidation reaction of carbocysteine is unknown. The enzyme, cysteine dioxygenase (cysteine oxidase) has been postulated to be responsible from structural

similarities between carbocysteine and L-cysteine, but to date all the published data indicate that carbocysteine is an activator (10 mM concentrations) and also an inhibitor (1 mM concentrations) of cysteine dioxygenase but not a substrate /28/. Little is known about the biochemistry of the *S*-oxidation of carbocysteine except that the reaction is inhibited by EDTA and superoxide dismutase and abolished by heat inactivation of the cytosolic fraction. The addition of Cu^{+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, SKF525A, iproniazid, hydroxylamine and the microsomal fraction had no affect on the *in vitro* rate of *S*-oxidation of carbocysteine /29/. The odds risk ratios for developing MND or PD if phenotyped as a PM for carbocysteine *S*-oxidase are 10.21 and 10.50. This should, however, be looked on as a susceptibility factor for these diseases, since 61% of PD and 62% of MND patients are not PMs of carbocysteine *S*-oxidation. Thus with this probe drug we were able to identify a subgroup of individuals with a defect in a phase I reaction which is a susceptibility factor for PD and MND. The fact that all patients with PD and MND are not PMs may be explained by the observation that both PD and MND are in fact syndromes /30/, a collection of diseases, characterised by the degeneration of the dopaminergic neurons of the substantia nigra in PD and the motor neurons (either upper, lower or both) in MND. Thus the identification of the enzyme(s) responsible for the *S*-oxidation of carbocysteine will enable a major advance to be made in the understanding of the aetiology of PD and MND associated with this metabolic defect.

The problem of metabolising cysteine-like drugs was further compounded when the metabolism of D-penicillamine (β,β-dimethyl D-cysteine) was investigated. The major urinary metabolite of D-penicillamine is inorganic sulphate /31/, with the *S*-methyl, *N*-acetyl and disulphide being regarded as minor urinary metabolites. The recovery of *S*-methyl-D-penicillamine in urine was increased 1.75- and 2.0-fold in patients with MND and PD. The urinary recovery of *N*-acetyl D-penicillamine and D-penicillamine metabolites was not significantly different from that of the controls for both PD and MND patients. This latter observation is of interest since the PM phenotype for carbocysteine *S*-oxidation has been implicated with the D-penicillamine-induced adverse reactions /32/. The fact that inorganic sulphate is the major urinary metabolite of D-penicillamine and presumably arises from either *S*-oxidation/desulphuration or desulphura-

tion/*S*-oxidation could be linked to the unidentified carbocysteine *S*-oxygenase. The increased sulphydryl methylation may be an attempt to remove this reactive chemical group due to the compromised *S*-oxidation pathway in patients with PD and MND.

The presence of XMEs in easily accessible tissues (erythrocytes and platelets) has allowed the investigation of the possible enzymic basis of a number of these *in vivo* observations as well as investigating other XMEs that could not be easily probed *in vivo* (Table 1b). The results from the *in vitro* investigation produced a somewhat 'mixed bag' of results (Table 3). Both 2-ME (classical TMT substrate) and D-penicillamine are substrates for erythrocyte TMT. The patients with PD and MND showed considerable differences in their respective *S*-methylation profiles. There was no significant difference between the patients with PD and controls using either substrate. This is in agreement with a previous report in the literature /33/. However, we had previously reported decreased 2-ME *S*-methylation in patients with PD /13/. The originally reported patients with PD, however, still showed significantly reduced 2-ME *S*-methylation compared to the controls, which has also been reported by other investigators /34/. Using D-pencillamine as substrate, there was no difference between the control and PD populations (Table 3).These results indicate that the increased urinary *S*-methyl D-penicillamine recovered was probably not due to disease-induced upregulation of the TMT gene or due to a possible polymorphism of the TMT gene. The most likely explanation for these *in vivo* results is that of a quantitative shift in the metabolic pathways caused by a defect in the *S*-oxidation pathway.

The patients with MND, however, showed significantly elevated TMT activity using either 2-ME or D-penicillamine as substrates. This could possibly be due to disease-associated gene induction of TMT activity in MND, since both *in vitro* enzyme activity and urinary recovery of *S*-methyl D-penicillamine were significantly increased in patients with MND.

Platelet SULT1A1/2 was assayed using 4-nitrophenol and paracetamol as substrates. No significant differences were seen with either substrate between the controls and the two neurodegenerative disease populations. A similar pattern of results was observed when platelet SULT1A3 was assayed using dopamine or paracetamol as substrates (Table 3). One conclusion that can be drawn from this study is that there is no sulphotransferase defect in patients with PD and MND

(Tables 2, 3). However, both PD and MND patients have been reported to have reduced plasma inorganic sulphate levels which could have implications for PAPS (3′-phosphoadenosine-5′-phosphosulphate) synthesis. The final *in vitro* investigation was platelet MAO activity using benzylamine, phenylethylamine, dopamine (3,4-dihydroxyphenylethylamine) and MPTP as substrates. There were no significant differences between the controls and MND patients using these four substrates. These results imply that in MND, increased or decreased MAO-B activity is not involved in the disease aetiology. The patients with PD, however, showed significantly elevated MAO-B activity using the non-ring hydroxylated substrates (benzylamine and phenylethylamine) compared to the controls, but significantly reduced platelet MAO-B activity using dopamine as substrate (Table 3). This leads to the possibility of altered substrate specificity or control of MAO-B in PD. The use of the known Parkinsonian pro-toxin MPTP, which is bioactivated by MAO-B to the mitochondrial complex I inhibitor MPP^+, was also investigated in the *in vitro* MAO-B assay (Table 3). No significant difference was seen in MAO-B activity between the controls and patients with PD when MPTP was used as a substrate. However, an association of the MAO-B allele 1 with PD has been reported /35/, indicating that an inherited variation of MAO-B activity may be involved in the genetic predisposition to PD.

CONCLUDING REMARKS

The results of this investigation into the functional genomics of XMEs in patients with PD and MND has highlighted some interesting problems:

1. The patient populations studied were undoubtedly mixed heterogeneous groups. Although to the clinician they have classical idiopathic PD and MND, the results from the *in vivo* and *in vitro* functional genomic investigations were clearly able to identify subgroups of patients with metabolism problems. These may be involved in the aetiology of these diseases as susceptibility factors.
2. The use of small numbers of patients can lead to misleading results. This is something that many investigators have been guilty of (including ourselves, the *in vitro S*-methylation of 2-ME in PD and the *in vivo* sulphation of paracetamol in PD and MND). This may

result in the reporting of increased or decreased metabolism of a compound which disappears on re-investigation of a larger group. However, using small groups can also potentially miss differences in the metabolism of xenobiotics. A tricky conundrum for the investigator to solve is what constitutes the correct number of individuals for a preliminary study.

To date the results indicate that larger numbers of patients should be investigated for:

(a) MAO-B activity *in vitro* in PD (K_m and V_{max} studies);

(b) NAT2 genotype/phenotyping in PD and MND;

(c) TMT activity in MND (K_m and V_{max} studies);

(d) to identify the enzyme(s) responsible for the *S*-oxidation of carbocysteine.

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