Debrisoquine hydroxylase gene polymorphism in familial Parkinson's disease

V Planté-Bordeneuve, M B Davis, D M Maraganore, C D Marsden, A E Harding

Abstract

Recent molecular genetic studies of the cytochrome *P*-450 system enzyme *CYP2D6*, which hydroxylates debrisoquine, have indicated an excess of mutant alleles in patients with Parkinson's disease compared with controls. This indicates that the *CYP2D6* locus confers genetic susceptibility to Parkinson's disease. *CYP2D6* polymorphism has been investigated in 48 patients with familial Parkinson's disease, from 22 families, and 88 of their unaffected relatives. An excess of *CYP2D6* mutant alleles in patients compared with healthy relatives was found only in subjects over the age of 60 years, presumably reflecting the age related prevalence of this disease. There was no difference in distribution of genotypes, however, between sib pairs concordant or discordant for Parkinson's disease. Linkage analysis, exclusively with affected family members, yielded negative lod scores. These data indicate that the *CYP2D6* locus is not the major determinant of genetic susceptibility in familial Parkinson's disease.

(*J Neurol Neurosurg Psychiatry* 1994;57:911–913)

A possible role for genetic factors in the aetiology of Parkinson's disease, thought for several years to be negligible on the basis of similar concordance rates for monozygotic and dizygotic twin pairs,[1] has recently attracted substantial interest. This has been fuelled by reports of two large pedigrees seemingly exhibiting autosomal dominant inheritance,[2,3] and studies of unaffected cotwins of patients with Parkinson's disease with PET which suggested a high incidence of subclinical Parkinson's disease in these subjects.[4] Given the existence of parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),[5] an attractive hypothesis for the aetiology of Parkinson's disease is one of genetic susceptibility to an environmental toxin. This has been explored by investigating metabolism of debrisoquine, a drug which is hydroxylated by an enzyme in the hepatic microsomal oxidative cytochrome *P*-450 system, specifically debrisoquine 4-hydroxylase (*CYP2D6*).

Impaired debrisoquine metabolism is found in 5% to 10% of caucasian populations; it is an autosomal recessive trait caused by mutations in the *CYP2D6* gene. Reports on the incidence of poor metabolisers in patients with Parkinson's disease provided conflicting results, possibly because of the limitations of pharmacokinetic assays.[6–9] This problem can be avoided by analysing *CYP2D6* genotypes directly. A G to A transition at the intron 3/exon 4 junction (allele B), a base pair deletion in exon 5 (allele A), and (rarely) a deletion of the entire gene, collectively account for 90% of poor metabolisers who are either homozygous or compound heterozygotes for one or two of these mutations.[10–12] Two recent studies by genotypic analyses have indicated an excess of poor metaboliser associated genotypes in Parkinson's disease compared with controls.[13,14] One showed an excess of allele B in patients, predominantly in heterozygotes,[13] and the other an increased frequency of poor metaboliser genotypes, but not heterozygotes.[14] We have investigated *CYP2D6* polymorphism in a group of families containing more than one member with Parkinson's disease to assess whether *CYP2D6* mutations segregate with the disease.

University Department of Clinical Neurology (Neurogenetics and Movement Disorders Sections), Institute of Neurology, London, UK
V Planté-Bordeneuve
M B Davis
D M Maraganore
C D Marsden
A E Harding

Correspondence to: Professor A E Harding, University Department of Clinical Neurology (Neurogenetics Section), Institute of Neurology, Queen Square, London WC1N 3BG, UK.

Received 6 September 1993 and in revised form 2 December 1993. Accepted 20 December 1993

Patients and methods

PATIENTS

Blood samples were obtained from members of 22 British and Irish families, selected on the basis of the index case having clinically typical Parkinson's disease and at least one affected relative. Inclusion criteria for index cases were: the presence of at least two of the features of tremor, rigidity, and bradykinesia; unilateral or asymmetric symptoms or signs at onset; and a positive response to levodopa. Nineteen of these families were reported previously, as were diagnostic and exclusion criteria and clinical classification of relatives.[15] A total of 136 people was recruited, including 29 with clinically definite Parkinson's disease, 19 clinically likely Parkinson's disease, and 88 unaffected relatives. There were 11 affected sib pairs and one sibship of 10 contained 5 patients with Parkinson's disease.[16] Thirty five of the patients from these families were included in the study reported by Smith and colleagues.[14] Control blood samples were from 69 unrelated healthy subjects of similar ethnic and geographical backgrounds. These were only roughly matched for age and sex as neither age nor sex influences the distribution of *CYP2D6* genotypes.

DNA ANALYSES

DNA was prepared from leucocytes by standard methods. The *CYP2D6* G to A transi-

Table 1 Distribution of CYP2D6 genotypes in families with Parkinson's disease and controls

Genotypes (B/A)*	Number of subjects							
	All subjects		Under 60 y		60 y or over		Index cases	Controls
	Affected	Unaffected	Affected	Unaffected	Affected	Unaffected		
WW/WW	26	61	11	36	15	25	13	53
BW/WW	17	19	8	16	9	3	6	14
BB/WW‡	2	6	1	5	1	1	2	2
BW/AW†‡	3	1	0	1	3	0	1	0
WW/AW	0	1	0	1	0	0	0	0
Total	48	88	20	59	28	29	22	69
χ^2 (p value)	7·0 (NS)		1·9 (NS)		8·5 (NS)		5·7 (NS)	
Poor metabolisers (%):	5 (10·4)	7 (7·9)	1 (5·0)	6 (10·2)	4 (14·3)	1 (3·4)	3 (13·6)	2 (2·9)
χ^2 (p value)	0·02 (NS)		ND		0·95 (NS)		1·92 (NS)	

* For example, BW/WW is heterozygous for the mutant B allele and homozygous normal (W = wild type) for the A allele.
† Haplotype analysis in pedigrees indicated that all these were compound heterozygotes.
‡ Poor metabolisers. ND = Not done; NS = not significant.

tion and base pair deletion were analysed in two separate polymerase chain reactions (PCRs), using respectively oligonucleotide primer pairs C + D and E + F described previously[14] and *Taq* polymerase (Promega). Thermal controller (Hybaid) settings were: 94°C for five minutes, then 30 cycles at 94°C for 30 seconds; 61°C for 30 seconds (C and D primers) or 59°C for 30 seconds (E and F primers); 72°C for 30 seconds; and 72°C for 10 minutes. Aliquots (10 μl) of PCR products were digested with the restriction enzymes *BstNI* (primers C and D) or *MspI* (primers E and F) and electrophoresis was performed through a 3·2% agarose gel. Amplification with primers C and D generates a 334 base pair (bp) fragment which is normally digested into two of 230 and 105 bp by *BstNI*; the restriction site is lost if the G to A transition at the junction of intron 3/exon 4 is present (mutant allele B). Primer E contains a one bp mismatch to the *CYP2D6* sequence, which creates a restriction site for *MspI* in the 268 bp PCR product in the presence of the single base pair deletion (mutant allele A). Both wild type alleles are termed W.

LINKAGE AND STATISTICAL ANALYSIS

Genotypes and allele frequencies were compared between controls and the 22 index cases of the Parkinson's disease families, using χ^2 analysis with Yates' correction for 2 × 2 tables. Similar comparisons were made between all patients and their unaffected relatives, once for all family members and again dividing them into two groups aged either under or over 60 years. The distribution of alleles in affected Parkinson's disease sib pairs was compared with that in unaffected/affected sib pairs from all the families investigated, paired without knowledge of their genotypes; the unaffected sib was the next eldest or next youngest in the sibship, depending on pedigree structure. No sibs were counted more than once, including those from the affected quintet from which a concordant and discordant pair were each selected randomly. Two point linkage analysis was performed with the MLINK (version V.O.3) program,[17] using only definitely and probably affected family members; unaffected subjects were assigned unknown disease state. Prevalence values for Parkinson's disease of 0·25 or 1% were assumed, based on United Kingdom data reported by Mutch *et al*[18] for those between the ages of 60 and 75 years.

Results

Table 1 shows the distribution of genotypes, determined by the presence or absence of allele B (G–>A transition) and the less frequent allele A (base pair deletion) in familial Parkinson's disease patients, their unaffected relatives, and controls. The proportion of index cases with poor metaboliser genotypes (mutant homozygotes or compound heterozygotes) was higher than in controls but this failed to reach significance at the 5% level. There was no difference between patients and their unaffected relatives. Table 2 illustrates the frequencies of mutant alleles in the groups

Table 2 Distribution of CYP2D6 alleles in families with Parkinson's disease and controls

Allele	Number of chromosomes							
	All subjects		Under 60 y		60 y or over		Index cases	Controls
	Affected	Unaffected	Affected	Unaffected	Affected	Unaffected		
G– > A transition (B):								
Wild type	72	144	30	91	42	53	33	120
B	24	32	10	27	14	5	11	18
Total	96	176	40	118	56	58	44	138
χ^2 (p Value)	0·95 (NS)		0·003 (NS)		4·4 (<0·05)		2·7 (NS)	
Base pair deletion (A):								
Wild type	93	174	40	116	53	58	43	138
A	3	2	0	2	3	0	1	0
Total	96	176	40	118	56	58	44	138
χ^2 (p Value)	ND		ND		1·4 (NS)		ND	

ND = not done; NS = not significant.

Table 3 Distribution of allele B genotypes in sib pairs

	Number of pairs	
Sib pair class	*PD/PD*	*PD/unaffected*
BB/BB	0	1
BB/BW	0	1
BB/WW	1	1
BW/BW	4	4
BW/WW	1	5
WW/WW	6	12
Total	12	24

$\chi^2 = 0{\cdot}67$; $p > 0{\cdot}5$.

studied. Allele B was significantly more frequent in patients than their unaffected relatives, but only when family members aged 60 years or more were studied. The frequency of allele A was low in patients, relatives, and controls. The distribution of classes of affected sib pairs defined by the allele B polymorphism was not significantly different from that in discordant sib pairs (table 3). Table 4 shows the results of two point linkage analysis. Cumulative lod scores were $-1{\cdot}791$ and $-0{\cdot}765$ at a recombination fraction of 0·001, assuming an age related population Parkinson's disease prevalence of 0·25% and 1% respectively.

Discussion

In this study of familial Parkinson's disease there was no significant excess of mutant *CYP2D6* alleles in unrelated index patients compared with unrelated controls. As in the report of Armstrong and colleagues,[13] there was no excess of poor metabolisers, despite the relatively low proportion of poor metabolisers in our controls. There was a trend in this direction and the number of subjects studied was inevitably smaller than in the larger study of predominantly sporadic Parkinson's disease.[14] These results do show an excess of the mutant allele B in patients with familial Parkinson's disease when compared with their unaffected relatives, but only in those over the age of 60 years. If real, this finding presumably relates to the age dependent prevalence of Parkinson's disease; it could be spurious given that multiple comparisons were made. The distribution of shared alleles between affected sib pairs was not significantly different from that in discordant pairs, although the number of affected sib pairs was small. The results of linkage analysis, which used only affected family members to avoid the problems of reduced penetrance of any possible Parkinson's disease susceptibility gene, excluded a susceptibility gene at the *CYP2D6* locus itself, and the presence of a tightly linked gene is unlikely. These data suggest that mutation at the *CYP2D6* locus is not the major genetic determinant of familial Parkinson's disease, but that one or more other genes are more important in this respect. The seemingly paradoxical finding of an association between a disease and a genetic polymorphism, but no evidence of significant segregation between the polymorphic locus and the disease in multiplex families, has been noted in other genetically complex disorders such as multiple sclerosis.[19] It is difficult to explain but probably represents the presence of several susceptibility genes. Unravelling the role of genetic factors in Parkinson's disease is likely to be complex given the potential for aetiological heterogeneity, both inherited and acquired.

Table 4 Cumulative lod scores for familial Parkinson's disease and CYP2D6 allele B polymorphism

Parkinson's disease prevalence	*Recombination fraction*							
	0	0·001	0·01	0·05	0·1	0·2	0·3	0·4
0·25%	$-\infty$	−1·79	−1·0	−0·04	0·33	0·44	0·29	0·09
1%	$-\infty$	−0·76	−0·49	0·10	0·37	0·43	0·27	0·08

We thank our colleagues, particularly Drs AJ Lees, SJ Wroe, NP Quinn, and MA Rice-Oxley, for referring patients, and the European Economic Community and the Brain Research Trust for financial support.

1 Ward CD, Duvoisin RC, Ince SE, Nutt JD, Eldridge R, Calne DB. Parkinson's disease in 65 pairs of twins and in a set of quadruplets. *Neurology* 1983;**33**:815–24.
2 Golbe LI, Di Iorio G, Bonavita V, Miller DC, Duvoisin RC. A large kindred with autosomal dominant Parkinson's disease. *Ann Neurol* 1990;**27**:276–82.
3 Degl'Innocenti F, Maurello MT, Marini P. A parkinsonian kindred. *Ital J Neurol Sci* 1989;**10**:307–10.
4 Burn DJ, Mark MH, Playford ED, *et al.* Parkinson's disease in twins studied with ^{18}F-DOPA and positron emission tomography. *Neurology* 1992;**42**:1894–900.
5 Jenner P, Schapira AHV, Marsden CD. New insights into the cause of Parkinson's disease. *Neurology* 1992;**42**: 2241–50.
6 Barbeau A, Cloutier T, Roy M, Plasse L, Paris S, Poirier J. Ecogenetics of Parkinson's disease: 4-hydroxylation of debrisoquine. *Lancet* 1985;**ii**:1213–6.
7 Benitez J, Ladero JM, Jiminez FJJ, *et al.* Oxidative polymorphism of debrisoquine in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1990;**53**:289–92.
8 Kallio J, Martilli RJ, Rinne UK, Sonninen V, Syvalahti E. Debrisoquine oxidation in Parkinson's disease. *Acta Neurol Scand* 1991;**83**:194–7.
9 Steiger MJ, Lledo P, Quinn NP, Marsden CD, Turner P, Jenner PG. Debrisoquine hydroxylation in Parkinson's disease. *Acta Neurol Scand* 1992;**86**:159–64.
10 Gough AC, Miles JS, Spurr NK, *et al.* Identification of the primary gene defect at the cytochrome *P*450 CYP2D6 locus. *Nature* 1990;**347**:773–6.
11 Hanioka N, Kimura S, Meyer UA, Gonzalez FJ. The human CYP2D6 locus associated with a common genetic defect in drug oxidation: a G_{1943} to A base change in intron 3 of a mutant CYP2D6 allele results in an aberrant 3' recognition site. *Am J Hum Genet* 1990;**47**: 994–1001.
12 Gaedigk A, Blum M, Gaedigk R, Eichelbaum M, Meyer UA. Deletion of the entire CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of the debrisoquine/sparteine polymorphism. *Am J Hum Genet* 1991;**48**:943–50.
13 Armstrong M, Daly AK, Cholerton S, Bateman DN, Idle JR. Mutant debrisoquine hydoxylation genes in Parkinson's disease. *Lancet* 1992;**339**:1017–8.
14 Smith CAD, Gough AC, Leigh PN, *et al.* Association between the CYP2D6-debrisoquine hydroxylase polymorphism and susceptibility to Parkinson's disease. *Lancet* 1992;**339**:1375–7.
15 Maraganore DM, Harding AE, Marsden CD. A clinical and genetic study of familial Parkinson's disease. *Mov Disord* 1991;**6**:205–11.
16 Sawle GV, Wroe S, Lees AJ, Brooks DJ, Frackowiak RSJ. The identification of presymptomatic parkinsonism: clinical and [^{18}F]Dopa positron emission tomography studies in an Irish kindred. *Ann Neurol* 1992;**32**:609–17.
17 Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for genetic linkage. *Proc Natl Acad Sci USA* 1984;**81**: 3443–6.
18 Mutch WJ, Dingwall-Fordyce I, Downie AW, Paterson JG, Roy SK. Parkinson's disease in a Scottish city. *BMJ* 1986;**292**:534–5.
19 Ebers GC, Paty DW, Stiller CR, Nelson RF, Seland TP, Larsen B. HLA-typing in multiple sclerosis sibling pairs. *Lancet* 1982;**ii**:88–90.