

# Expression polymorphism of the blood–brain barrier component P-glycoprotein (*MDR1*) in relation to Parkinson's disease

Taku Furuno<sup>a</sup>, Maria-Teresa Landi<sup>b</sup>, Mauro Ceroni<sup>c</sup>, Neil Caporaso<sup>b</sup>, Ilaria Bernucci<sup>b</sup>, Giuseppe Nappi<sup>c</sup>, Emilia Martignoni<sup>d</sup>, Elke Schaeffeler<sup>a</sup>, Michel Eichelbaum<sup>a</sup>, Matthias Schwab<sup>a</sup> and Ulrich M. Zanger<sup>a</sup>

Because drug transporters such as P-glycoprotein, the product of the multidrug resistance (*MDR1*) gene, contribute to the function of the blood–brain barrier, we hypothesized that differences in their expression could affect the uptake of neurotoxic xenobiotics, thereby modulating interindividual susceptibility for neurological disorders such as Parkinson's disease. In a pilot case–control study comprising 95 Parkinson's disease patients (25 early-onset patients with onset age  $\leq$  45 years) and 106 controls we analysed the three common *MDR1* polymorphisms, 3435C>T in exon 26, 2677G>T,A in exon 21, and –129T>C in exon 1b. There were no statistically significant associations between any of these polymorphisms and Parkinson's disease. However, a distribution pattern consistent with our hypothesis was observed in that the frequency of the 3435T/T genotype, which had previously been associated with decreased P-glycoprotein expression and function, was highest in the early-onset Parkinson's disease group (36.0%), second-highest in the late-onset Parkinson's disease group (22.9%), and lowest in the control group (18.9%). Furthermore, we confirmed that the *MDR1* exon 21 and

exon 26 polymorphisms are in significant linkage disequilibrium since the [2677G, 3435C] and [2677T, 3435T] haplotypes were far more frequently observed than expected. In conclusion, *MDR1* and other drug transporters represent plausible candidates as Parkinson's disease risk genes. Larger studies are required to confirm this role in the etiology of Parkinson's disease. *Pharmacogenetics* 12:529–534 © 2002 Lippincott Williams & Wilkins

*Pharmacogenetics* 2002, 12:529–534

**Keywords:** ABC-transporters, genetic polymorphism, morbus parkinson, multi-drug resistance gene, P-glycoprotein, pharmacogenetics

<sup>a</sup>Dr Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Auerbachstr 112, 70376 Stuttgart, Germany, <sup>b</sup>National Cancer Institute, NIH, Bethesda, MD, 20892 USA, <sup>c</sup>Istituto Neurologico IRCCS Mondino, University of Pavia, Pavia, Italy and <sup>d</sup>University of Piemonte Orientale, Novara, Italy

Correspondence to Dr Margarete Fischer-Bosch-Institut für Klinische Pharmakologie Auerbachstr. 112, D-70376 Stuttgart, Germany  
Tel: +49-711-81 01 37 04; fax: +49-711-85 92 95;  
e-mail: uli.zanger@ikp-stuttgart.de

Supported by grant 01 GG 9846 from the German Federal Ministry of Education and Science and by the Robert Bosch Foundation, Stuttgart, Germany.

Received 5 April 2002  
Accepted 6 June 2002

## Introduction

Parkinson's disease is one of the most common neurodegenerative disorders in Western countries with an estimated incidence of 20 new cases per 100 000 individuals per year and a prevalence ranging from 70–170 per 100 000 [1]. Parkinson's disease develops most commonly as a sporadic form, but rare familial forms inherited as autosomal dominant or recessive traits are also known and their investigation has led to the identification of several candidate genes that appear to be related to the disease [2,3]. Less is known about the etiology of sporadic Parkinson's disease, but epidemiological studies suggest that both genetic and environmental factors play a role [4]. The identification of the Parkinsonian neurotoxin 1-methyl-4-phenylpyridinium (MPP(+)), as an illicit drug contaminant [5], has greatly stimulated the search for environmental risk factors, revealing, among others, exposure to pesticides, rural living, or well water drinking as being associated with Parkinson's disease [6,7]. Strikingly, it was recently

shown that chronic exposure to the insecticide rotenone, a powerful mitochondrial electron transport inhibitor commonly used on farms and in lakes, reproduces both behavioural and neuropathological features of Parkinson's disease in rats [8].

If environmental chemicals can cause syndromes resembling neurological disorders such as Parkinson's disease, host factors that contribute to variability in their uptake, metabolic activation or inactivation and distribution in the body can be expected to modulate individual risk. Genetic polymorphisms of xenobiotic metabolism enzymes involved in the biotransformation of innumerable exogenous substances have therefore been intensely investigated as possible risk factors of Parkinson's disease [9,10]. According to recent meta-analyses, a number of enzyme polymorphisms showed significant association with Parkinson's disease, although their pathophysiologic role remains to be determined [11–13].

An important category of proteins that regulate the chemical traffic of endogenous and exogenous substances across biological membranes and may thus constitute possible risk factors for sporadic Parkinson's disease are the numerous ATP-binding cassette (ABC)-transporter proteins [14,15]. P-glycoprotein (P-gp or ABCB1), the product of the multi-drug resistance gene (*MDR1*), functions as an ATP-dependent drug efflux pump with a broad substrate specificity including a large number of drugs and xenobiotics [16]. P-gp is not only expressed in drug-resistant tumor cells, which lead originally to its discovery, but is present in virtually all cell types and tissues that have a role as protective barriers against the environment, including epithelial cells of the intestine, biliary ducts, renal proximal tubules, placental trophoblasts, as well as the endothelial cells of brain capillaries, where it forms a functional component of the blood-brain barrier [17,18]. In fact, expression of P-gp has been demonstrated to be a critical factor in preventing entry of pesticides and drugs into the central nervous system of mice [19,20], Collie dogs [21] and humans [22]. Recently it became clear that P-gp expression is at least in part influenced by genetic polymorphisms in the human *MDR1* gene [23-29]. In particular, a common silent 3435C>T polymorphism in exon 26 was associated with decreased P-gp expression in various tissues. Although interindividual variability among genotypes appears to be substantial, on average about a 2-fold difference in expression levels between CC and TT genotypes was observed in intestinal epithelial cells [24] and in kidney proximal tubular cells [27], and slower efflux from leukocytes of the P-gp substrate rhodamine [28] and possibly of antiretroviral agents from cells susceptible to human immunodeficiency virus-1 infection [29] was found.

In addition, a 2677G>T,A polymorphism that leads to amino acid changes in exon 21, as well as a -129T>C polymorphism in the untranslated exon 1b were found to be associated with lower P-gp expression in placental trophoblasts [26]. However, there are a number of controversial aspects as the 2677G>T (Ala893Ser) variant was reported to be associated with decreased P-gp expression in placental trophoblasts [26], whereas it was found to be more active in an in-vitro digoxin transport system [25]. It is also unclear how the silent 3435C>T base change could affect changes in expression and/or function. A direct effect on splicing or translation is principally possible, another possibility is linkage disequilibrium with one or more other mutations. Previous studies reported partial linkage disequilibrium for the 2677G>T and 3435C>T polymorphisms [25,26].

Based on the functional role of P-gp as a neuroprotective barrier, we hypothesized that the *MDR1*-polymorphism, via altered P-gp expression or function in

brain capillaries, would affect the intracellular concentrations of potentially neurotoxic substances. Alleles associated with decreased expression or function would therefore be expected to increase susceptibility for Parkinson's disease and/or lead to earlier onset of disease symptoms. To test this hypothesis, we determined *MDR1* genotypes and allele frequencies in Parkinson's disease patients and in a control group.

## Materials and methods

### Study population

A total of 100 idiopathic, unrelated white Italian Parkinson's disease patients were identified at the Neurological Institute Mondino, Pavia, Italy between 1992 and 1995. The diagnosis of Parkinson's disease was established by neurologists skilled in movement disorders, according to the United Kingdom Parkinson's Disease Society Brain Bank Criteria [30]. Parkinsonisms resulting from other degenerative conditions or secondary parkinsonian syndromes related to drugs, metabolic disorders, or exposure to toxins were not considered. As onset of the disease we considered the time of the first symptoms of Parkinson, retrospectively assessed by both the patient and neurologists skilled in movement disorders. The patients were divided in two groups according to the age at onset of the disease. Early onset Parkinson's disease (EOPD) group included subjects with age at onset  $\leq 45$  years, while patients with a disease onset  $> 45$  years were defined as late onset Parkinson's disease (LOPD). Due to inability to confirm the diagnosis or lack of important data, five patients were excluded from the analysis.

Of the 95 Parkinson's disease patients finally included, 25 patients had EOPD (19 males, 6 females; age range, 31-65 years; mean age,  $50.0 \pm 7.3$  years; mean age at onset  $41.3 \pm 6.9$  years), and 70 patients had LOPD (41 males, 29 females; age range, 51-84 years; mean age,  $65.1 \pm 7.4$  years; mean age at onset,  $57.1 \pm 7.4$  years). The control group consisted of 106 unrelated individuals, randomly recruited from hospital clinics of the same geographic area as the patients with Parkinson's disease (56 males, 50 females; mean age,  $52.4 \pm 14.7$  years). Parkinson's disease or other progressive neurological disorders were ruled out in the controls and it was assured that they were not related to the Parkinson's disease patients. A questionnaire including information on residence, occupational exposure, smoking habits and drug consumption was administered to all study participants. None of the controls was taking drugs.

The study was approved by the University of Pavia ethics committee and all participants provided written informed consent.

### Genotyping

Genomic DNA was prepared from leukocytes isolated from whole blood samples. Analyses of the 3435C>T polymorphism in exon 26 and the 2677G>T,A polymorphism in exon 21 were carried out using denaturing high performance liquid chromatography (DHPLC) assays as reported by Hitzl *et al.* [28]. Briefly, the respective exon 26 and exon 21 *MDR1* gene fragments were amplified by PCR using primers described before [24]. The PCR products were denatured at 95 °C for 5 min, cooled down to 65 °C at -1 °C/min, and then applied to a preheated reversed phase column (DNA Sep<sup>®</sup> Cartridge, Transgenomic). Elution was achieved with a linear acetonitril gradient (flow rate 0.9 ml/min) consisting of buffer A (0.1 M triethylammonium acetate; TEAA) and buffer B (0.1 M TEAA, 25% acetonitrile). Values for the gradient ranges (buffer B component indicated) and separation times are as follows: 52–61%, 6.8 min for exon 26 and 52–61%, 4.5 min for exon 21. The oven temperature was 62 °C for exon 26 and 56 °C for exon 21. Heteroduplex formation was detected from the melting profile in comparison to wild-type and mutant controls which were confirmed by sequencing. All samples were reanalysed by adding equal amounts of the wild-type PCR product before denaturation to detect homozygous mutants.

Analysis of the -129T>C polymorphism in exon 1b was carried out using PCR–restriction fragment length polymorphism (RFLP). After amplification using newly designed primers (5'-AGTCATCTGTGGTGAGGC TG-3' and 5'-AACGGCCACCAAGACGTGA-3'), the 215-bp PCR products were digested with *Msp*AI. The wild-type allele contains one *Msp*AI site and digestion produces 74- and 141-bp fragments. The mutation (-129T>C) creates an additional *Msp*AI site resulting in three fragments of 32, 74 and 109 bp.

### Statistical analysis

Associations between polymorphism data and Parkinson's disease were analysed by the chi-square test or Fisher's exact test, where appropriate. The distribution of the genotypes in the controls was tested for consistency with Hardy–Weinberg equilibrium using a chi-square statistic test. Adjusted odds ratios (ORs), 95% confidence intervals (CIs), and tests for trend were computed by use of multiple logistic regression models. All the variables assessed by the questionnaire were not associated with the polymorphisms or case status, and thus were not included in the models. Statistical significance was defined as  $P < 0.05$ .

### Results

Table 1 shows the distribution of *MDR1* genotypes at the three polymorphic sites for all Parkinson's disease patients and after stratification into EOPD and LOPD subgroups in comparison to the control group. All genotype frequencies observed in controls and in patients were in agreement with Hardy–Weinberg distribution based on the observed allele frequencies (data not shown). The frequency of the 3435T/T genotype was highest in the EOPD group (36.0%), second-highest in the LOPD group (22.9%), and lowest in the control group (18.9%) ( $P = 0.08$ , test for trend). The frequency of the 3435C/C genotype showed the opposite trend and was highest in controls (26.4%), second-highest in the LOPD patients (21.4%), and lowest in EOPD patients (20.0%). The frequency of the 3435T/T genotype was thus nearly 2-fold higher in the EOPD group as compared to the controls, leading to an odds ratio of 2.4 (95% confidence intervals, 0.93–6.26;  $P = 0.10$ ). A similar pattern was seen for the 3435T allele frequency, and both the 3435T/T genotype as well as the 3435T allele were also more frequent in all Parkinson's disease patients compared to controls, but

**Table 1** Single locus *MDR1* genotypes in Parkinson's disease patients and in controls

Position	Genotype	All PD (N = 95)		EOPD (N = 25)		LOPD (N = 70)		Controls (N = 106)	
		N (%)	CI	N (%)	CI	N (%)	CI	N (%)	CI
3435 (exon 26)	CC	20 (21.1)	0.13–0.31	5 (20.0)	0.07–0.41	15 (21.4)	0.13–0.33	28 (26.4)	0.18–0.36
	CT	50 (52.6)	0.42–0.63	11 (44.0)	0.24–0.65	39 (55.7)	0.43–0.68	58 (54.7)	0.45–0.64
	TT	25 (26.3)	0.18–0.36	9 (36.0)	0.18–0.58	16 (22.9)	0.14–0.35	20 (18.9)	0.12–0.28
2677 (exon 21)	GG	28 (29.5)	0.21–0.40	4 (16.0)	0.05–0.36	24 (34.3)	0.23–0.47	37 (35.0)	0.26–0.45
	GA	3 (3.2)	0.01–0.09	1 (4.0)	0.00–0.20	2 (2.8)	0.00–0.10	3 (2.8)	0.01–0.08
	AA	0 (0.0)	0.00–0.04	0 (0.0)	0.00–0.14	0 (0.0)	0.00–0.05	0 (0.0)	0.00–0.03
	GT	40 (42.1)	0.32–0.53	16 (64.0)	0.43–0.82	24 (34.3)	0.23–0.47	42 (39.6)	0.30–0.50
	AT	3 (3.2)	0.01–0.09	0 (0.0)	0.00–0.14	3 (4.3)	0.01–0.12	3 (2.8)	0.01–0.08
	TT	21 (22.1)	0.14–0.32	4 (16.0)	0.05–0.36	17 (24.3)	0.15–0.36	21 (19.8)	0.13–0.29
-129 (exon 1b)	TT	92 (96.8)	0.91–0.99	24 (96.0)	0.80–1.00	68 (97.1)	0.90–1.00	100 (94.3)	0.88–0.98
	TC	3 (3.2)	0.01–0.09	1 (4.0)	0.00–0.20	2 (2.8)	0.00–0.10	6 (5.7)	0.02–0.12
	CC	0 (0.0)	0.00–0.04	0 (0.0)	0.00–0.14	0 (0.0)	0.00–0.05	0 (0.0)	0.00–0.03

Position numbers are for the published mRNA sequence of *MDR1* (GenBank accession number M14758). Genotype frequencies are given in % with 95% confidence intervals (CI) for all patients with Parkinson's disease (PD), patients with early-onset (EOPD), late-onset (LOPD), and for controls.

these differences were not statistically significant (Tables 1 and 2). The overall allele frequencies were in good agreement with those of former studies (Table 3).

Similar patterns were observed for the frequency distributions of genotypes and alleles with respect to the 2677G>T,A and the -129T>C polymorphisms. The 2677G/G genotype was about 2-fold less frequent among EOPD patients than among the controls (Table 1). The 2677G allele frequency was highest in controls, second-highest in LOPD, and lowest in EOPD, and the opposite pattern was seen for the 2677T allele (Table 2). The similar frequency distribution patterns observed for 2677 and 3435 alleles and genotypes suggested linkage disequilibrium between the two polymorphisms. Indeed, significant although incomplete linkage between these two sites was observed. Of 48 individuals with homozygous 3435C/C genotype, 40

(83.3%) were 2677G/G homozygotes (expected: 15,  $P < 0.0001$ ); of 45 homozygous 3435T/T subjects, 33 (73.3%) were 2677T/T (expected: 9,  $P < 0.0001$ ); and of 108 C/T heterozygotes for 3435, 72 were also 2677G/T heterozygotes (Table 4). Thus, [2677G/3435C] and [2677T/3435T] were the most common haplotypes among this Italian Caucasian population, in agreement with a recent analysis in European Americans, where these two haplotypes have been termed alleles *MDR1\*1* and *MDR1\*2*, respectively [24].

## Discussion

In agreement with our hypothesis, we found the interesting tendency that the 3435T/T genotype is more common among Parkinson's disease (especially EOPD) patients than among controls. The tendency of a higher frequency of the low-expression allele among Parkinson's disease patients was further supported by the

**Table 2 Allele frequencies of MDR1 polymorphisms in Parkinson's disease patients and in controls**

Position	Allele	all PD (N = 95)		EOPD (N = 25)		LOPD (N = 70)		Controls (N = 106)	
		%	CI	%	CI	%	CI	%	CI
3435 (exon 26)	C	47.4	0.40–0.55	42.0	0.28–0.57	49.3	0.41–0.58	53.8	0.47–0.61
	T	52.6	0.45–0.60	58.0	0.41–0.70	50.7	0.42–0.59	46.2	0.39–0.53
2677 (exon 21)	G	52.1	0.48–0.59	50.0	0.36–0.64	52.9	0.44–0.61	56.1	0.49–0.63
	A	3.2	0.01–0.07	2.0	0.00–0.11	3.6	0.01–0.08	2.8	0.01–0.06
	T	44.7	0.38–0.52	48.0	0.34–0.63	43.6	0.35–0.52	41.0	0.34–0.48
-129 (exon 1b)	T	98.4	0.95–1.00	98.0	0.81–1.00	98.6	0.95–1.00	97.2	0.94–0.99
	C	1.6	0.00–0.05	2.0	0.00–0.11	1.4	0.00–0.05	2.8	0.01–0.06

Polymorphic positions are based on the published mRNA sequence of MDR1 (GenBank accession number M14758). Allele frequencies are given in % with 95% confidence intervals (CI) for all patients with Parkinson's disease (PD), patients with early-onset (EOPD), late-onset (LOPD), and for controls.

**Table 3 Allele frequencies of MDR1 exon 21 and exon 26 polymorphisms according to recent studies**

Reference	Population	N	3435 (exon 26)		2677 (exon 21)		
			C	T	G	A	T
This study	Italian/controls	106	0.54	0.46	0.56	0.03	0.41
	Italian/overall	201	0.51	0.49	0.54	0.03	0.43
[33]	German	537	0.50	0.50	n.d.	n.d.	n.d.
[25]	Eur. Am.	37	0.46	0.54	0.54	n.d.	0.45
[34]	German	461	0.46	0.54	0.56	0.02	0.42
[26]	Japanese	100	0.58	0.42	0.43	0.18	0.39

n.d. not determined

**Table 4 Observed and expected number of individuals with given two loci MDR1 genotypes (overall population, N = 201)**

		2677 (exon 21) genotype						total
		GG	GA	AA	GT	AT	TT	
3435 (exon 26) genotype	CC	40 (15.2)	5 (1.7)	0 (0)	2 (24.0)	0 (1.3)	1 (9.5)	48
	CT	21 (29.5)	1 (3.2)	0 (0)	72 (46.6)	6 (2.6)	8 (18.4)	108
	TT	4 (14.4)	0 (1.6)	0 (0)	8 (22.7)	0 (1.3)	33 (8.9)	45

Expected numbers (in parentheses) were calculated on basis of the observed frequencies of the single locus genotypes in all individuals.

opposite trend for the 3435C/C genotype, whereas the frequencies of 3435C/Ts were similar between Parkinson's disease patients and controls (Table 1). Furthermore, the distribution of the 2677G>T,A polymorphism also showed a similar tendency in that the frequency of wild-type homozygotes was 2-fold higher in controls than in EOPD, whereas the 2677G/T genotype was significantly more frequent among EOPD patients. However, none of these differences reached statistical significance, with the exception of the 2677G/T frequency difference between EOPD and controls ( $P = 0.04$ ), possibly because of the small study size. The overall result is thus negative and agrees with one previous study showing similar levels of immunodetectable P-gp and multidrug resistance protein (MRP) in lymphocytes of Parkinson's disease patients and controls [15].

It should be noted that the variant human *MDR1* alleles so far known are associated with rather modest expression and functional differences even between homozygous genotypes. This situation is different from that of other enzyme polymorphisms, such as CYPs 2D6 and 2C19. Consequently, the *MDR1* polymorphisms currently available for genetic diagnostics are unlikely to predict the true variability of this transporter in the blood-brain barrier. Based on the results of several studies showing about 2-fold lower expression for 3435TT as compared to CC in different tissues [24,26–29], a similar difference may be assumed for P-gp expression in brain capillaries. On the other hand, it could also be possible or it may even be likely that idiopathic Parkinson's disease is a heterogeneous condition, to which a number of different environmental neurotoxins contribute, which may be substrates for different transporters. It has been reported, for example, that MPP<sup>+</sup> is a poor substrate for P-gp [31], i.e. another transporter may be involved in its elimination from brain, e.g. of the MRP family, the organic anion-transporting polypeptide family, or the organic cation transporter family (for review see [14]). Further research on transporters and identification of potentially neurotoxic substrates may thus cast new light on mechanisms and genetic factors influencing susceptibility to Parkinson's disease.

Our study also provided new data on the frequency of *MDR1* polymorphisms and the linkage between them. The frequency of the 3435C>T polymorphism is known to be quite different among the different ethnic populations [32,33]. Our frequency data in a northern Italian population are similar to that of other European populations [25,32–34]. Our data also support differences in the frequency of the exon 21 polymorphism at 2677, the A allele of which is rare in our Italian and in other European populations (about 3%), but was much more frequent among Japanese (about 18%; [26]). Kim

*et al.* [25] and Tanabe *et al.* [26] reported partial linkage between the exon 26 and exon 21 polymorphisms. Our data clearly show that the 2677G and A alleles are linked to the 3435C allele, whereas the 2677T allele is linked to 3435T in this Northern Italian population (Table 4). The extent of linkage appeared to be somewhat less tight as reported for Japanese (~94%) where also linkage between 2677A and 3435T was observed, contrary to our observations [26].

The –129T>C polymorphism in exon 1b, which had also been suggested to be associated with the decreased P-gp expression level in the placenta, was discovered in Japanese populations [26]. According to our study, the –129C allele was about 2- to 3-fold less frequent in Italians (~3%) than in Japanese (6–8%). The linkage between the –129C allele and 2677A or T observed in Japanese was not present in our Italian population, where –129C allele appeared to be linked to 2677G. However, because of the relative rarity of the –129 polymorphism in Caucasians, these observations need to be confirmed. A systematic extended haplotype analysis in different populations could shed light on the mechanisms and controversial data regarding *MDR1* genotype–phenotype relationships.

## Acknowledgements

We are grateful to Dr Laura Godi for the selection of the study cases, and to the subjects who participated in the study. Excellent technical assistance by Igor Liebermann and Brigitte Körner-Shresta is gratefully acknowledged.

## References

- 1 Ben-Shlomo Y, Sieradzan K. Idiopathic Parkinson's disease: epidemiology, diagnosis and management. *Br J Gen Pract* 1995; **45**:261–268.
- 2 Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, *et al.* Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 1997; **276**:2045–2047.
- 3 Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, *et al.* Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998; **392**:605–608.
- 4 Maimone D, Dominici R, Grimaldi LM. Pharmacogenomics of neurodegenerative diseases. *Eur J Pharmacol* 2001; **413**:11–29.
- 5 Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 1983; **219**:979–980.
- 6 Koller W, Vetere-Overfield B, Gray C, Alexander C, Chin T, Dolezal J, *et al.* Environmental risk factors in Parkinson's disease. *Neurology* 1990; **40**:1218–1221.
- 7 Semchuk KM, Love EJ, Lee RG. Parkinson's disease and exposure to agricultural work and pesticide chemicals. *Neurology* 1992; **42**:1328–1335.
- 8 Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 2000; **3**:1301–1306.
- 9 Landi MT, Ceroni M, Martignoni E, Bertazzi PA, Caporaso NE, Nappi G. Gene-environment interaction in parkinson's disease. The case of CYP2D6 gene polymorphism. *Adv Neurol* 1996; **69**:61–72.
- 10 Checkoway H, Farin FM, Costa-Mallen P, Kirchner SC, Costa LG. Genetic polymorphisms in Parkinson's disease. *Neurotoxicology* 1998; **19**:635–643.
- 11 Christensen PM, Gotzsche PC, Broesen K. The sparteine/debrisoquine (CYP2D6) oxidation polymorphism and the risk of Parkinson's disease: a meta-analysis. *Pharmacogenetics* 1998; **8**:473–479.

- 12 Rostami-Hodjegan A, Lennard MS, Woods HF, Tucker GT. Meta-analysis of studies of the CYP2D6 polymorphism in relation to lung cancer and Parkinson's disease. *Pharmacogenetics* 1998; **8**:227–238.
- 13 Tan EK, Khajavi M, Thornby JI, Nagamitsu S, Jankovic J, Ashizawa T. Variability and validity of polymorphism association studies in Parkinson's disease. *Neurology* 2000; **55**:533–538.
- 14 Ayrton A, Morgan P. Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica* 2001; **31**:469–497.
- 15 Le Couteur DG, Davis MW, Webb M, Board PG. P-glycoprotein, multi-drug-resistance-associated protein and Parkinson's disease. *Eur Neurol* 2001; **45**:289–290.
- 16 Fromm MF, Eichelbaum M. The pharmacogenomics of human P-glycoprotein. In: Licinio J, Wong ML, eds. *Pharmacogenomics: The search for individualized therapies*. Wiley-VCH, Weinheim 2002, pp. 159–178.
- 17 Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* 1987; **84**:7735–7738.
- 18 Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci USA* 1989; **86**:695–698.
- 19 Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, et al. Disruption of the mouse *MDR1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* 1994; **77**:491–502.
- 20 Schinkel AH, Wagenaar E, Mol CA, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Invest* 1996; **97**:2517–2524.
- 21 Mealey KL, Bentjen SA, Gay JM, Cantor GH. Ivermectin sensitivity in collies is associated with a deletion mutation of the *mdr1* gene. *Pharmacogenetics* 2001; **11**:727–733.
- 22 Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM, et al. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest* 1998; **101**:289–294.
- 23 Mickley LA, Lee JS, Weng Z, Zhan Z, Alvarez M, Wilson W, et al. Genetic polymorphism in MDR-1: a tool for examining allelic expression in normal cells, unselected and drug-selected cell lines, and human tumors. *Blood* 1998; **91**:1749–1756.
- 24 Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, John A, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* 2000; **97**:3473–3478.
- 25 Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; **70**:189–199.
- 26 Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, et al. Expression of P-glycoprotein in human placenta: Relation to genetic polymorphism of multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther* 2001; **297**:1137–1143.
- 27 Siegsmond M, Brinkmann U, Schaeffeler E, Weirich G, Schwab M, Eichelbaum M, et al. Association of the P-glycoprotein transporter MDR1 C3435T polymorphism with the susceptibility to renal epithelial tumors. *J Am Soc Nephrol* 2002; **13**:1847–1854.
- 28 Hitzl M, Drescher S, van der Kuip H, Schaeffeler E, Fischer J, Schwab M, et al. The C3435T mutation in the human *MDR1* gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics* 2001; **11**:293–298.
- 29 Fellay J, Marzolini C, Meaden ER, Back DJ, Buclin T, Chave JP, et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002; **359**:30–36.
- 30 Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992; **55**:181–184.
- 31 Staal RG, Yang JM, Hait WN, Sonsalla PK. Interactions of 1-methyl-4-phenylpyridinium and other compounds with P-glycoprotein: relevance to toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Brain Res* 2001; **910**:116–125.
- 32 Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A, et al. *MDR1* pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics* 2001; **11**:217–221.
- 33 Schaeffeler E, Eichelbaum M, Brinkmann U, Penger A, Asante-Poku S, Zanger UM, et al. Increased frequency of the P-glycoprotein high-expression genotype C3435T of the *MDR1* polymorphism in Africans. *Lancet* 2001; **358**:383–384.
- 34 Cascorbi I, Gerloff T, John A, Meisel C, Hoffmeyer S, Schwab M, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* 2001; **69**:169–174.