ELECTRONIC LETTER

*MDR*1, the blood–brain barrier transporter, is associated with Parkinson's disease in ethnic Chinese

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Parkinson's disease is the second most common neurodegenerative disease after Alzheimer's disease. It is characterised by bradykinesia, rigidity, resting tremor, and postural instability.[1] It is a genetically heterogeneous disorder. Pathogenic mutations in several genes—including *α-synuclein*, *Parkin*, *UCH-L1* (ubiquitin-C terminal hydrolase-L1) and *DJ*-1—have previously been identified in rare monogenic forms of this disease showing autosomal dominant, autosomal recessive, or maternal transmission, with or without genetic anticipation.[2 3] The more common, sporadic form of Parkinson's disease appears to result from an interaction between genetic and environmental factors.[4] Polymorphisms in several genes, including those implicated in familial forms of the disease such as *α-synuclein*[5] and *Parkin*,[6 7] are also reported to be associated with the sporadic form.[8]

Genetic susceptibility to sporadic Parkinson's disease was also found to be modulated by genes involved in xenobiotic management. A meta-analysis of 84 association studies of 14 genes showed that polymorphisms in four genes are significantly associated with the disease.[9] These genes are either responsible for xenobiotic metabolism, such as *NAT*2[10 11] and *GST*T1,[12] or may interact with environmental agents, such as monoamine oxidase (*MAO*B).[13] Poor metaboliser alleles of the cytochrome P450 xenobiotic metabolism enzyme, CYP2D6, may also be associated with increased risk of Parkinson's disease.[14–20] Furthermore, there may be sex effects in the association of *CYP*2D6 mutant alleles with Parkinson's disease.[21]

These genetic association studies corroborate epidemiological studies, which have long suggested that Parkinson's disease is associated with exposure to certain environmental xenobiotics. Although most of the specific agents remain to be identified, rural living, well water consumption, industrialisation, and herbicide/pesticide exposure have been implicated as potential risk factors.[1 22 23]

Another category of genes that may influence susceptibility to Parkinson's disease is the ATP binding cassette (ABC) superfamily of transporter genes which regulate the bioavailability of xenobiotics within critical tissues and cells in the body, of which the *MDR*1 multidrug transporter or P-glycoprotein is the best characterised member. Unlike drug metabolising enzymes, whose major drug metabolising functions occur in the liver, the *MDR*1 transporter is expressed at the interface of major organs. This pattern of distribution suggests that the *MDR*1 transporter regulates the traffic of drugs and xenobiotics in the body at two levels: its expression in the epithelial cells of the gut serves as a first initial barrier regulating the absorption of xenobiotics into the body, while its expression at the blood–brain and blood–germ cell/fetal interface serves as a second barrier controlling the uptake of xenobiotics into these sensitive tissues.[24]

The importance of the MDR1 transporter as a component of the blood–brain barrier is evident in knockout mouse studies. *Mdr*1a(−/−) mice were found to accumulate toxic levels of the anticancer drug, vinblastine, in the brain.[25] Also, loperamide—an antidiarrhoeal narcotic analogue that normally does not enter the central nervous system (CNS)—was found to enter the brain of *mdr*1a(−/−) mice, causing them to develop abnormal behaviour characteristic of toxicity to CNS permeable opiates (for example morphine).[26] Hence, we hypothesised that functional polymorphisms in the *MDR*1 gene may compromise its blood–brain barrier transporter function, increase accessibility of neurotoxic xenobiotics to the brain, and result in increased susceptibility to Parkinson's disease.

Several single nucleotide polymorphisms (SNPs) have been identified in the *MDR*1 gene, of which two (e21/2677(G/T/A) and e26/3435(C/T)) have been reported to be associated with differences in *MDR*1 expression and function, although the functional significance remains unclear. The non-synonymous SNP e21/2677(G/T/A) was reported to change the efflux of digoxin in cells in vitro in one study,[27] but did not alter the efflux of several substrates in another study that used a different experimental system.[28] The synonymous SNP e26/3435(C/T) has variously been associated with differences in MDR1 protein expression and plasma drug concentration,[27 29–31] with drug induced side effects,[32] and with drug response.[33] Recently, these two SNPs and a third one, e1/-129(T/C), were examined in two case–control studies of approximately 100 patients with Parkinson's disease and

Key points

- Seven single nucleotide polymorphisms (SNPs) spanning ~100 kb of the *MDR*1 gene were examined in 206 Chinese patients with Parkinson's disease and 224 matched normal controls.
- Three SNPs—e12/1236(C/T), e21/2677(G/T/A), and e26/3435(C/T)—showed a significant association with Parkinson's disease. In particular, e12/1236T, e21/2677T, and e26/3435T, or haplotypes containing these alleles, were found to be over-represented in the matched normal controls compared with the Parkinson patients.
- The significant effects of these SNPs were primarily observed in men and in patients with age of onset ⩾60 years; they were not associated with significant risk for Parkinson's disease in women or in patients with a younger age of onset (⩽55 years).
- It appears that the MDR1 transporter is a significant modulator of susceptibility to Parkinson's disease among male ethnic Chinese ⩾60 years of age.

matched normal controls.[34 35] No statistical significance was found between any of these SNPs and Parkinson's disease.

In this study, we examined seven SNPs as well as haplotypes of these SNPs spanning ~100 kb in potentially functional regions of the *MDR*1 gene (that is, promoter region, coding regions, and 3'UTR) for an association with Parkinson's disease. We found a significant association between Parkinson's disease and the SNPs e12/1236(C/T), e21/2677(G/T/A), and e26/3435(C/T) (p values between 0.0367 and 0.00067), or haplotypes of these SNPs ($p<0.05$), in the Chinese population.

METHODS

Study population

All patients with Parkinson's disease and controls in this study were ethnic Chinese from Singapore. The Chinese in Singapore are predominantly descendents of migrants from south China. Individuals identified from the health screening programme in Singapore with no evidence of neurodegenerative disease on clinical examination were selected to serve as controls for the study. The diagnosis of Parkinson's disease was made by neurologists specialising in movement disorders according to the United Kingdom Parkinson's disease brain bank criteria.[36] DNA was isolated from blood samples collected from 206 patients with Parkinson's disease and 224 controls matched for age, sex, and ethnic group (table 1).

Ethical approval was obtained from the Singapore General Hospital research ethics committee.

Genotyping

The seven SNPs spanning ~100 kb of the *MDR*1 gene are located in five potentially functional genomic regions (promoter, exons 12, 21, 26, and 28) (fig 1). The five genomic segments were amplified in a single polymerase chain reaction, and all seven SNPs were genotyped by multiplex minisequencing as previously described.[37]

Data analyses

Genotype frequencies for the various SNPs in Parkinson's disease patients and controls were assessed for deviation from Hardy–Weinberg equilibrium using Pearson's χ^2 test.[38] A log-linear model embedded within the EM algorithm was used to estimate haplotype frequencies and haplotype–disease association.[39 40] The analyses assumed Hardy-Weinberg equilibrium but allowed for linkage disequilibrium. A likelihood ratio test was used to assess whether haplotype–disease association models fitted better than models assuming no haplotype–disease association. As the likelihood ratio test assessed models rather than particular haplotypes, we also estimated odds ratios (OR) for each haplotype to quantify the strength and direction of the association of individual haplotypes, using the more prevalent haplotypes as reference. We obtained 95% confidence intervals (CI) of the odds ratios by the profile likelihood approach; a 95% CI that excluded the value of 1 indicated a significant relation between a particular haplotype and Parkinson's disease risk.[41 42] The EM algorithm estimation was carried out using the Stata program.[41] All probability (p) values were two sided, and a p value smaller than 0.05 was considered significant.

SNPs with frequencies below 5% were excluded from the haplotype–disease association studies. In supplementary analyses, we examined the conditional independency of the excluded SNPs from Parkinson's disease given the flanking SNPs by a likelihood ratio test,[39] to determine whether the inclusion of these SNPs could improve the haplotype–Parkinson's disease (haplotype–PD) association models given the flanking SNPs.

In subset analyses we further explored whether the association of the various alleles/haplotypes in the *MDR*1 gene with Parkinson's disease differed between categories of sex and age of onset. As the average age of onset of Parkinson's disease is around 60 years (table 1), early onset was defined as developing the disease at or before the age of

Table 1 Characteristics of the study population

	Normal controls		Parkinson's disease	
Total number analysed	224		206	
Age (years)*	65.4 (9.4)		66.3 (9.6)	
Age range (years)	39 to 93		40 to 92	
Age of onset (years)*	–		60.5 (10.7)	
Age of onset range (years)	–		32 to 85	
	Male	**Female**	**Male**	**Female**
Number	119	105	110	96
Age (years)*	63.5 (9.7)	67.5 (8.6)	64.2 (9.6)	68.8 (9.0)
Age range (years)	39 to 88	45 to 93	40 to 84	47 to 92
Age of onset (years)*	–		57.2 (10.1)	64.0 (10.1)
Age of onset range (years)	–		32 to 81	33 to 85
No of individuals with age of onset ⩾60 years	–		45	65
No of individuals with age of onset ⩽55 years	–		41	16

*Mean (SD).

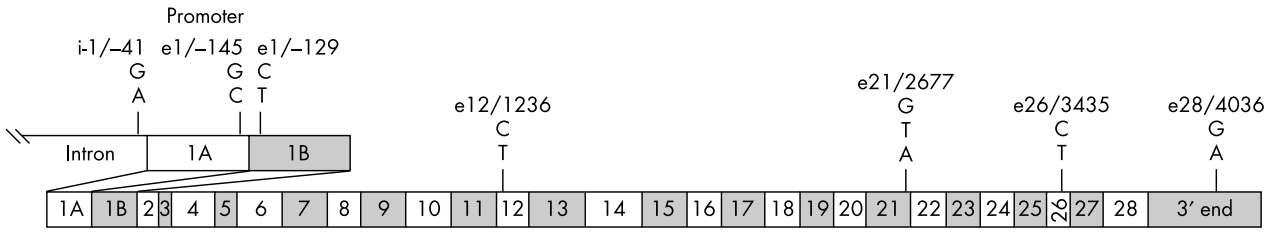

Figure 1 Schematic diagram showing relative positions of the SNP sites in the promoter and exons of the *MDR*1 gene.

55, while late onset was defined as developing the disease at or after the age of 60. A gap of four years between 56 and 59 was not analysed, to allow for uncertainty in the ascertainment of the exact age of onset of some of the patients. Odds ratios and their confidence intervals were estimated separately in the different sex and age of onset groups. A sensitivity analysis was also carried out whereby we restricted the analysis of haplotype–disease association to subjects with phase-known haplotypes only. A logistic regression was used to estimate the odds ratio of disease.

RESULTS

As the genetic basis for complex disorders including Parkinson's disease is still unclear, there could be extensive allelic variation at any disease locus, resulting in multiple susceptibility alleles of independent origin present in the

Table 2 Association of single nucleotide polymorphisms (SNPs) or haplotypes of SNPs with Parkinson's disease

		Overall				
SNP/haplotype	Allele/ haplotype*	p Value	Freq control†	Freq PD‡	OR	95% CI
i-1/-41(A/G)	A	0.72381	408	378	–	
	G		40	34	0.91748	0.5663 to 1.4837
e12/1236(C/T)	T	**0.0367**	292	240	–	
	C		156	172	1.3414	**1.0218 to 1.7658**
e21/2677(G/T/A)	T		200	134	–	
	A	**0.00067**	62	58	1.39617	0.9217 to 2.12152
	G		186	220	1.76531	**1.317 to 2.3651**
e26/3435(C/T)	T	**0.00074**	183	123	–	
	C		265	289	1.62241	**1.2231 to 2.1611**
e28/4036(A/G)	A	0.66059	330	298	–	
	G		118	114	1.0698	0.794 to 1.4459
i-1/-41(A/G)-e12/1236(C/T)	A-T	0.15113	276	225	–	
	A-C		132	153	1.45195	**1.0449 to 1.9184**
e12/1236(C/T)-e21/2677(G/T/A)	T-T		196	130	–	
	T-G	**0.00147**	87	109	1.88933	**1.321 to 2.7232**
	C-A		53	57	1.60856	**1.0367 to 2.507**
	C-G		99	111	1.69684	**1.997 to 2.4193**
e21/2677(G/T/A)-e26/3435(C/T)	T-T	**0.00617**	172	116	–	
	G-C		177	215	1.80882	**1.3353 to 2.4658**
e26/3435(C/T)-e28/4036(A/G)	T-A		157	108	–	
	C-A	**0.00917**	173	190	1.60502	**1.1347 to 2.2757**
	C-G		92	99	1.56348	**1.0687 to 2.2779**
i-1/-41(A/G)-e12/1236(C/T)-e21/2677(G/T/A)	A-T-T		184	120	–	
	A-T-G	**0.01012**	81	104	1.96018	**1.3399 to 2.8866**
	A-C-A		33	43	2.03391	**1.1996 to 3.4717**
	A-C-G		98	106	1.65987	**1.1605 to 2.4052**
e12/1236(C/T)-e21/2677(G/T/A)-e26/3435(C/T)	T-T-T		171	113	–	
	T-G-C	**0.01106**	87	105	1.83153	**1.252 to 2.7257**
	C-G-C		89	110	1.87579	**1.2676 to 2.7477**
e21/2677(G/T/A)-e26/3435(C/T)-e28/4036(A/G)	T-T-A		145	103	–	
	G-C-A	**0.00512**	133	166	1.7656	**1.2344 to 2.5448**
	G-C-G		45	49	1.54064	0.9326 to 2.5572
i-1/-41(A/G)-e12/1236(C/T)-e21/2677(G/T/A)-e26/ 3435(C/T)	A-T-T-T	0.10405	161	103	–	
	A-T-G-C		82	99	1.90435	**1.2869 to 2.8341**
	A-C-A-C		33	43	2.04396	**1.2056 to 3.5209**
	A-C-G-C		88	106	1.88818	**1.2907 to 2.7658**
e12/1236(C/T)-e21/2677(G/T/A)-e26/3435(C/T)-e28/ 4036(A/G)	T-T-T-A		143	102	–	
	T-G-C-A		55	75	1.93216	**1.2151 to 3.0868**
	T-G-C-G	**0.04321**	33	29	1.23882	0.6484 to 2.3129
	C-G-C-A		78	91	1.64406	**1.0853 to 2.5237**
	C-G-C-G		11	20	2.43982	0.9435 to 7.1189
i-1/-41(A/G)-e12/1236(C/T)-e21/2677(G/T/A)-e26/ 3435(C/T)-e28/4036(A/G)	A-T-T-T-A	0.47511	136	94	–	
	A-T-G-C-A		51	74	2.08936	**1.2976 to 3.4032**
	A-T-G-C-G		30	24	1.12511	0.5361 to 2.2761
	A-C-A-C-G		25	35	2.00016	**1.086 to 3.7267**
	A-C-G-C-A		78	84	1.55423	**1.0018 to 2.4346**
	A-C-G-C-G		11	22	2.81636	**1.1161 to 9.3852**

*Data for the alleles of the five SNPs are shown. Only relevant haplotypes that have significant CI values in either tables 2, 3, or 4 are shown. Values in bold are significant.
†Number of chromosomes containing a particular allele in control population.
‡Number of chromosomes containing a particular allele in Parkinson's disease population.
CI, confidence interval; freq, frequency; OR, odds ratio; PD, Parkinson's disease.

Table 3 Effect of sex on the association of single nucleotide polymorphisms (SNPs) or haplotypes of SNPs in the *MDR1* gene with Parkinson's' disease

		Female					Male				
SNP/haplotype	**Allele/ haplotype**	**p Value**	**Freq control**	**Freq PD**	**OR**	**95% CI**	**p Value**	**Freq control**	**Freq PD**	**OR**	**95% CI**
i-1/-41(A/G)	A	0.9668	196	179	–		0.6267	212	199	–	
	G		14	13	1.0167	0.4528 to 2.232		26	21	0.86049	0.4639 to 1.5798
e12/1236(C/T)	T	0.1912	139	115	–		0.1021	153	125	–	
	C		71	77	1.310769	0.8756 to 1.9713		85	95	1.36793	0.9351 to 1.9931
e21/2677(G/T/A)	T	0.1998	92	69	–		**0.0015**	108	65	–	
	A		29	25	1.149345	0.6196 to 2.1281		33	33	1.66138	0.941 to 2.9528
	G		89	98	1.468101	0.9576 to 2.2536		97	122	2.08964	**1.3957 to 3.1425**
e26/3435(C/T)	T	0.1618	83	63	–		**0.0009**	100	60		
	C		127	129	1.338052	0.8938 to 2.0228		138	160	1.9322	**1.3057 to 2.8756**
e28/4036(A/G)	A	0.1670	148	147	–		0.0600	182	151	–	
	G		62	45	0.730769	0.4624 to 1.1361		56	69	1.485	0.9804 to 2.2449
i-1/-41(A/G)-e12/1236(C/T)	A-T	0.6001	133	109	–	–	0.3117	143	116	–	–
	A-C		63	70	1.352262	0.8716 to 2.0865		69	83	1.47178	0.9716 to 2.2364
e12/1236(C/T)-e21/2677(G/T/A)	T-T	0.5543	91	68	–		**0.0008**	105	62	–	
	T-G		46	46	1.356151	0.7999 to 2.2938		41	63	2.59005	**1.553 to 4.3758**
	C-A		26	24	1.212953	0.6349 to 2.3216		27	33	2.0926	**1.1362 to 3.8989**
	C-G		43	52	1.600788	0.9567 to 2.6824		56	59	1.80188	**1.105 to 2.935**
e21/2677(G/T/A)-e26/3435(C/T)	T-T	0.6236	78	59	–		**0.0029**	95	57	–	
	G-C		84	94	1.480759	0.9428 to 2.3309		93	122	2.17695	**1.42 to 3.3418**
e26/3435(C/T)-e28/4036(A/G)	T-A	0.1622	71	55	–		**0.0052**	86	52	–	
	C-A		77	92	1.54442	0.935 to 2.5759		96	99	1.69206	**1.0509 to 2.756**
	C-G		50	37	0.954989	0.5446 to 1.6637		42	61	2.39309	**1.4302 to 4.0662**
i-1/-41(A/G)-e12/1236(C/T)- e21/2677(G/T/A)	A-T-T	0.7510	87	63	–		**0.0175**	97	57	–	
	A-T-G		43	45	1.436822	0.8363 to 2.4752		39	59	2.59609	**1.5161 to 4.4975**
	A-C-A		19	21	1.488114	0.7266 to 3.0982		14	23	2.85191	**1.3312 to 6.422**
	A-C-G		43	48	1.522141	0.8978 to 2.5983		55	59	1.85694	**1.1243 to 3.0972**
e12/1236(C/T)-e21/2677(G/T/A)-e26/3435(C/T)	T-T-T	0.7280	78	58	–		**0.0038**	93	55	–	
	T-G-C		45	42	1.240787	0.727 to 2.1228		42	63	2.54487	**1.5209 to 4.3241**
	C-G-C		38	52	1.835104	1.0824 to 3.1754		51	59	1.95541	**1.1839 to 3.2614**
e21/2677(G/T/A)-e26/3435(C/T)-e28/4036(A/G)	T-T-A	0.2079	64	52	–		**0.0047**	81	50	–	
	G-C-A		56	80	1.739484	1.0123 to 2.9961		77	88	1.84233	**1.1298 to 3.0296**
	G-C-G		28	14	0.611446	0.2579 to 1.3278		17	34	3.64414	**1.6523 to 7.2689**
i-1/-41(A/G)-e12/1236(C/T)- e21/2677(G/T/A)-e26/3435(C/T)	A-T-T-T	0.9632	75	53	–		0.1612	86	50	–	
	A-T-G-C		43	40	1.341026	0.7595 to 2.4776		39	59	2.6128	**1.4849 to 4.5873**
	A-C-A-C		19	21	1.511609	0.7262 to 3.1824		14	22	2.6491	**1.2262 to 5.9862**
	A-C-G-C		38	48	1.795518	0.9749 to 3.1911		50	59	2.0291	**1.2127 to 3.4638**

Table 3 Continued

SNP/haplotype	Allele/ haplotype	Female					Male				
		p Value	Freq control	Freq PD	OR	95% CI	p Value	Freq control	Freq PD	OR	95% CI
e12/1236(C/T)-e21/2677(G/T/A)-e26/3435(C/T)-e28/4036(A/G)	T-T-T-A		64	51	–			78	51	–	
	T-G-C-A		25.2	35	1.7483	0.8809 to 3.5168		29.4	41.5	2.18	**1.1666 to 4.1128**
	T-G-C-G	0.7215	20.4	6.6	0.4092	0.0767 to 1.1701	**0.0188**	12.6	22.2	2.7151	**1.1824 to 6.8876**
	C-G-C-A		30.4	44.7	1.8451	0.9874 to 3.5098		47.4	45.9	1.493	0.8456 to 2.6651
	C-G-C-G		7.6	7.2	1.1821	0.2401 to 4.6633		3.3	12.4	5.7776	**1.2864 to 93.8129**
i-1/-41(A/G)-e12/1236(C/T)- e21/2677(G/T/A)-e26/3435(C/T)- e28/4036(A/G)	A-T-T-T-A		62.2	47.9	–			73.5	44.1	–	
	A-T-G-C-A		25.2	37	1.90872	0.9618 to 3.944		26	38.1	2.4498	**1.2536 to 4.8135**
	A-T-G-C-G	0.9839	17.5	2.4	0.1801	0.0037 to 1.0173	0.6324	12.5	21	2.8043	**1.09 to 7.1264**
	A-C-A-C-G		13.6	17	1.6276	0.7056 to 3.897		11.2	16.7	2.4791	**1.0362 to 6.2267**
	A-C-G-C-A		31.2	38.3	1.5909	0.7891 to 3.1378		47.8	47.4	1.6576	0.913 to 3.0238
	A-C-G-C-G		7.5	9.3	1.6211	0.3125 to 6.898		3.1	12.8	6.9369	**1.5434 to 114.829**

Data for the alleles of the five SNPs are shown. Only relevant haplotypes that have significant CI values in either tables 2, 3, or 4 are shown. Values in bold are significant.
CI, confidence interval; freq, frequency; OR, odds ratio; PD, Parkinson's disease.

population.[43–45] It has been suggested that analysis of haplotypes rather than individual SNPs may be more advantageous in the presence of multiple susceptibility alleles at a single disease locus.[43] In this study, we examined the association of individual SNPs as well as SNP haplotypes with Parkinson's disease in ethnic Chinese.

Pearson's χ^2 test showed that all seven SNPs in our study population were consistent with the Hardy–Weinberg equilibrium assumption (each $p>0.05$).

Association of *MDR*1 SNPs and their haplotypes with Parkinson's disease

As shown in table 2, the C allele of SNP e12/1236(C/T) (OR 1.341 (95% CI, 1.022 to 1.766); $p = 0.0367$), the G allele of SNP e21/2677(G/T/A) (OR 1.765 (1.317 to 2.365); $p = 0.00067$), and the C allele of SNP e26/3435(T/C) (OR 1.622 (1.223 to 2.161); $p = 0.00074$) were individually significantly associated with a higher risk of developing Parkinson's disease. These three SNPs have previously been shown to be in tight linkage disequilibrium in the Chinese population.[46] Calculated p values for all the possible haplotypes containing the above SNPs showed significant associations between these SNP combinations and Parkinson's disease ($p = 0.04321$ to 0.00147), except for three combinations containing SNP i-1/-41(A/G) (i-1/-41(A/G)-e12/1236(C/T) ($p = 0.1511$), i-1/-41(A/G)-e12/1236(C/T)-e21/2677(G/T/A)-e26/3435(C/T) ($p = 0.1041$), and i-1/-41(A/G)-e12/1236(C/T)-e21/2677(G/T/A)-e26/3435(C/T)-e28/4036(A/G) ($p = 0.4751$)) (table 2). Even so, some specific haplotypes within these three SNP combinations were individually found to be associated with an increased risk of Parkinson's disease (table 2).

SNPs e1/-145(C/G) and e1/-129(T/C) were excluded from the haplotype–association analyses as the minor alleles of these SNPs occur at less than 5% frequency. To evaluate whether the inclusion of these two SNPs would improve the haplotype–PD association models, we undertook conditional independence tests of the two SNPs from Parkinson's disease, given the flanking SNPs by the likelihood ratio test. It was found that these two SNPs did not improve the haplotype–PD association model significantly (each $p>0.05$).

A sensitivity test using only phase-known haplotypes yielded similar results as EM estimated haplotype frequencies (data not shown), suggesting that the EM estimated haplotype frequencies were reliable.

Sex differences in risk determination

The characteristics of male and female Parkinson's disease patients in our study population were found to be different. The women tended to be older and to have a later age of disease onset than the men ($p<0.05$) (table 1). We proceeded to examine whether there are sex specific associations between SNPs/haplotypes of the *MDR*1 gene and Parkinson's disease. Our results showed that only haplotypes e12/1236C-e21/2677G-e26/3435C (OR 1.835 (95% CI, 1.082 to 3.175) and e21/2677G-e26/3435C-e28/4036A (OR 1.739 (1.012 to 2.996) were significantly associated with Parkinson's disease in women (table 3). In contrast, most of the *MDR*1 SNPs and haplotypes that were significant in table 2 were also significant in men (table 3). Only SNP e12/1236C, and haplotypes i-1/-41A-e12/1236C, e12/1236C-e21/2677G-e26/3435C-e28/4036A, and i-1/-41A-e12/1236C-e21/2677G-e26/3435C-e28/4036A were not significantly associated with Parkinson's disease in men, although their association with the disease in the overall population was significant. In addition, haplotypes e21/2677G-e26/3435C-e28/4036G (OR 3.644 (1.652 to 7.269), e12/1236T-e21/2677G-e26/3435C-e28/4036G (OR 2.715 (1.182 to 6.888), e12/1236C-e21/2677G-e26/3435C-e28/4036G (OR 5.778 (1.286 to

Table 4 Effect of age of onset on the association between single nucleotide polymorphisms (SNPs) or SNP haplotypes in the *MDR1* gene and Parkinson's disease

		⩽55 years old					⩾60 years old				
SNP/haplotype	Allele/ haplotype	p Value	Freq control	Freq PD	OR	95% CI	p Value	Freq control	Freq PD	OR	95% CI
i-1/-41(A/G)	A	**0.0100**	58.000	108.000	–		0.8720	318.000	205.000	–	
	G		14.000	8.000	0.307	0.125 to 0.758		22.000	15.000	1.058	0.521 2.074
e12/1236(C/T)	T	0.9741	43	69	–		0.0608	226	129	–	
	C		29.000	47.000	0.992	0.550 to 1.806		114.000	91.000	1.334	0.950 to 1.896
e21/2677(G/T/A)	T		27	40	–			156	73	–	
	A	0.1299	17.000	16	0.635	0.268 to 1.479	**0.0102**	41.001	30.000	1.564	0.898 to 2.693
	G		28.002	60.000	1.446	0.740 to 2.819		143.000	117.000	1.748	**1.209 to 2.534**
e26/3435(C/T)	T	0.9351	24	38	–		0.0061	144	68	–	
	C		48.000	78.000	1.0261	0.5531 to 1.9159		196.000	152.000	1.6421	**1.1482 to 2.5341**
e28/4036(A/G)	A	0.6112	49.0013	83	–		0.9778	250	162	–	
	G		23.000	33.000	0.847	0.443 to 1.622		90.000	58.000	0.995	0.676 to 1.453
i-1/-41(A/G)-e12/1236(C/T)	A-T	0.2503	36.2	66.3	–		0.0754	219.7	123.7	–	
	A-C		21.800	41.700	1.045	0.531 to 2.097		98.300	81.300	1.470	**1.005 to 2.143**
e12/1236(C/T)-e21/2677(G/T/A)	T-T		27	37.2	–			152.3	71.6	–	
	T-G	**0.0489**	12.200	31.800	1.892	0.820 to 4.559	0.0774	69.000	56.000	1.719	**1.095 to 2.720**
	C-A		13.200	16.000	0.880	0.365 to 2.180		36.600	28.600	1.664	0.943 to 2.945
	C-G		15.800	28.200	1.294	0.581 to 2.916		73.700	61.000	1.759	**1.134 to 2.742**
e21/2677(G/T/A)-e26/3435(C/T)	T-T	0.1575	21.8	37	–		0.0562	135.5	61.6	–	
	G-C		26.910	60.000	1.314	0.652 to 2.644		134.500	112.300	1.836	**1.241 to 2.732**
e26/3435(C/T)-e28/4036(A/G)	T-A		16.59	30.7	–			126.2	61.6	–	
	C-A	0.8473	32.400	52.300	0.871	0.388 to 1.913	**0.0423**	123.800	100.4	1.657	**1.081 to 2.583**
	C-G		15.600	25.700	0.891	0.369 to 2.137		72.200	51.600	1.465	0.910 to 2.352
i-1/-41(A/G)-e12/1236(C/T)- e21/ 2677(G/T/A)	A-T-T		23.1	36.1	–			148.1	67.8	–	
	A-T-G	0.0954	9.900	29.700	1.916	0.786 to 4.992	0.4225	67.200	54.500	1.771	**1.100 to 2.843**
	A-C-A		6.000	12.200	1.299	0.411 to 4.532		24.900	22.400	1.961	**1.019 to 3.793**
	A-C-G		15.500	28.600	1.184	0.527 to 2.709		70.100	57.600	1.792	**1.127 to 2.849**
e12/1236(C/T)-e21/2677(G/T/A)- e26/ 3435(C/T)	T-T-T		21.8	34.3	–			134.2	61.7	–	
	T-G-C	0.2693	12.500	31.600	1.611	0.686 to 3.919	0.3340	69.1	56	1.765	**1.069 to 2.820**
	C-G-C		14.400	28.400	1.248	0.536 to 2.936		65.200	56.200	1.872	**1.177 to 3.078**
e21/2677(G/T/A)-e26/3435(C/T)- e28/ 4036(A/G)	T-T-A		14.7	30.6	–			116.8	56.2	–	
	G-C-A	0.5064	22.400	46.000	0.988	0.425 to 2.277	0.0225	94.000	89.000	1.963	**1.250 to 3.106**
	G-C-G		4.500	14.000	1.504	0.452 to 5.929		40.100	23.500	1.221	0.639 to 2.279
i-1/-41(A/G)-e12/1236(C/T)- e21/ 2677(G/T/A)-e26/3435(C/T)	A-T-T-T		20.7	33.1	–			130	57.6	–	
	A-T-G-C	0.6496	9.800	29.600	2.017	0.717 to 5.255	0.8822	66.400	50.200	1.704	**1.045 to 2.976**
	A-C-A-C		6.200	11.105	1.185	0.361 to 4.063		25.600	20.400	1.979	**1.013 to 3.873**
	A-C-G-C		14	28.7	1.4227	0.5391 to 3.4622		63.5	57.8	2.0584	**1.1659 to 3.369**

Table 4 Continued

SNP/haplotype	Allele/ haplotype	⩽55 years old					⩾60 years old				
		p Value	Freq control	Freq PD	OR	95% CI	p Value	Freq control	Freq PD	OR	95% CI
e12/1236(C/T)-e21/2677(G/T/A)- e26/3435(C/T)-e28/4036(A/G)	T-T-T-A		15	29.3	–			114.9	55.7	–	
	T-G-C-A		8.500	24.500	1.481	0.515 to 4.531		41.600	37.400	1.851	**1.034 to 3.377**
	T-G-C-G	0.6580	4.500	7.400	0.830	0.182 to 3.675	0.4147	27.400	14.400	1.075	0.462 to 2.393
	C-G-C-A		13.900	21.300	0.784	0.300 to 2.089		53.200	52.600	2.046	**1.159 to 3.540**
	C-G-C-G		0.000	6.800				12.100	8.300		
i1/-41(A/G)-e12/1236(C/T)- e21/2677(G/T/A)-e26/3435(C/T) e28/4036(A/G)	A-T-T-T-A		15.9	28.5	–			109.7	52.9	–	
	A-T-G-C-A		6.500	24.300	2.092	0.646 to 8.328		39.800	37.000	1.924	**1.060 to 3.570**
	A-T-G-C-G		3.700	5.700	0.864	0.171 to 4.612		26.600	13.000	1.005	0.385 to 2.490
	A-C-A-C-G	0.9934	4.1	7.6	1.0229	0.2504 to 4.691	0.9718	20.8	18.5	1.8464	0.8583 to 3.9307
	A-C-G-C-A		14.100	20.000	0.783	0.300 to 2.540		53.500	49.000	1.901	**1.017 to 3.527**
	A-C-G-C-G		0.000	7.900				10.900	8.300		

Data for the alleles of the 5 SNP are shown. Only relevant haplotypes that have significant CI values in either tables 2, 3, or 4 are shown. Values in bold are significant.
CI, confidence interval; freq, frequency; OR, odds ratio; PD, Parkinson's disease.

93.813), and i-1/-41A-e12/1236T-e21/2677G-e26/3435C-e28/4036G (OR 2.804 (1.090 to 7.126) were significantly associated with Parkinson's disease in men but not overall (table 3).

Role of SNPs/haplotypes in the *MDR*1 gene in later onset of Parkinson's disease

Interesting observations were made when we examined the age of onset specific association of SNPs/haplotypes in the *MDR*1 gene with Parkinson's disease. While the promoter SNP i-1/-41(A/G) was found not to be associated with Parkinson's disease in our overall or sex specific analyses, the low frequency G allele of this SNP was found to be significantly associated ($p = 0.01$), with a decreased risk of developing Parkinson's disease at or before the age of 55 years (OR 0.307 (95% CI, 0.125 to 0.758) (table 4). Conversely, SNPs e21/2677(G/T/A) ($p = 0.0102$), e26/3435(C/T) ($p = 0.0061$), and SNP combinations e26/3435(C/T)-e28/4036(A/G) ($p = 0.0423$) and e21/2677(G/T/A)-e26/3435(C/T)-e28/4036(A/G) ($p = 0.0225$) were associated with increased risk of developing Parkinson's disease at or after age 60, with SNPs e21/2677G (OR 1.748 (1.209 to 2.534)) and e26/3435C (OR 1.642 (1.148 to 2.354)), and haplotypes e26/3435C-e28/4036A (OR 1.657 (1.081 to 2.583)) and e21/2677G-e26/3435C-e28/4036A (OR 1.963 (1.250 to 3.106)) being associated with the increased risk (table 4). Some haplotypes that include either or both of the SNPs e21/2677(G/T/A) and e26/3435(C/T) were also associated with an increased risk of developing Parkinson's disease (table 4). Curiously, although SNPs i1/-41(A/G) and e12/1236(C/T) were not individual risk factors, the haplotype i-1/-41A-e12/1236C (OR 1.470 (1.005 to 2.143)) was significantly associated with increased risk of late onset Parkinson's disease (table 4).

Overall, the results from table 4 suggest that SNP i-1/-41(A/G) may be associated with decreased risk for developing Parkinson's disease at or before the age of 55, while SNPs e21/2677(G/T/A) and e26/3435(C/T) and haplotypes containing these SNPs are associated with later onset disease (⩾60 years).

DISCUSSION

Environmental xenobiotics have been implicated in the development of Parkinson's disease, a complex genetically heterogeneous disorder.[1 22 23] The blood–brain barrier plays an important role in regulating the traffic of environmental xenobiotics in the brain, and individual differences in the ''quality'' of this barrier may influence the susceptibility to Parkinson's disease. The MDR1 multidrug transporter represents an important component of the blood–brain barrier and has been shown to regulate the uptake of drugs and xenobiotics into this sensitive organ.[25 26 47] It is conceivable that polymorphisms which alter the expression levels or transport ability of this transporter could result in altered susceptibility to neurotoxic substances and thus alter the genetic threshold for the development of Parkinson's disease.

Two recent case–control studies have examined the role of *MDR*1 gene polymorphisms (SNPs e1/-129(T/C), e21/2677(G/T/A), and e26/3435(C/T)) in Parkinson's disease development. The studies involved approximately 100 white Italian and Polish patients and 100 controls from the same geographical regions.[34 35] No significant associations between these SNPs and Parkinson's disease were detected. However, our present study of 206 Chinese patients and 224 controls showed that three SNPs—e12/1236(C/T) ($p = 0.0367$), e21/2677(G/T/A) ($p = 0.00067$), and e26/3435(C/T) ($p = 0.00074$), all in tight linkage disequilibrium with each other[46]—are significantly associated with an altered risk of developing Parkinson's disease (table 2). The odds ratios of the haplotypes that were associated with Parkinson's disease

were not very high. These observations are, however, consistent with the widely held view that Parkinson's disease is a complex disorder involving the interaction of multiple genes with different environmental factors, whereby the individual contribution of each causative gene may not be large.

We recently found strong evidence of positive selection for the e21/2677T and e26/3435T alleles in the Chinese, but only marginal evidence for this in white Americans (Tang K, Wong L, Lee E, *et al*, *Human Molecular Genetics* (in press)). The Chinese samples in that study were from anonymised umbilical cord blood from Chinese neonates, and allele frequencies of the seven SNPs were found to be very similar to those in the present study. When we used cord blood DNA samples as controls to compare against the Parkinson's disease samples, we obtained a similar, statistically significant association between Parkinson's disease and these two SNPs (data not shown). The strong evidence of a recent positive selection for the T alleles of these two SNPs supports our current observation that these alleles are significantly underrepresented in patients with Parkinson's disease compared with unaffected controls, suggesting that the T alleles of these SNPs may confer better protection for the brain against xenobiotic insults in the Chinese population.

It is possible that the earlier Italian and Polish association studies did not detect a significant statistical association because of their limited sample size. There may be another reason why neither study was able to detect a significant association between any *MDR*1 SNPs and Parkinson's disease. If we assume that the Italian and Polish subjects[34 35] were genetically similar to white Americans, their *MDR*1 haplotype and LD profiles may not favour the detection of associations. Our observation of only marginal evidence of recent positive selection in white Americans compared with the Chinese supports this hypothesis. Nonetheless, it remains to be determined whether the white Italians and Poles are in fact similar to white Americans in their underlying genetic architecture at this locus.

It is possible that either SNP e21/2677(G/T/A) or e26/3435(C/T) could be potential causal SNPs as they had much lower p values than SNP e12/1236(C/T). Consistent with our observation that individuals carrying the G allele at the non-synonymous SNP e21/2677(G/T/A) have a higher risk of developing Parkinson's disease, the *MDR*1 transporter carrying the e21/2677G allele—coding for Ala at amino acid position 893—has been shown to be a less effective transporter than one carrying the T allele (Ser 893).[27] The synonymous SNP e26/3435(C/T) appears to be associated with altered MDR1 transporter expression and function. While several reports found that the T allele is associated with lower *MDR*1 expression,[29 30 33 48] resulting in lower efflux or higher plasma levels of drugs and xenobiotics,[29 30] others have reported lower drug plasma concentration in individuals carrying the T allele.[27 31 33] Most of these studies examined only SNP e26/3435(C/T) without taking into account the underlying haplotype and linkage disequilibrium architecture of the study population. Detailed characterisation of the genetic and evolutionary history of the entire *MDR*1 gene in each study population, and the influence of recent events in the history of each population on linkage disequilibrium and the likelihood of detecting an association, could resolve these conflicting reports. Our data showing an association between e26/3435T and a lower risk of developing Parkinson's disease support observations that the T allele alters MDR1 function, resulting in a greater efflux of drugs or xenobiotics. Although SNP e26/3435(C/T) is a synonymous SNP and does not result in an amino acid change, there are several possible explanations for this observation. The observed correlation with e26/3435T could reflect either differential codon usage of the C or T allele at the wobble position of the isoleucine codon, or allele specific differences in RNA folding,[49] sometimes influencing RNA processing[50] or splicing,[51 52] or differences in translation control[53] and regulation.[54] It is also possible that neither SNP e21/2677(G/T/A) nor e26/3435(C/T) represents the causal SNP, but that they are merely in strong linkage disequilibrium with an unobserved causal SNP. A strong association of these two SNPs with Parkinson's disease could suggest that the linked causal variant resides within a region defined by strong LD.

An interesting observation was made when male and female patients with Parkinson's disease were investigated independently—the *MDR*1 gene appears to play a more important role in determining risk of developing the disease in men than in women (table 3). This is consistent with the view that the MDR1 transporter regulates the accumulation of neurotoxic xenobiotics in the brain to modulate the risk of developing Parkinson's disease. As older women in urban Singapore are primarily home makers while men often work out of doors, it is conceivable that the observed greater risk for Parkinson's disease in men compared with women is related to increased exposure to environmental susceptibility factors among men, given the same genetic risk factors in the two sexes.

When patients with Parkinson's disease were compared on the basis of their age at disease onset, we found that several polymorphisms in the *MDR*1 gene seemed to play a greater role in later onset disease (≥60 years) (table 4). One hypothesis is that, in individuals with particular *MDR*1 genotypes (for example, e12/1236C, e21/2677G, e26/3435C) and haplotypes, the blood–brain barrier allows neurotoxic xenobiotics easier access and gradual accumulation in the brain, eventually leading to Parkinson's disease. Conversely, individuals with the alternative alleles (that is, e12/1236T, e21/2677T and e26/3435T) are better protected from xenobiotic insults and hence from Parkinson's disease. In contrast, early onset Parkinson's disease is probably a result of other genetic factors and hence is less dependent on genetic variation at the *MDR*1 locus.

The promoter SNP i-1/-41(A/G), which resides in a putative CCAAT box, was found to influence the risk of Parkinson's disease in patients with a younger age of onset ($p = 0.01$) (table 4). The G allele of this SNP appeared to protect individuals from Parkinson's disease (OR 0.307 (95% CI, 0.125 to 0.758)). This observation, however, should be interpreted cautiously, given the low frequency (<10%) of i-1/-41G in the general population and the resultant sample sizes in this comparison.

Conclusions

We have produced strong statistical evidence that particular alleles and haplotypes of *MDR*1 SNPs—e12/1236(C/T), e21/2677(G/T/A), and e26/3435(C/T)—are important risk factors for the development of Parkinson's disease in ethnic Chinese, especially in men, through sex associated lifestyle differences, and in individuals with a later age of onset (≥60 years). The wide variations in allele frequencies of the *MDR*1 SNPs (especially SNP e12/1236(C/T), e21/2677(G/T/A), and e26/3435(C/T)) among different ethnic populations[46] may account for the differences in the ability to detect an association between *MDR*1 and Parkinson's disease in other ethnic groups, especially if the increase in relative risk is small.

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