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CASE REPORT

Single nucleotide polymorphisms in the multidrug resistance 1 gene in Korean epileptics

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Summary

Purpose: P-glycoprotein 170 encoded by the multidrug resistance 1 (*MDR1*) gene exports various antiepileptic drugs out of the CNS, which leads to multidrug resistance. This study was performed to elucidate the relationship between single nucleotide polymorphisms (SNPs) in the *MDR1* gene and drug resistance in Koreans with epilepsy.
Subjects and methods: Three SNPs at nucleotide position 1236 in exon 12, 2677 in exon 21 and 3435 in exon 26 of the *MDR1* gene were genotyped in 207 Korean epileptics. Subjects were classified according to whether they had drug-resistant (RS group; $N = 99$) or drug-responsive epilepsy (RP group; $N = 108$). The frequencies of genotype and haplotype were compared between the RS and RP groups.
Results: The frequencies of genotype and haplotype in the RS group were not statistically different from those in the RP group.
Conclusions: In Korean epileptics, there was no significant relationship between three known SNPs in *MDR1* and drug resistance. And there was no association of *MDR1* haplotype based on above three sites with pharmacoresistance.

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Introduction

Drug-resistant epilepsy causes recurrent convulsions and side-effects that are related to the use of multiple drugs, which sometimes result in death. Drug-resistant epilepsy is estimated to affect about 30% of the epileptic population despite the availability of several newly developed antiepileptic drugs.[1] Although efforts to predict pharmacoresistance have revealed several risk factors,[1,2] the mechanism of resistance remains unknown. Recently, a genetic approach to studying drug-resistant epilepsy raised the possibility that the pathogenic mechanism of pharmacoresistance in epilepsy might be revealed, which would facilitate the treatment of drug-resistant epilepsy.[3–5]

Pharmacogenetic assessments of drug-resistant epilepsy[4] have focused on drug-metabolizing enzymes, such as the cytochrome P450 family of enzymes; drug transporters, such as multidrug resistance transporters; and drug targets. Transporter proteins play an important role in drug disposition. Among these proteins, P-glycoprotein 170, which is the product of the ATP-binding cassette subfamily b member 1 (*ABCB1*), also known as the multidrug resistance 1 (*MDR1*) gene, is the most extensively studied.[4] P-glycoprotein 170 is an energy-dependent efflux pump that exports several antiepileptic drugs,[4,6] including carbamazepine,[7] phenytoin,[8] phenobarbital, lamotrigine, and felbamate.[9]

Single nucleotide polymorphisms (SNPs) in the *MDR1* gene have been described, including T1236C in exon 12, G2677T/A in exon 21, and C3435T in exon 26. These SNPs have been reported to confer drug resistance upon patients with various diseases.[10–12] Initially, Siddiqui et al.[3] studied the association between multidrug-resistant epilepsy and the C3435T polymorphism. They demonstrated that patients with drug-resistant epilepsy were more likely to have the CC than the TT genotype, although the frequency of the CT genotype did not differ significantly between patients with drug-resistant epilepsy and patients with drug-responsive epilepsy.[3] However, similar consecutive studies by Tan et al.[13] and Sills et al.[14] failed to corroborate this association[3] between the C3435T polymorphism in the human *MDR1* gene and pharmacoresistant epilepsy.

As previous studies of *MDR1* polymorphisms in normal volunteers revealed significant differences in the genotype or allele frequencies among different ethnic groups,[15–17] studies such as that of Siddiqui et al.[3] should be replicated in other ethnic groups, especially in Asian populations with genotype frequencies that differ from those of Caucasians. The present study was performed to elucidate the relationship between *MDR1* SNPs and drug resistance in Korean epileptics.

Subjects and methods

Subjects

Approximately 400 patients who had been diagnosed at three different epilepsy clinics (Chonnam National University Hospital, Mirae Children's Hospital, and Gwangju Christian Hospital, Gwangju, Korea) were recruited for the study over 5 months (June–October, 2004) to elucidate the relationship between the *MDR1* SNPs and drug-resistant epilepsy. The research review board of Chonnam National University Hospital (Gwangju, Korea) approved the study, and all of subjects provided informed consent. Medical records were reviewed retrospectively for responsiveness to antiepileptic drugs by neurologists and pediatric neurologists at the three epilepsy clinics. Among this initial group, 207 patients who met the criteria described below were enrolled. Patients with drug-resistant epilepsy (60 men plus 39 women; median age = 15 years; range = 2–51 years) were classified into the RS group, while patients with drug-responsive epilepsy (57 men plus 51 women; median age = 13 years; range = 3–62 years) were classified into the RP group.

Drug resistance was defined as the occurrence of at least four seizures during the previous year for patients who were being treated with more than two primary antiepileptic drugs at the maximally tolerable daily doses. Cases of pseudorefractory epilepsy that resulted from inappropriate drug selection or poor compliance with medication were excluded. Subjects who had undergone surgical treatment for drug-resistant epilepsy were classified as exhibiting drug resistance irrespective of the outcome of the surgery. Drug responsiveness was defined as a complete absence of seizures for at least 1 year prior to the date of the latest follow-up visit.

Genotyping of SNP sites in the *MDR1* gene

To obtain genomic DNA, blood samples were drawn after obtaining informed consent from the patient (or guardian in the case of a child). Genomic DNA was isolated from the blood using standard methods.

Three known polymorphic sequences in the *MDR1* gene were amplified by polymerase chain reaction (PCR). The index polymorphic sequence strings were identified in exons 12 (nucleotide position 1236), 21

Table 1 Genotyping of polymorphic sequence strings of *MDR1*

Nucleotide position	Exon	Nucleotide sequence		5′ primer	3′ primer
		Wild type	Mutant type		
1236	12	agggTctga	agggCctga	5′ ATCCTGTGTC TGTGAATTGC 3′	5′ TCAGAAAGATGT GCAATGTG 3′
2677	21	aggtGctgg	aggtActgg aggtTctgg	5′ TCAGAAAATAG AAGCATGAGTTG 3′	5′ AGCAGTAGGGAG TAACAAAATAAC 3′
3435	26	agatCgtga	agatTgtga	5′ACATTCAAAGTGT GCTGGTC 3′	5′ ACTATAGGCCAG AGAGGCTG 3′

(2677), and 26 (3435) (Table 1). Appropriate forward and reverse primer sets for each string were prepared (Table 1), and PCR was carried out under the following conditions. Genomic DNA (100–300 ng) was amplified in a volume of 50 μl of enzyme storage buffer B (Promega, USA), 10 pmol of each primer, 2.5 mM $MgCl_2$, 200 μM deoxynucleotide triphosphates, and 1.25 units of *Taq* DNA polymerase (Promega). The PCR conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 30 s, annealing for 45 s at 55 °C for exon 12, 56 °C for exon 21, and 57 °C for exon 26, an extension step at 72 °C for 1 min, and a final extension step at 72 °C for 7 min. The PCR products were analyzed on standard 2% agarose gels stained with ethidium bromide (0.5 μg/ml). All of the purified PCR products were sequenced directly using BigDye Terminator Chemistry (PE Biosystems, USA), and three sites in the *MDR1* sequence were inspected.

While PCR amplification of the *MDR1* SNPs in the present study was successful in most cases, some of the PCR products were inadequate and were excluded from the initial analysis (i.e., for position 1236 in exon 12, two PCR products in the RS group and one in the RP group; for position 2677 in exon 21, one in the RP group; and for position 3435 in exon 26, eight in the RP group).

Statistical analysis

Deviations of genotype frequencies

The genotype frequencies at each nucleotide position in *MDR1* were assessed for deviations from Hardy–Weinberg equilibrium using the χ^2-test in the population genetics data analysis program Popgen32.

Comparisons of genotype and haplotype frequencies

The genotype and haplotype frequencies were compared between the RS and RP groups using the Pearson χ^2-test. If the minimum expected count was <5, Fisher's exact test was used to compare the frequencies. Logistic regression with reference to the RP group was used to estimate the potential influence of each genotype on the degree of pharmacoresistance. Statistical significance was accepted as $P < 0.05$. The SPSS 11.0 and MedCalc 7.4 programs were used for these analyses.

Estimation of haplotype frequencies

Samples in which at least one locus could not be genotyped were excluded from the haplotype frequency calculation (i.e., two samples in the RS group and nine in the RP group).

The haplotype frequencies were estimated based on an expectation maximization (EM) algorithm, leading to maximum-likelihood estimates of molecular haplotype frequency under the assumption of Hardy-Weinberg proportions.[18] The EM algorithm estimated the haplotype frequencies from the genotype data. An EM linkage utility program was used to estimate the haplotype frequencies.

Results

Genotype frequencies of *MDR1* SNPs and drug responsiveness

Three different genotypes, TT, TC, and CC, were identified at position 1236 in exon 12 (Table 2). There were no significant deviations from Hardy–Weinberg equilibrium in any group (for the RS group, χ^2/*P*-value = 1.76/0.18; for the RP group, χ^2/*P*-value = 0.19/0.66). The genotype frequencies of TT, TC and CC are listed in Table 2. The genotype frequencies in the RS group were not statistically different from those in the RP group.

Six different genotypes, GG, GT, GA, TA, TT, and AA, were identified at position 2677 in exon 21 (Table 3). There were no significant deviations from Hardy–Weinberg equilibrium in any group (for the RS group, χ^2/*P*-value = 2.08/0.56; for the RP group, χ^2/*P*-value = 3.40/0.33). For the RS versus RP groups, the frequencies of each genotype are listed

Table 2 Genotype frequencies at nucleotide position 1236 in exon 12 of *MDR1*

Genotype	Genotype frequency, *N* (%)		*P* value	Odds ratio	95% C.I.
	RS [*N*, 97]	RP [*N*, 107]			
TT	39 (40.2)	36 (33.6)	0.41	1.32	0.75–2.35
TC	40 (41.2)	54 (50.5)	0.23	0.69	0.40–1.20
CC	18 (18.6)	17 (15.9)	0.75	1.21	0.58–2.50

N, total number; RS, patients with drug-resistant epilepsy; RP, patients with drug-responsive epilepsy.

Table 3 Genotype frequencies at nucleotide site 2677 in exon 21 of *MDR1*

Genotype	Genotype frequency, *N* (%)		*P* value	Odds ratio	95% C.I.
	RS [*N*, 99]	RP [*N*, 107]			
GG	19 (19.2)	17 (15.9)	0.66	1.26	0.61–2.58
GT	33 (33.3)	40 (37.4)	0.64	0.84	0.47–1.48
GA	22 (22.2)	21 (19.6)	0.77	1.17	0.60–2.29
TA	12 (12.1)	15 (14.0)	0.84	0.85	0.37–1.91
TT	11 (11.1)	9 (8.4)	0.68	1.36	0.54–3.44
AA	2 (2.0)	5 (4.7)	0.49	0.42	0.08–2.22

N, total number; RS, patients with drug-resistant epilepsy; RP, patients with drug-responsive epilepsy.

in Table 3. The genotype frequencies in the RS group were not statistically different from those in the RP group.

Three different genotypes, CC, CT, and TT, were identified at position 3435 in exon 26 (Table 4). There were no significant deviations from Hardy–Weinberg equilibrium in any group (for the RS group, χ^2/*P*-value = 1.47/0.23; for the RP group, χ^2/*P*-value = 1.49/0.22). The genotype frequencies in the RS group were not statistically different from those in the RP group, as shown in Table 4.

Haplotype frequencies of *MDR1* SNPs and drug responsiveness

The haplotype frequencies estimated from the genotype data were compared between the two groups (Table 5). There were 12 possible *MDR1* haplotypes and the frequencies of each of the *MDR1* haplotypes were not statistically different between the two groups.

Discussion

The brain is protected by a unique barrier called the blood–brain barrier (BBB). But the BBB represents a serous obstacle in achieving appropriate drug concentrations in the CNS.[19] The P-glycoprotein of an efflux transporter in the BBB is a 170-kDa transmembrane phosphoglycoprotein that is encoded by the *MDR1* gene, which is located on chromosome 7 and consists of 28 exons.[6,20] The P-glycoprotein expression levels in different tissues are not constant, but rather change in response to various agents and environmental factors.[20] In epileptics, P-glycoprotein is overexpressed in endothelial cells of the BBB and is expressed at sites at which P-glycoprotein is not expressed in normal subjects, e.g., in astrocytes and neurons.[21–23]

Mutations in *MDR1* influence the expression or function of P-glycoprotein in normal tissues.[24] Approximately 28 SNPs have been identified in *MDR1*, among which the SNPs of T1236C in exon 12, G2677A/T in exon 21, and C3425T in exon 26 are the most commonly reported in normal subjects

Table 4 Genotype frequencies at nucleotide site 3435 in exon 26 of *MDR1*

Genotype	Genotype frequency, *N* (%)		*P* value	Odds ratio	95% C.I.
	RS [*N*, 99]	RP [*N*, 100]			
CC	47 (47.5)	45 (45.0)	0.83	1.10	0.63–1.93
CT	46 (46.5)	48 (48.0)	0.94	0.94	0.54–1.64
TT	6 (6.1)	7 (7.0)	0.98	0.86	0.28–2.65

N, total number; RS, patients with drug-resistant epilepsy; RP, patients with drug-responsive epilepsy.

Table 5 Haplotype frequencies of three single nucleotide polymorphisms in the *MDR1* gene

Haplotype			Haplotype frequency (%)		*P* value	Odds ratio	95% C.I.
T1236C	G2677T/A	C3435T	RS [*N*, 97]	RP [*N*, 99]			
T	G	C	20.78	19.49	0.96	1.09	0.54–2.21
T	G	T	1.07	4.68	0.28	0.20	0.02–1.71
T	T	C	10.40	9.13	0.95	1.15	0.45–2.96
T	T	T	17.12	15.74	0.95	1.10	0.52–2.33
T	A	C	1.06	0.59	0.67	1.02	0.06–16.56
T	A	T	10.40	8.95	0.92	1.15	0.45–2.96
C	G	C	19.91	17.24	0.77	1.18	0.57–2.42
C	G	T	0.00	0.00			
C	T	C	16.54	16.12	0.91	1.02	0.48–2.19
C	T	T	0.79	1.44	0.80	1.02	0.06–16.56
C	A	C	1.94	6.62	0.21	0.28	0.06–1.37
C	A	T	0.00	0.00			

N, total number; RS, patients with drug-resistant epilepsy; RP, patients with drug-responsive epilepsy.

from different ethnic groups.[15–17] The G2677A/T SNP in exon 21 is usually associated with an amino acid conversion from Ala to Thr and to Ser, respectively.[15] On the other hand, the T1236C SNP in exon 12 is located in non-coding regions and the C3435T SNP in exon 26 does not lead to a change in the amino acid sequence.[15] Nevertheless, silent SNPs may play important roles in drug resistance, since they may be in linkage disequilibrium with non-silent SNPs.[3,15,25]

The mutation at nucleotide position 3435 in exon 26 of *MDR1* is the most frequently studied polymorphism in relation to multidrug resistance.[3,10–12] Siddiqui et al.[3] have reported an association between multidrug resistance in epileptics and the C3435T polymorphism in the drug transporter gene *ABCB1*. These authors demonstrated that patients with drug-resistant epilepsy had a higher frequency of the CC genotype (27.5%) in comparison with drug-responsive epileptics or nonepileptic controls (15.7 or 18.5%, respectively) and a lower frequency of the TT genotype (19.5, 29.6, and 23.5% for drug-resistant epileptics, drug-responsive epileptics, and controls, respectively).[3] Although Siddiqui et al.[3] included Caucasian and Asian patients in their study (187 versus 13 in the drug-resistant group; 110 versus 5 in the drug-responsive group, respectively), the authors suggested that the results were similar when the analysis was restricted to the Caucasian patients. In contrast to the results of Siddiqui et al.,[3] Tan et al.[13] (subjects: 98.5% Caucasians in a total population of 609 persons) and Sills et al.[14] (subjects: 400 Caucasians in Scotland) showed no significant association between the CC genotype and drug-resistant epilepsy. In addition, we failed to find any differences in genotype frequencies between Koreans with drug-resistant and drug-responsive epilepsy. As Tan et al.[13] regarded the initial positive study[3] as having a tendency to overestimate effect size, their subsequent replication attempts involved larger sample sizes (401 drug-resistant and 208 drug-responsive subjects). Although the current study also involves low numbers of subjects, it replicates the previous study carried out by Siddiqui et al.[3] in showing that Asians (Koreans only) have a different linkage disequilibrium than Caucasians.

The mutations at nucleotide position 2677 in exon 21 and at nucleotide position 1236 in exon 12 of *MDR1* confer drug resistance in several different disease states, although the data available for epilepsy are insufficient.[10–12] In addition, for the haplotype analysis based on polymorphisms at the above three sites, only some of the data are relevant to epilepsy.[26,27] Although our study in Koreans lacks sufficient sample size to avoid analysis bias and needs to be corroborated in other ethnic groups, it reveals no differences in the genotype and haplotype frequencies for these three SNPs between patients with drug-resistant and drug-responsive epilepsy. Despite these negative results, the importance of these SNPs cannot be ignored, as they may be in linkage disequilibrium with causal variants.

The identification of mutations in the *MDR1* gene is expected to result in the prediction of therapeutic outcomes and improvements in therapeutic efficacy, although SNPs or mutations alone cannot explain these factors.[3,12] In patients who exhibit predicted drug resistance, a more intensive treatment regimen combined with the administration of P-glycoprotein inhibitors may be applied. Moreover, *MDR1* mutations may reveal the characteristics of specific MDR1 variants, which could lead to the development of inhibitors that are specific for individual variants.[12]

Studies such as ours and that of Siddiqui et al.[3] have been carried out under the assumptions that SNPs in *MDR1* influence responses to antiepileptic drugs and that a direct relationship exists between the *MDR1* genotype and the MDR1 expression level in the brain. Even though these assumptions remain to be substantiated, future studies of surgical cases will allow these issues to be addressed.

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