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Multidrug Resistance 1 (MDR1) Gene Polymorphisms in Childhood Drug-Resistant Epilepsy

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Abstract

Despite considerable progress in the pharmacotherapy of epilepsy, more than 30% of patients are reported to be resistant to antiepileptic drugs. Multidrug resistance 1 (MDR1) gene could play a role in drug resistance in epilepsy. In this study, the authors investigated the association between the MDR1 gene polymorphisms, C3435T and G2677AT, and drug resistance epilepsy by using polymerase chain reaction/restriction fragment length polymorphism and pyrosequencing methods in a group of 39 patients with drug-resistant epilepsy and 92 controls. No associations were found between the polymorphisms of the MDR1 gene and drug-resistant epilepsy. Haplotype analysis showed no significant association. Compound genotype analysis showed that CC3435/GG2677 was significantly higher in the control group compared to the patient group. In conclusion, MDR1 polymorphisms investigated in this study are not associated with antiepileptic drug resistance, but the CC3435/GG2677 compound genotype might have an effect on antiepileptic drug response.

Keywords

drug-resistant epilepsy, MDR1 gene, polymorphism

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Epilepsy, which affects 1% to 2% of the population, is the most common neurological disorder characterized by recurrent, unprovoked epileptic seizures. Despite considerable advances in the treatment of epilepsy, approximately 30% of patients have proven to be resistant to antiepileptic drugs.[1] Following the detection of insufficient levels of intraparenchymal antiepileptic drug concentrations in the presence of adequate antiepileptic drug serum levels, multidrug transporters are taken into consideration as a candidate mechanism underlying drug resistance.[2]

P-glycoprotein, the most recently investigated multidrug transporter protein, was first isolated from drug-resistant tumor cells in 1973.[3] P-glycoprotein is encoded by the MDR1 (multidrug resistance) gene, which is a member of the adenosine triphosphate binding cassette transporter family. The MDR1 gene is located at 7q21.1 and contains 28 exons and 28 introns.[4]

Overexpression of MDR1 in medically intractable partial lobe epilepsy was first described in 1995.[5] Compared to endothelial cells in normal brain tissue, endothelial cells in epileptic brain tissue show higher P-glycoprotein expression levels.[6]

Recent studies have shown that polymorphisms in the MDR1 gene are responsible for different expression profiles of P-glycoprotein. To date, more than 50 polymorphisms have been identified within the MDR1 gene.[7] C3435T, G2677AT, and T1236C are the most commonly investigated polymorphisms. The majority of these were found to have effects on the expression and function of P-glycoprotein. It is reported that a polymorphism in exon 26 of the MDR1 gene, at a position 3435(C3435T), changes the P-glycoprotein expression levels in the duodenal cells and also affects intestinal absorption of digoxin, a substrate for MDR1.[8] Several studies have demonstrated associations between drug-resistant epilepsy and the MDR1 gene polymorphisms. However, others have failed to confirm this association.[9-13]

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Table 1. Primer Sequences for C3435T and G2677AT Polymorphisms Designed for Enzyme Digestion and Pyrosequencing

Single-Nucleotide Polymorphism Name	Method	Primer Sequence	Annealing Temperature
C3435T	MboI enzyme digestion	Forward (F): 5′-TGATGGCAAAGA AATAAAGCGA-3′ Reverse (R): 5′-TGACTCGATGAA GGCATGTATGT-3′	55°C
	Pyrosequencing	F: 5′-TGAGAACATTGCCTATGGAGA CA-3′ R: 5′-TTAGGCAGTGACTCGATGAAG G-3′ Sequencing primer: 5′-CTTTGCCCTC AC-3′	62°C
G2677A	BsrI enzyme digestion	F: 5′-TTTGCAGGCTATAGGTTCCAG-3′ R: 5′-GTTTGACTCACCTTCCCAG-3′	55°C
G2677T	BanI (BshNI) enzyme digestion	F: 5′-TGACTCGATGAAGGCATGTAT GT-3′ R: 5′-TTTAGTTTGACTCACCTTCCC G-3′	55°C
G2677AT	Pyrosequencing	F: 5′-CTGGACAAGCACTGAAAGATA AGA-3′ R: 5′-AATGGCCTGAAAACTGAAAAA GTC-3′ Sequencing primer: 5′-TTAGTTTGA CTCACCTTCC-3′	62°C

In this study, we aimed to investigate the possible contribution of 2 common polymorphisms of the MDR1 gene to drug resistance in patients with intractable epilepsy.

Materials and Methods

Participants

Thirty-nine antiepileptic drug-resistant children with epilepsy (14 females, 25 males) attending Ege University Medical Faculty Child Neurology Department and 92 healthy individuals were involved in this study. Informed consent was obtained from the parents of the patients and controls. The study was approved by Ege University's Ethical Committee. Drug-resistant epilepsy was defined as having 2 or more seizures while using 2 or more antiepileptic drugs within a 2-year-period. The children's ages ranged between 6 and 18. The mean ages of the children in the study group and control group were 12.05 $\pm$ 6.5 and 18.3 $\pm$ 3.5, respectively. The mean age of onset of the seizures was 3.5 $\pm$ 2.6 years. The average duration of the seizures was 8.6 $\pm$ 4.4 years. Children having syndromic epilepsy were excluded from the study as other genes may be involved in the pathogenesis of epilepsia. Seizures were classified as generalized or partial; 17 patients had generalized seizures, and 22 had partial seizures.

Genotyping

DNA was isolated from 2 mL of venous blood by using the KingFisher DNA isolation kit. Genotyping of C3435T and G2677AT single-nucleotide polymorphisms was performed by amplification of known sequences using polymerase chain reaction (PCR) with forward and reverse primers, as listed in Table 1. For C3435T polymorphism detection, a 193-bp PCR product was digested into 49 bp and 144 bp with 5 U of MboI restriction enzyme at 37°C for 1.5 hours. A 193-bp PCR product was digested with MboI enzyme, producing 49-bp and 144-bp digest products. The T allele is resistant to enzyme digestion. For G2677T, a 226-bp PCR product was digested with 5 U of BanI (BshNI) restriction enzyme at 37°C for 1 hour, and for G2677A polymorphism, a 222-bp PCR product was digested with BsrI restriction enzyme at 65°C for 1 hour. A 226-bp PCR product was digested with BanI restriction enzyme, bearing 3 different alleles: A, T, and G. The T allele was resistant to enzyme digestion, whereas PCR products having A or G allele were digested into 200 and 26 bp. To distinguish the A allele, the amplified samples were digested again with BsrI restriction enzyme. G and T alleles were resistant to digestion, whereas the A allele was digested into 208 and 14 bp.

All samples were also analyzed by pyrosequencing with the PSQ96 instrument (Biotage AB, Uppsala, Sweden). Primers for pyrosequencing were designed with Pyrosequencing Assay Design Software Version 1.0 (Biotage AB), as listed in Table 1.

The allele and genotype frequencies were determined by using the statistical analysis program SPSS Version 14.0 (SPSS, Inc, an IBM Company, Chicago, Illinois). Allele and genotype frequencies of the cases were compared to the controls by using X2 analysis. Linkage disequilibrium and haplotype frequencies were determined using Helixtree Version 5.0.0 software (Golden Helix, Bozeman, Montana).

Results

The mean age of onset of epilepsy was 3.5 $\pm$ 2.6 years (range, 3 months to 9 years). Fourteen patients were given at least 2 antiepileptic drugs, and the median number of antiepileptic drugs was 2.72 (range, 2-4). Motor and mental retardation were present in all cases. The etiologic spectrum of the symptomatic epilepsy group was hypoxic ischemic encephalopathy in 25 of 39 cases (64%), cerebral developmental malformations in 8 cases (20.5%), chronic encephalopathy in 3 cases (8%), hippocampal sclerosis in 2 cases (5%), and neurometabolic disorders in 1 case (2.5%).

C3435T polymorphism was genotyped in 38 children with antiepileptic drug-resistant epilepsy. G2677AT polymorphism was genotyped in 35 antiepileptic drug-resistant epileptic children. The distribution of genotypes for these 2 polymorphisms

Table 2. Frequencies of Genotypes of C3435T and G2677AT Polymorphisms in the MDR1 Gene

		Patient, n (%)	Control, n (%)	Total, n (%)	χ^2	*P* Value
C3435T	CC	6 (15.8)	26 (29.9)	32 (25.6)	2.758	.09
	CT	20 (52.6)	37 (42.5)	57 (45.6)	1.088	.29
	TT	12 (31.6)	24 (27.6)	36 (28.8)	0.205	.65
G2677AT	GA	0	1 (1.1)	1 (0.8)	0.440	.50
	GG	8 (21.1)	29 (30.5)	37 (27.8)	1.213	.27
	GT	19 (50)	37 (38.9)	56 (42.1)	1.360	.24
	TA	1 (2.6)	0	1 (0.8)	2.518	.11
	TT	10 (26.3)	28 (29.5)	38 (28.5)	0.132	.71

Table 3. Allele Frequencies of C3435T and G2677AT Polymorphisms in the MDR1 Gene

		Patient, n (%)	Control, n (%)	χ^2	*P* Value
C3435T	C allele	32 (42.1)	89 (51)	1.73	.18
	T allele	44 (57.9)	85 (49)		
G2677AT	G allele	35 (46)	96 (51)	0.37	
	T allele	40 (54)	93 (49)		

Table 4. Combined Genotype Analysis of C3435T and G2677AT Polymorphisms

C3435T/G2677AT	Patient, n (%)	Control, n (%)	Total, n (%)	χ^2	*P* Value
CC/GG	4 (10.5)	24 (27.6)	28 (22.4)	4.428	.03
CC/GT	2 (5.2)	1 (1.2)	3 (2.4)	1.910	.16
CC/GA	0	1 (1.2)	1 (0.8)	0.440	.50
CT/GG	3 (7.9)	3 (3.4)	6 (4.8)	1.144	.28
CT/GT	15 (39.6)	28 (32.2)	43 (34.4)	0.622	.43
CT/TT	1 (2.6)	6 (6.9)	7 (5.6)	0.910	.34
CT/TA	1 (2.6)	0	1 (0.8)	2.307	.13
TT/GG	1 (2.6)	0	1 (0.8)	2.307	.13
TT/GT	2 (5.2)	3 (3.4)	5 (4)	0.226	.63
TT/TT	9 (23.8)	21 (24.1)	30 (24)	0.002	.95
Total	38	87	125		

Table 5. Combined Haplotype Analysis of the C3435T and G2677AT Polymorphisms

C3435T/G2677AT	Patient (%)	Control (%)	χ^2	*P* Value	Odds Ratio	Confidence Interval
C/G	0.3742	0.4644	0.0167	.89	0.69	0.39-1.22
T/T	0.4823	0.4529	0.0017	.96	0.13	0.65-1.96
C/T	0.0447	0.0413	0.0001	.99	1.09	0.28-4.26
T/G	0.0853	0.0356	0.0217	.88	2.53	0.71-8.99
C/A	0.0135	0.0058	0.0031	.95	2.35	0.11-51.54
Total	1	1				

in patients and controls was consistent with Hardy-Weinberg equilibrium (both $P > .05$).

The frequencies of genotypes for both polymorphisms were not different in either patients or controls (Table 2). The allelic distribution was also evaluated in patient and control groups, and no significant differences were found between the groups (Table 3). Combined genotype analysis was performed, and the combined genotype $CC^{3435}GG^{2677}$ was found to be significantly higher in the control group than in patients with drug-resistant epilepsy ($P = .03$; Table 4). Haplotype analysis was performed, and no significant haplotype patterns were found (Table 5).

As brain development is not completed within the first years of infancy, we aimed to exclude the potential role of immaturity underlying the mechanism of drug resistance in epilepsy. Therefore, we reevaluated patients whose epileptic seizures began after the first year of age. The CC^{3435} genotype was found to be significantly higher in patients whose epileptic

Table 6. Frequencies of Genotypes of C3435T and G2677AT Polymorphisms in Patients Whose Epileptic Seizures Began After the First Year of Age and Controls

		Patients (Age of Onset >1 Year), n (%)	Control, n (%)	Total, n (%)	χ^2	*P* Value	Odds Ratio	Confidence Interval
C3435T	CC	2 (8)	26 (29.9)	28 (25)	3.86	.03	0.20	0.05-0.93
	CT	12 (48)	37 (42.5)	49 (43.8)	0.24	.62	1.24	0.51-3.04
	TT	11 (44)	24 (27.6)	35 (31.2)	2.44	.14	2.06	0.82-5.17
G2677AT	GA	0	1 (1.1)	1 (0.8)	0.440	.50		
	GG	5 (20)	29 (30.5)	34 (28.3)	1.213	.27		
	GT	11 (44)	37 (38.9)	48 (40)	1.360	.24		
	TT	9 (36)	28 (29.5)	37 (30.9)	0.132	.71		

Table 7. Allele Frequencies of C3435T and G2677AT Polymorphisms in Patients Whose Epileptic Seizures Began After the First Year of Age and Controls

		Patient (Age of Onset >1 Year), n (%)	Control, n (%)	χ^2	*P* Value	Odds Ratio	Confidence Interval
C3435T	C allele	16 (32)	89 (51)	5.72	.02	2.23	1.45-4.32
	T allele	34 (68)	85 (49)				
G2677AT	G allele	21 (42)	96 (51)	1.22	.34	1.43	0.76-2.68
	T allele	29 (58)	93 (49)				

Table 8. Combined Haplotype Analysis of the C3435T and G2677AT Polymorphisms in Patients Whose Epileptic Seizures Began After the First Year of Age and Controls

C3435T/ G2677AT	Patient (Age of Onset >1 Year), %	Control, %	χ^2	*P* Value	Odds Ratio	Confidence Interval
C/G	0.2726689	0.4643986	7.90	.004	0.43	0.24-0.78
T/T	0.5326689	0.4529043	1.27	.52	1.38	0.79-2.40
C/T	0.0473311	0.04134856		1	1.15	0.30-4.45
T/G	0.1473311	0.03560143	7.51	.016	4.68	1.42-15.44
C/A		0.00574712				

seizures began after the first year in comparison to the control group (Table 6). When allele frequencies were analyzed in the same age group, the T^{3435} allele was significantly higher in patients, whereas the C^{3435} allele was higher in the control group (Table 7). Combined haplotype analysis of this age group revealed that the $C^{3435}G^{2677}$ haplotype was significantly frequent in the control group, and the $T^{3435}G^{2677}$ haplotype was frequent in the patient group (Table 8).

Discussion

Almost 30% of epileptic patients using antiepileptic drugs are considered to have drug resistance.[1] The mechanism underlying the drug resistance in epileptic patients still remains unclear. P-glycoprotein, expressed in the blood-brain barrier, is the most commonly investigated drug transporter in drug-resistant patients.[6,14] A number of polymorphisms that affect the expression levels of P-glycoprotein have been determined to be associated with poor response to antiepileptic drugs.[9-11,15]

The synonymous single-nucleotide polymorphism C3435T (rs1045642) in exon 27 of the multidrug resistance 1 gene was the first single-nucleotide polymorphism that was reported to be associated with drug resistance in epileptic patients.[9] In this report, the CC genotype of this polymorphism was found to be significantly higher in patients with drug-resistant epilepsy, whereas the TT genotype was significantly lower in the same group.[9] However, several studies failed to confirm the association between the C3435T polymorphism and drug-resistant epilepsy.[10,11,16] Some studies also failed to confirm the association between C3435T, G2677A, and C1236T polymorphism

and drug-resistant epilepsy in the pediatric patient group.[12,13] The reverse results were observed in the Han Chinese population, with a higher frequency of the TT genotype associated with drug-resistant epilepsy.[15] These results were supported by Hung et al.[17] In our study group, we did not find any association between C3435T polymorphism and the response to antiepileptic drugs in epileptic patients. The genotype distribution in the patient group studied was 15.8% CC, 52.6% CT, and 31.6% TT, in accordance with Sills et al.[11] The frequencies of the C allele (51%) and the T allele (49%) in our healthy control group are consistent with the frequencies reported in whites.[8,18] When we compare the genotype frequency of the patients whose epileptic seizures began after the first year of age to the genotype frequencies of the control group, we found that the CC genotype was significantly higher in the control group, and the T allele was significantly higher in the patient group. It was previously reported that the immature brain differs from the mature brain in the basic mechanisms of epileptogenesis and propagation of seizures.[19] Regarding this, we hypothesized that genetic influence on drug resistance is more evident in patients when brain maturation is complete. Our results were concordant with genotype distribution in Asian populations, in whom the TT genotype is associated with drug-resistant epilepsy.[15,17]

The second polymorphism in multidrug resistance 1 gene G2677AT (rs2032582) that has been widely investigated is a nonsynonymous polymorphism in exon 12 resulting in alanine to threonine or serine amino acid substitution, respectively. The G2677AT polymorphism was found to be linked to the C3435T polymorphism in a number of studies.[20,21] As shown in Table 3, 92.3% of controls with CC at position 3435 were homozygous for the G allele at position 2677, showing a linkage between these 2 polymorphisms as in previous studies.[22] When we compared the genotype distribution of the G2677AT polymorphism in the patients to the control group, we found no significant differences between the groups, in accordance with Zimprich et al.[23] The genotype frequencies of C3435T and G2677AT polymorphisms in our patients whose epileptic seizures began after the first year of age and controls did not differ significantly. Variant allele A, mostly found in Asian populations, was not observed in our population, whereas the G and T allele frequencies were similar to those of whites.[23]

The C allele at 3435 position and the G allele at 2677 position were reported to be linked in several studies.[9,24] Carrying homozygous mutant alleles for both polymorphisms was shown to be associated with an altered drug disposition.[25] The combined genotype CC3435/GG2677 was found to be significantly higher in our control group, suggesting the influence of the C3435T and G2677AT polymorphisms on drug response (Table 5). A combined haplotype analysis found that the C/G haplotype was significantly higher in the control group than in patients whose epileptic seizures began after the first year of age.

Among the transporters, P-glycoprotein is the most important protein that affects drug disposition in tissues. In recent years, polymorphisms within the multidrug resistance 1 gene were found to be associated with the expression levels of P-glycoprotein. Besides single-polymorphism analysis, combined genotype or haplotype analysis is more meaningful in the functioning of P-glycoprotein. We conclude that the alleles C3435 and G2677 can influence the occurrence of drug resistance in epileptic patients. The age of onset should be taken into consideration during polymorphism studies as some polymorphisms may be associated with the early onset of a disease, whereas others may be associated with a late onset. More studies performed in larger and different ethnic groups are needed to support this hypothesis.

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Contributors

AA performed the molecular analysis and prepared the manuscript. FO and CO developed the project and revised the manuscript. HT and SG followed up the patients and participated in the selection of the cases according to the diagnosis. SP and MS organized the molecular analysis.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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