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Original article

Multidrug resistance 1 (MDR1) 3435C/T genotyping in childhood drug-resistant epilepsy

Semra Saygi [a,*], Fusun Alehan [a], Fatma Belgin Atac [b], Ilknur Erol [a], Hasibe Verdi [b], Remzi Erdem [c]

[a] Department of Pediatrics, Division of Child Neurology, Baskent University Faculty of Medicine, Ankara, Turkey
[b] Department of Medical Biology, Baskent University School of Medicine, Ankara, Turkey
[c] Department of Pharmacology, Baskent University, Faculty of Medicine, Adana, Turkey

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Abstract

Introduction: A mutation at nucleotide position 3435 in exon 26 of the multidrug resistance 1 (MDR1) gene is the most frequently studied polymorphism in relation to multidrug resistance. However, there are conflicting data as to whether the CC or TT genotype of the 3435C>T polymorphism is associated with drug resistance. *Methods and results:* We investigated the association between this polymorphism in drug-resistant childhood epilepsy by comparison with drug-responsive patients. In total, 59 patients with drug-resistant epilepsy, defined as having four or more seizures within a 12-month period while using three or more AEDs, 60 children with drug-responsive epilepsy who had remained seizure-free for 12 months on their current AED regimen and 76 healthy children were involved in this study. Genotype frequencies in drug-resistant patients were as follows: 32.2% CC, 44.1% CT, 23.7% TT; in the drug-responsive group: 20.0% CC, 50.0% CT, 30.0% TT; in the control group: 24.3% CC, 50.0% CT, 25.7% TT. Comparison of drug-resistant and drug-responsive patients revealed no significant difference in genotype frequency. The findings of the epilepsy patients were not significantly different from those of the healthy control subjects. *Conclusions:* Our study does not support any significant association between the MDR1 polymorphism and drug-resistant childhood epilepsy.

Keywords: Antiepileptic drug treatment; Intractable epilepsy; MDR1 polymorphism

1. Introduction

Epilepsy is the most common neurologic disorder. It is characterized by recurrent unprovoked epileptic seizures. It is estimated that approximately one third of patients with newly diagnosed epilepsy are resistant to currently available medications [1]. The mechanism of drug resistance remains unknown. Recent studies in epilepsy patients have suggested that the multidrug resistance 1 (MDR1) gene may contribute to drug resistance in epilepsy. The MDR1 gene attracted initial attention because of its demonstrated influence on cellular resistance to anticancer drugs [2]. Drawing on parallels between drug resistance in cancer and epilepsy, a number of preliminary reports have documented brain overexpression of MDR1 in epileptic tissue from patients with multidrug-resistant epilepsy [3–5].

The human MDR1 gene has been localized to chromosome 7p21.1 and encodes P glycoprotein (P-gp), a transporter that is involved in the export of substances from the interior of the cell. P-gp has been found to be expressed in the blood-tissue barriers of the liver, kidneys, intestine, blood–brain barrier, endothelium, pla-

* Corresponding author. Address: Güzelyalı mah. Turgut Özal Bulvarı, Dosteller apt. kat:5 daire 9, 01170 Adana, Turkey. Tel.: +90 3222359542; fax: +90 3122157597.
E-mail address: semra_saygi@yahoo.com (S. Saygi).

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centa, ovaries, testes, and salivary glands. The substrate specificity of P-gp is wide and includes anticancer and antihypertensive drugs as well as antivirals, antibiotics, antiepileptics and others [6,7].

The tissue localization and substrate specificity of P-gp suggest its importance in drug absorption and elimination [8]. Because this transporter can limit the penetration into, and retention within, the brain of drugs, it may thus influence drug treatment outcome. Mutations in MDR1 affect the expression or function of P-gp, by reducing antiepileptic drug (AED) accumulation in the seizure foci, and accordingly a link to drug-resistant epilepsy has been hypothesized. Additionally Siddiqui et al. (2003) reported that the homozygous C variant, which is associated with higher expression and increased activity of P-gp, is more common in patients with pharmacoresistant epilepsy [9].

Several studies have confirmed a link between the MDR1 3435C > T polymorphism and drug resistance but other studies in different populations failed to corroborate these findings [10]. Although the etiology and treatment are different in pediatric and adult epilepsy groups, most studies of MDR1 polymorphism in relation to multidrug resistance have not attempted to distinguish children from adults. Thus we aimed to evaluate the correlation between C3435T polymorphisms in the MDR1 gene and childhood drug-resistant epilepsy.

2. Materials and methods

2.1. Participants

The study included children and adolescents (2–18 years of age) in whom epilepsy was diagnosed and AED therapy was begun at the Baskent University Faculty of Medicine, Division of Child Neurology, as well as 76 healthy children and adolescents (2–17 years of age). All the patients had been evaluated by the same epileptologist (F.A.) during this period. All information was obtained by reviewing medical records to extract the following information; gender, age at onset of seizure, initial seizure type, seizure frequency, history of febrile/neonatal seizure/status epilepticus, perinatal history, neurodevelopmental status, family history of seizure disorders, neurological examination, mental status, brain imaging findings, EEG records and etiology of the epilepsy. Scalp EEG recordings were obtained in all patients using a 21-channel system with the standard 10–20-electrode placement system for diagnosis and classification of epilepsy. We excluded children whose seizures were poorly controlled but in whom serum drug concentrations were low or compliance was poor.

2.2. Definitions

Seizures were classified according to the ILAE classification of epileptic seizures, and epilepsy and epilepsy syndromes were classified using ILAE classification [11,12]. Classification of the seizure types was based on seizure descriptions and EEG findings according to the ILAE definitions. Drug-resistant epilepsy was defined as having four or more seizures within a 12 month period while using three or more antiepileptic drugs. Patients who had remained seizure-free for $\geqslant$12 months on their current AED regimen were classified as responders.

2.3. Genotyping

All participants provided written informed consent and the ethics committee of Baskent University, Ankara, Turkey, approved the study protocol. Peripheral blood samples were drawn from all participants. Genomic DNA was extracted from peripheral blood leukocytes by means of a high pure polymerase chain reaction (PCR) template preparation kit (Roche Diagnostics GmbH, Mannheim, Germany). MDR1 3435C/T genotype was determined by real-time PCR assay using a LightCycler® Real-Time PCR System (Roche Molecular Biochemicals, Mannheim, Germany). Genomic DNA amplification was performed with primers 5′-TgTTTTCAgCTgCTTgATgg and 5′-CATgCTCCC AggCTgTTTAT. Fluorescent detection was performed using hybridization probes 5′-LC-Red640-AAggAggC-CAACATACATgCC–ph and 5′-AAgAgATCGT-gAgggCA–Flu (TIB Molbiol, Berlin, Germany). Melting temperatures were 56.0 °C for 3435C and 46.0 °C for 3435T. The CYP2D6 genotype was determined using a LightCycler® with primers and probes obtained from TIB MOLBIOL (Cat. No: 40-0181-16).

2.4. Statistical analysis

Statistical package for social sciences (SPSS) 15.0 was used to carry out all analyses. For primer analysis we compared genotype and allelic frequencies between the study groups with the Chi-square test. Associations between independent risk factors and intractable epilepsy were evaluated using multivariate logistic regression analysis including demographic and clinical variables.

3. Results

In total 59 patients (30 male, 29 female, age range 2–16 years, median age of 6 years) were defined as drug-resistant, 60 (33 male, 27 female, age range 2–18 years, median age of 9.5 years) were defined as drug-responsive children with epilepsy and 76 healthy children (35 male, 41 female, age range 2–18 years, median age of 8 years) were involved in this study. The mean ages of the children in the drug-responsive group, drug-resistant group and control group were 9.3 ± 4.9, 6.7 ± 4.2, and