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ScienceDirect
Epilepsy & Behavior 11 (2007) 112–117
Epilepsy & Behavior
www.elsevier.com/locate/yebeh

Association between *ABCB1* C3435T polymorphism and drug-resistant epilepsy in Han Chinese

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Received 22 November 2006; revised 26 March 2007; accepted 12 April 2007
Available online 22 May 2007

Abstract

There is accumulating evidence to suggest that overexpression of efflux drug transporters at the blood–brain barrier, by reducing antiepileptic drug (AED) accumulation in the seizure foci, contributes to drug resistance in epilepsy. P-glycoprotein, encoded by the *ABCB1* gene, is the most studied drug transporter. There are conflicting data as to whether the CC genotype of the *ABCB1* 3435C > T polymorphism is associated with drug resistance in Caucasian patients with epilepsy. We investigated this association in ethnic Chinese. *ABCB1* 3435C > T was genotyped in 746 Han Chinese patients with epilepsy and 179 controls. Patients with drug-resistant epilepsy were more likely to have the TT genotype compared with those with drug-responsive epilepsy (16.7% vs 7.4%, odds ratio = 2.5, 95% confidence interval = 1.4–4.6, $P = 0.0009$). Our results contrast with those of studies of Caucasians, and highlight the complexity of the possible role of this polymorphism in AED response in different ethnic populations.

Keywords: Epilepsy; Drug resistance; *ABCB1*; P-glycoprotein; Pharmacogenetics; Genetic polymorphisms; Antiepileptic drugs; Chinese; MDR1; Drug transporters

1. Introduction

Despite antiepileptic drug (AED) treatment, 30% of patients continue to have seizures [1]. There is accumulating evidence to suggest that overexpression of efflux drug transporters at the blood–brain barrier, by reducing AED accumulation in the seizure foci, contributes to drug resistance in epilepsy [2–4]. P-glycoprotein (P-gp), encoded by the *ABCB1* (or *MDR1*) gene, is the most studied drug transporter. A common single-nucleotide polymorphism (SNP), 3435C > T in exon 26 of *ABCB1*, although synonymous, has been found to be associated with altered P-gp level and activity in duodenal enterocytes, affecting drug absorption [5]. It has been hypothesized that the 3435CC genotype, possibly by increasing P-gp expression and/or function in the brain, might be associated with drug resistance in epilepsy. As the demonstration of such an association could potentially influence drug choice in patients with different genotypes, a number of studies have been performed in both Caucasian [6–10] and non-Caucasian [11–14] subjects to test this hypothesis. The results of these studies have been conflicting and inconclusive. It is important to investigate whether the same association (or lack thereof) may be observed in different ethnic groups due to considerable ethnic variation in the frequencies of the 3435C > T genotypes [15], and because genetic variants may have inconsistent effects across different ethnic groups

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doi:10.1016/j.yebeh.2007.04.013

[16]. It is also important that, to avoid bias and chance finding, genetic association studies be repeated in different patient cohorts [17].

The relationship between the *ABCB1* 3435C > T polymorphism and drug response of patients with epilepsy remains controversial. Therefore, we investigated whether the 3435C > T SNP of *ABCB1* might be associated with drug response among patients with epilepsy of Chinese ethnicity.

2. Methods

2.1. Subjects

This multicenter study was performed in Hong Kong. The study was approved by the ethics committees of the participating hospitals. Patients were recruited from the neurology/epilepsy clinics and inpatient wards of four major regional hospitals, serving a total population of 2.5 million (representing 35% of the whole population of the city). Patients with epilepsy at least 15 years of age and of Han Chinese ethnicity (based on self-reported ancestry) were eligible for inclusion if they had been receiving AED treatment for at least a year. Exclusion criteria included poor compliance with AED therapy, unreliable record of seizure frequency, significant psychiatric comorbidity, history of pseudoseizures, alcohol or illicit drug abuse, and presence of progressive or degenerative neurological or systemic disorders.

After written informed consent was obtained from the patients or their guardians, a standardized questionnaire was administered to collect demographic details and information on seizure types and frequency, past medical history, AED history, concomitant drug history, and relevant family history. Seizures and epilepsy syndromes were classified according to international guidelines [18,19]. A 5-mL venous blood sample was taken for DNA extraction and genotyping. One hundred seventy-nine DNA samples extracted from cord bloods of unidentified neonates from a DNA repository at the Chinese University of Hong Kong served as controls. Subject information and genotype data were identified by a coded ID to protect privacy and to ensure that the genotyping was done "blind".

2.2. Phenotyping

Response to treatment was assessed by the recruiting neurologists in each center who were blind to the genotype results. Three phenotypic groups were defined: drug-responsive, drug-resistant, and active. Patients who had not experienced any seizure for at least a year up to the date of recruitment, and received a stable dose of an AED, were considered to have drug-responsive epilepsy [1]. Patients who had had an average of one seizure or more per month over the previous year despite treatment with two or more AEDs at therapeutic dosages and/or serum drug concentrations were considered to have drug-resistant epilepsy. In this context, AEDs must have failed primarily because of inadequate efficacy instead of adverse effects. To address what is frequently encountered in clinical practice, patients whose seizure status did not fulfill these criteria, that is, who had failed only one AED and/or had one or more seizures per year but less than one per month, were considered to have "active" epilepsy to represent an intermediate group in terms of drug response. Patients who underwent epilepsy surgery for refractory epilepsy were classified as having drug-resistant epilepsy, regardless of their postoperative seizure control status.

2.3. Genotyping

Patients were genotyped using a PCR-RFLP assay to analyze the 3435C > T polymorphism of the *ABCB1* gene as described previously [20]. To confirm accuracy, genotyping of all the drug-resistant and drug-responsive patients was repeated by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) on a Sequenom Mass Array system. Any discrepancies between the two methods were resolved by direct sequencing using an Applied Biosystems 3100 capillary sequencer.

2.4. Population stratification

To detect any unsuspected population stratification, randomly selected polymorphisms on eight different chromosomes were selected from HapMap and typed using MALDI-TOF.

2.5. Statistical analysis

For the primary analysis, we compared genotype and allelic frequencies between patients with drug-resistant and drug-responsive epilepsy with the χ^2 test. Where deviation from Hardy–Weinberg equilibrium existed, we applied the method proposed by Schaid and Jacobsen to correct for any effect on the Type 1 error rate and power when comparing allele frequencies [21]. Logistic regression was used to adjust for age, sex, and epilepsy syndrome. For the secondary analysis, χ^2 for trend was used to assess whether a dose–phenotype effect existed for genotype and level of drug resistance. P values $\leqslant 0.05$ (two-sided) were considered statistically significant.

3. Results

Seven hundred forty-six (221 drug-resistant, 297 drug-responsive, and 228 active) patients were recruited. Their mean age was 36.2 years (SD = 16.2); 49.9% were male. Epilepsy was classified as idiopathic in 18.4% of patients, cryptogenic in 31.4%, and symptomatic in 46.6%, and was unclassifiable in 3.6%. Consistent with previous observational data [1], compared with the "active" and drug-responsive groups, the drug-resistant group had a larger proportion of patients with symptomatic epilepsy syndromes and a smaller proportion of patients with idiopathic syndromes (Table 1). The mean duration of epilepsy was 16.5 years (SD = 12). The median number of AEDs that failed among drug-resistant patients was 3 (range: 2–9), and their mean monthly seizure frequency was 11 (SD = 47.3). The distributions of 3435C > T genotypes in the controls and drug-resistant patients were consistent with Hardy–Weinberg equilibrium (both $P > 0.05$), but deviation from equilibrium was observed for the active and drug-responsive groups and for the patient cohort as a whole (all $P < 0.05$) (Table 2).

To examine the possibility that drug-responsive and drug-resistant groups were covertly stratified, eight random polymorphisms across the genome were genotyped (Table 3). None were significantly associated with drug responsiveness. All were in Hardy–Weinberg equilibrium in both the drug-resistant and drug-responsive groups.

Patients with drug-resistant epilepsy were more likely to have the TT genotype at *ABCB1* 3435 compared with those with drug-responsive epilepsy (16.7% vs 7.4%, odds ratio [OR] = 2.5, 95% confidence interval [CI] = 1.4–4.6, $P = 0.0009$). To account for deviation from Hardy–Weinberg equilibrium in the drug-responsive group, we applied the method proposed by Schaid and Jacobsen to compare