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The potential implication of *SCN1A* and *CYP3A5* genetic variants on antiepileptic drug resistance among Egyptian epileptic children

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ABSTRACT

Purpose: Despite the advances in the pharmacological treatment of epilepsy, pharmacoresistance still remains challenging. Understanding of the pharmacogenetic causes is critical to predict drug response hence providing a basis for personalized medications. Genetic alteration in activity of drug target and drug metabolizing proteins could explain the development of pharmacoresistant epilepsy. So the aim of this study was to explore whether *SCN1A c.3184 A/G* (rs2298771) and *CYP3A5*3* (rs776746) polymorphisms could serve as genetic based biomarkers to predict pharmacoresistance among Egyptian epileptic children.

Methods: Genotyping of *SCN1A c.3184 A/G* and *CYP3A5*3* polymorphisms using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was performed in 65 healthy control subjects and 130 patients with epilepsy, of whom 50 were drug resistant and 80 were drug responsive.

Results: There was a significant higher frequency of the AG genotype ($p = 0.001$) and G allele ($p = 0.006$) of *SCN1A* polymorphism in epileptic patients than in controls. Also their frequency was significantly higher in drug resistant patients in comparison with drug responders ($p = 0.005$ and 0.054 respectively). No significant association between *CYP3A5*3* polymorphism and drug-resistance was found.

Conclusions: Overall, results confirmed the claimed role of *SCN1A c.3184 A/G* polymorphism in epilepsy and moreover in development of pharmacoresistance among Egyptian epileptic children. *CYP3A5*3* variants have no contributing effect on pharmacoresistance among Egyptian epileptic children.

1. Introduction

Epilepsy is a debilitating life-long neurological disorder. Despite advances in the therapeutic options, about one third of patients still remain pharmacoresistant [1]. The concept of pharmacoresistance extends beyond being a side effect of treatment to represent a huge burden to patients and societies [2]. The fact that patient's genotype affect its phenotype, suggests that variations in drug response could be explained, at least in part, by personal genetic variations [3]. Polymorphic genes encoding drug targets (*SCN1A c.3184 A/G*) and drug-metabolizing enzymes (*CYP3A5*3*) could be utilized to predict antiepileptic drugs (AEDs) outcome [4].

SCN1A gene encodes the alpha subunit of voltage gated sodium channel type 1 that initiates firing of brain neurons [5,6]. These ion channels are molecular targets for many AEDs [7] which block ionic conductance through these channels [4,8]. The architecture of these channels reveals a large pore-forming α subunit associated with two smaller β subunits [5,9] and evidence suggests that the channel blocking AEDs do so mainly by binding α subunit [8]. Previous studies documented most of de novo mutations in the sodium-channel gene *SCN1A* cause severe myoclonic epilepsy of infancy, also the association of *SCN1A* mutations with familial epilepsy [10–13] and moreover with drug effectiveness [12,14–16]. The protein coding missense *c.3184 A/G* polymorphism of *SCN1A* results in p.Thr1067Ala change [17]. This polymorphism could affect the concerted gating of these channels by altering their structure-function relationship rendering them

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insensitive to the blocking effect of AEDs so that the question of why some patients respond to AEDs while others do not might be answered by this polymorphism [12].

Another issue that could be incriminated in pharmacoresistant epilepsy is drug metabolism. AEDs are primarily metabolized by phase I oxidative *CYP3A* subfamily [18–21]. Being the chief members, *CYP3A4* and *CYP3A5* account for the *CYP3A* activity [3,22–24]. In contrast to *CYP3A4*,*CYP3A5* gene is highly polymorphic with interindividual variability in expression and catalytic activity [3,10,23,25,26] resulting in variable metabolism of its substrates [27,28], so *CYP3A5* genetic polymorphism could modulate response to AEDs [3]. This variability could be attributed to the functional variant *CYP3A5*3* (rs776746) in intron 3 resulting in splicing defect and truncated *CYP3A5* protein of no catalytic activity [29–34].

Thus in this study we genotyped the *SCN1A c.3184 A/G* and *CYP3A5*3* polymorphisms in Egyptian epileptic patients either drug sensitive or drug resistant, to evaluate the association of these polymorphisms with epilepsy and their role in resistance to AEDs.

2. Patients and methods

2.1. Patients and setting

This study included 130 Egyptian epileptic children treated with AEDs, 80 patients are drug responders (55 males and 25 females) with mean age of 8.34 ± 2.95 years and 50 patients are drug resistant (25 males and 25 females) with mean age of 7.43 ± 2.95. All epileptic children were recruited from the Pediatrics Department, Menoufia University Hospital, Egypt. The subjects were seen over a period of 6 months in the pediatric neurology clinic sequentially until the study was filled. The patients were considered to be drug responsive if they had not experienced any type of seizures for a minimum of 1 year after receiving AEDs. Drug-resistant epilepsy was defined as uncontrolled seizures over a year, despite attempts to treat with three or more different AEDs. The antiseizure medications were chosen according to the type of seizures and epilepsy syndrome. Polypharmacy was determined by numbers of medication ever used, not only at enrollment into the study. Any patients suspected of poor compliance or with known *SCN1A* mutations were excluded from the study. Clinical data and medical history of each patient were also collected. The controls consisted of 65 age and gender matched healthy children (36 males and 29 females) with mean age of 7.48 ± 2.50 with negative individual and family history, as regards epilepsy and febrile convulsions. The controls were recruited from outpatient general pediatric clinic after being treated for simple infections, as common cold. All patients and/or their parents gave their written consent to participate in the study. The protocol was approved by the Menoufia University Ethics Committees.

2.2. DNA analysis

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood (QIAamp DNA Blood Mini Kit, QIAGEN).

2.2.1. Genotyping of SCN1A c.3184 A/G polymorphism [10]

Fragments containing polymorphic sites were amplified with the following forward and reverse primers {F: 5′-TGCACAAAG-GAGTAGCTTATG-3′ and R: 5′-AGTCAAGATCTTTCCCAATTTCAG-3′} by PCR in a final volume of 25 μL containing 10 μL genomic DNA, 12.5 μL DreamTag green PCR master mix (Thermo Scientific), 1 μL of each primer and 0.5 μL of DNase/RNase free water. PCR conditions were as follows: a denaturing step at 95 °C for 5 min, then 40 cycles at 94 °C for 30 s, 57 °C for 1 min, 72 °C for 1 min, and a final incubation at 72 °C for 7 min. After amplification, The *SCN1A c.3184 A/G* polymorphism was identified by *PvuII* restriction enzyme (A allele; 168 bp, G allele; 145 and 23 bp separated in 2% agarose gel containing ethidium bromide).

2.2.2. Genotyping of CYP3A5*3 polymorphism [26]

The genomic DNA was amplified with the use of specific forward and reverse primers {F: 5′-CTTTTAAGAGCTCTTTTGTCTCTC A-3′ and R: 5′-CCAGGAAGCCAGACTTTGAT-3′}. The 25 μL PCR mixture for reaction contained 10 μL genomic DNA, 12.5 μL DreamTag green PCR master mix (Thermo Scientific), 1 μL of each primer and 0.5 μL of DNase/RNase free water. After denaturation at 94 °C for 5 min, the amplification was carried out for 40 cycles at 94 °C for 30 s, at 56 °C for 1 min, and at 72 °C for 1 min. The terminal elongation was carried out at 72 °C for 7 min. After amplification the PCR product (200 bp) was cleaved by the restriction enzyme DdeI (Fermentas), obtaining fragments of 129, 107, 71, and 22 bp for allele *1, as well as 107, 71, and 22 bp for allele *3. The amplified fragments of DNA were separated in 2% agarose gel containing ethidium bromide.

2.3. Statistical analysis

Results were collected, tabulated, statistically analyzed by statistical package SPSS version 20 (SPSS Chicago., Inc.). Mann–Whitney test and Kruskal–Wallis test were used for nonparametric variables. Chi-Squared (χ^2) and Fisher's exact test were used for qualitative variables. p value ≤ 0.05 was considered significant.

3. Results

Out of the 130 children with epilepsy, drug-resistant epilepsy was diagnosed in 50 children and drug-responsive epilepsy in 80 children. The mean age of the drug-resistant patients was 7.43 ± 2.95 years vs. drug responders, in whom it was 8.34 ± 2.95 years (Table 1). Among the 130 epileptic children epilepsy was more frequent in male in comparison to female (61.5% vs.38.5% respectively). Mean age of onset for seizures in drug-resistant was

Table 1
Distribution of the studied patients groups and controls regarding their age and gender.

	Patients				Controls (n = 65)		Test	p value
	Drug responders (n = 80)		Drug resistant (n = 50)					
Age (Y)								
Mean ± SD	8.34 ± 2.95		7.43 ± 2.95		7.48 ± 2.50		Kruskal–Wallis = 4.41	0.110
Gender: no, %								
Female	25	31.2	25	50.0	29	44.6	χ^2 = 5.16	0.075
Male	55	68.8	25	50.0	36	55.4		