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Common variants of *ATP1A3* but not *ATP1A2* are associated with Chinese genetic generalized epilepsies

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ABSTRACT

Objective: *ATP1A2* and *ATP1A3* are genes that code for catalytic subunits of Na/K-ATPases, which play important roles in the basal electrophysiological states of nerve cells. The aim of this study was to investigate whether genetic polymorphisms of *ATP1A2* and *ATP1A3* influence susceptibility to genetic generalized epilepsies (GGEs) and the efficacy of anti-epileptic drugs in a Chinese population.
Method: Six *ATP1A2* tagged single-nucleotide polymorphisms (tagSNPs) and two *ATP1A3* tagSNPs were were genotyped by allele-specific MALDI–TOF mass spectrometry in 484 Chinese GGE patients (280 drug-responsive and 204 drug-resistant patients) and 284 healthy controls.
Results: Significant differences were found in the frequencies of the *ATP1A3* rs8107107 C allele and the CC genotype between the GGEs and the healthy controls (11% vs. 15%, odds ratio (OR) = 0.807 (0.68–0.960), $p = 0.021$ and 0.4% vs. 3.2%, OR = 0.121 (0.026–0.565), $p = 0.002$, respectively). The frequency of the rs8107107 CT + CC genotype was significantly lower among the GGE patients than among the healthy controls (15% vs. 26.8%, OR = 0.327 (0.248–0.942), $p = 0.001$). No significant differences in the frequencies of six *ATP1A2* tagSNPs or *ATP1A2* haplotypes were found between the GGEs and the healthy controls. No tagSNPs were involved in anti-epileptic drug resistance.
Conclusion: Our findings demonstrated that common variants of *ATP1A3* but not *ATP1A2* were associated with the susceptibility to GGEs in a Chinese population, which indicates that the *ATP1A3* gene plays a significant role in the pathophysiology of genetic generalized epilepsies.

1. Introduction

Epilepsy is a chronic neurological disease that causes abnormal electrical activity in the brain that leads to involuntary changes in body movement, function, sensation, and behaviors [1]. The growing body of accepted evidence demonstrates that 0.3% of the entire population is affected by genetic generalized epilepsies (GGEs), which accounts for approximately 30% of all of the patients who suffer from epilepsies [2]. The majority of generalized epilepsies are benign, self-limited or easily treatable disorders and are believed to be largely genetic in origin [2]. Genetic generalized epilepsies are thought to be polygenic and primarily include childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME) and epilepsy with generalized tonic–clonic seizures alone (EGTCS) [3].

Currently, the majority of the known bona fide disease genes for epilepsy encode voltage-gated or ligand-gated ion channels, such as *SCN1A*, *KCNQ2*, *CHRNA4*, *GABRA1*, and GRIN2A [4,5]. Mutations in *ATP1A2* and *ATP1A3* have been reported to be associated with other neurological diseases, such as familial hemiplegic migraine (FHM) [6,7], alternating hemiplegia of childhood [8–10], bipolar disorders [11], and rapid-onset dystonia–parkinsonism (RDP) [12]. *ATP1A1*, *ATP1A2* and *ATP1A3* are categorized as genes that code for the catalytic subunits of Na/K-ATPases. Na/K-ATPases hydrolyze adenosine triphosphate (ATP) to drive the transport of potassium into cells and sodium out of cells [13]. There are three α subunits of Na/K-ATPases [14], including the α-2 subunit (which is primarily present in neurons) and the α-3

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subunit (which is preferentially present in glial cells) [14,15] that are present in brain tissue.

It has been reported that *ATP1A2* mutations are associated with febrile seizures in an FHM2 family and hemiplegic migraine with epilepsy [16–21]. Mutations in *ATP1A2* might change the role of the ATPase enzyme in the regulation of the neuronal membrane potential and hence neuronal firing to induce seizures [22,23]. In a mouse mutagenesis screen, the *ATP1A3* mutation was found to be related to seizures, which indicates the functional significance of the Na/K-ATPases α-3 isoform in the control of epileptiform activity and seizure behavior [24]. Overall, the abnormal function of Na/K-ATPases significantly affects the function of the brain.

One-third of patients continuously suffer from seizures despite efforts to identify optimal treatment regimens of one or more drugs [2]. The majority of drug resistance research has focused on the transporters, targets, metabolism and enzymes that are associated with the elimination of the drug. However, different types of AEDs affect distinct drug transporters and drug targets, exhibit distinct drug metabolism and are eliminated by different enzymes. The actual mechanism of drug resistance remains unknown. The Na and K gradients across the cellular plasma membrane are determined by the activity of Na/K-ATPases and facilitate the generation of action potentials and influence neurotransmitter release and reuptake [25–28]. Thus, Na/K-ATPases establish the basal electrophysiological state of nerve cells. Nerve cells in different electrophysiological basal states might respond differentially to anti-epileptic drugs. We hypothesized that mutations in the ATP1A2 or ATP1A3 genes that lead to dysfunctions of Na/K-ATPases would affect the basal electrophysiological states of nerve cells and thus result in the failure of anti-epileptic drugs.

To our knowledge, no clinical studies have illustrated the relationships of the common variants of *ATP1A2* and *ATP1A3* with susceptibility to GGEs or anti-epileptic drug efficacy in the Chinese population. Thus, we analyzed six tagSNPs of *ATP1A2* and two tagSNPs of *ATP1A3* in 484 Chinese GGE patients and 284 healthy controls to further investigate whether the common variants of *ATP1A2* and *ATP1A3* are involved in the etiology of GGEs and AED drug resistance.

2. Methods

2.1. Subjects

The GGEs patients and healthy controls in this study were recruited from Xiangya hospitals, the Second Xiangya Hospital of Central South University, Hunan Provincial People's Hospital, and the Third Hospital of Huaihua City. The GGE patients included 185 patients with childhood absence epilepsy (CAE), 95 patients with juvenile absence epilepsy (JAE), 124 patients with juvenile myoclonic epilepsy (JME), and 80 patients with generalized tonic–clonic seizures alone (EGTCS). The diagnostic classification of the GGE was performed according to the revised version of the 'Classification of Epilepsies and Epileptic Syndromes' of the International League Against Epilepsy and standardized protocols (available at: http://portal.ccg.uni-koeln.de/ccg/research/epilepsy-genetics/sampling-procedure) [29,30]. The exclusion criteria included poor compliance with AEDs, unreliable or lacking records of seizure frequency, severe adverse drug reactions, a history of drug abuse, the presence of progressive or degenerative neurological or systemic disorders, and hepatic or renal failure. The clinical data, including seizure types and frequencies, drug history and relevant family history, were collected according to a standardized questionnaire. 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 resistance was defined as having at least four seizures during the previous year while trying at least three antiepileptic medications at maximal tolerated doses [31,32]. This study was performed according to the principles of the Declaration of Helsinki (revision of Edinburgh 2000). This clinical study (registration number: ChiCTR-TCH-0000813) was approved by the Chinese Clinical Trail Register. The study protocol was approved by the Ethics Committee of Xiangya School of Medicine and the Ethics Committee of the Institute of Clinical Pharmacology of Central South University. All adult patients and the parents of all children patients provided written consent to participate in this research.

2.2. Genotyping

DNA was isolated from 3-milliliter whole blood samples using the phenol-chloroform extraction method. TagSNPs were identified across the regions of the *ATP1A2* and *ATP1A3* genes (27.82 kilobase pairs [kbp], 27.65 kbp, and 27.64 kbp; HapMap Data Rel 24/phaseII Nov. 08 on NCBI B36 assembly dbSNP b126) with Haploview (http://www.broad.mit.edu/mpg/haploview). Linkage disequilibrium (LD) data for the SNPs with minor allele frequencies (MAFs) ≧0.1 among the Han Chinese in Beijing, China from the International HapMap Project (http://www.hapmap.org) were applied with taggers to identify the SNP-tagging clusters with LDs with r^2s > 0.8. MassArray (Sequenom, San Diego, CA) was used to genotype all eight of the tagSNPs via allele-specific MALDI–TOF mass spectrometry. The primers and multiplex reactions were designed using the RealSNP.com website.

2.3. Statistical analyses

The SPSS software package (Version 13.0 for Windows; SPSS, Chicago, IL, USA) was used for the statistical analyses. Hardy–Weinberg equilibriums were tested with χ^2 tests or Fisher's exact tests as applicable to the case–control samples. Nonparametric K–S test was used to detect whether data followed the normal distribution. Age and sex were compared between the GGE patients and the healthy controls with Student's t-tests or chi-square analyses. The relationships of the various genotypes and alleles with the susceptibilities to GGEs were examined with χ^2 tests. Comparisons of the genotypic and allelic frequencies between the drug-resistant and drug-responsive patients were performed with binary logistic regression after adjusting for age and sex. Linkage disequilibriums and haplotypes were analyzed using Haploview. Bonferroni's method was used to correct for multiple comparisons. Statistical significance was accepted at $p < 0.05$.

3. Results

A total of 484 Chinese GGE patients (296 males, 188 females, age: 18.3 ± 12.1 years) and 284 healthy controls (180 males, 104 females, age: 18.6 ± 12.2 years) were enrolled in our study. The demographic and clinical characteristics of the patients are shown in Table 1. Nonparametric K–S test results showed that the sex in patients and healthy controls were all of normal distribution. No significant differences in sex or age between the groups were observed. Across all of the 484 Chinese GGE patients, the following GGE subtypes were observed: CAE (38.3%), JAE (19.6%), JME (25.6%), and EGTCS (16.5%). Six tagSNPs in *ATP1A2* and two on *ATP1A3* were selected based on a comprehensive study of all of the tagSNPs that were identified across the entire ATP1A2 and ATP1A3 gene regions in the HapMap data as assessed with the Haploview software (Table 2, Fig. 1).

We examined the frequencies of these eight tagSNPs in the 484 Chinese GGE patients and the 284 healthy controls (Table 3). The investigated SNPs were all in Hardy–Weinberg equilibrium in the case and controls groups. The *ATP1A3* rs8107107 allele and genotype were associated with the Chinese GGE population. The frequency of the rs8107107 C allele was significantly lower among the Chinese GGE population than among the healthy controls (11% vs. 15%, OR = 0.807 (0.678–0.960), $p = 0.021$). Moreover, there was a significant difference in the frequencies of the CC genotype between the two groups (0.4% vs. 3.2%, OR = 0.121 (0.026–0.565), $p = 0.002$). However, after correcting for multiple comparisons with Bonferroni's method, only the *ATP1A3* rs8107107 CC genotype was significantly associated with GGE