Journal of the Neurological Sciences xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Absence of association between major vault protein (MVP) gene polymorphisms and drug resistance in Chinese Han patients with partial epilepsy

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ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 13 September 2015
Accepted 22 September 2015
Available online xxxx

Keywords:
Major vault protein
Epilepsy
Drug resistance
Polymorphism
Haplotype
Chinese Han

ABSTRACT

Drug resistance in epilepsy is common despite many antiepileptic drugs (AEDs) available for treatment. The development of drug resistant epilepsy may be a result of multiple factors. Several previous studies reported that the major vault protein (MVP) was significantly increased in epileptogenic brain tissues resected from patients with partial-onset seizures, indicating the possible involvement of MVP in drug resistance. In this article, we aimed to identify the association between single nucleotide polymorphisms (SNPs) of MVP gene and drug resistance of partial epilepsy in a Chinese Han population. A total of 510 patients with partial-onset seizures and 206 healthy controls were recruited. Among the patients, 222 were drug resistant and 288 were responsive. The selection of tagging SNPs was based on the Hapmap database and Haploview software and the genotyping was conducted on the Sequenom MassARRAY iPLEX platform. For the selected loci rs12149746, rs9938630 and rs4788186 in the MVP gene, there was no significant difference in allele or genotype distribution between the drug resistant and responsive groups, or between all of the patients and healthy controls. Linkage disequilibrium between any two loci was detected but there was no significant difference in haplotype frequency between the drug resistant and responsive groups. Our results suggest that MVP genetic polymorphisms and haplotypes may not be associated with drug resistance of partial epilepsy in the Chinese Han population.

1. Introduction

Epilepsy is a common neurological disease with challenging therapeutic strategies. Despite the availability of many antiepileptic drugs (AEDs), about 30% of patients still experience recurrent seizures while taking therapeutic doses of the medications [1]. Previous investigations suggested that a major cause of AED resistance is the efflux of drug molecules by multidrug resistant transporters (MDTs) such as P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP), leading to insufficient drug concentration in the epileptic foci [2].

The major vault protein (MVP) is a vital component and marker of the vault, a large-sized hollow and barrel-shaped ribonucleoprotein existing mainly in the cytoplasm of eucaryotic cells. The vault consists of multiple copies of three proteins including MVP, telomerase-associated protein 1, and poly-(ADP-ribose) polymerase [3]. The MVP/vault may act as a vesicular transporter to play a role similar to the above mentioned MDTs. MVP was initially reported to be involved in chemotherapy resistance of various tumors and was considered as a poor predictor of prognosis [4]. Subsequent studies revealed MVP as a putative marker for drug resistance in partial epilepsy. In the brain resections of patients with drug resistant partial epilepsy, MVP was overexpressed ectopically in the lesional neurons of various etiologies, including hippocampus sclerosis (HS), focal cortical dysplasia (FCD), and dysembryoplastic neuroepithelial tumor [5]. Likewise, MVP upregulation was noted in the capillary endothelium of the HS tissue in chronic temporal lobe epilepsy (TLE) patients [6]. In a recent article on refractory frontal lobe epilepsy, dramatically increased MVP level was observed in the epileptogenic tissues resected from the patients' frontal cortex, and pathological examination revealed FCD, gliosis and neuronal degeneration [7]. Moreover, P-gp, BCRP, and MVP were found to be colocalized in the microvascular endothelium in human epileptogenic brain tissue, suggesting the possibility of MVP acting in concert with other MDTs to induce active efflux of AEDs across the blood brain barrier (BBB), impeding the penetration of AEDs into the target region [8]. However the exact mechanism by which MVP contribute to drug resistance in partial epilepsy is not entirely clear. Studies have found that in some populations the specific single nucleotide polymorphism

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http://dx.doi.org/10.1016/j.jns.2015.09.363
0022-510X/

(SNP) in genes such ABCB1 rs1045642 for P-gp, ABCC2 rs717620 for MRP2 leading to altered cellular function of MDTs may be associated with non-response of AEDs [9,10]. Therefore, to better understand the function of MVP in drug resistance, we here took a genetic approach and investigated the association between MVP gene polymorphism and drug resistance of partial epilepsy in a Chinese Han population.

2. Materials and methods

2.1. Patients and healthy controls

All subjects were unrelated Chinese Han individuals recruited from Xiangya Hospital, Central South University, Changsha, China, from March 2010 to December 2013. A total of 510 patients with partial-onset seizures and 206 healthy controls were enrolled. Patients with drug or alcohol abuse, medication non-compliance, unreliable seizure history, severe systemic or co-morbidities were excluded. The diagnosis and classification of the patients were based on the guidelines proposed by International League Against Epilepsy (ILAE). The seizure types, symptoms, and AED schedules of each patient were valued and provided by the treating neurologists. Drug resistance or unresponsiveness to AEDs was defined as the failure of complete seizure control after at least two well-tolerated and appropriately dosed AEDs (monotherapies or in combination). Drug responsiveness was defined as being completely seizure free for the longer of one year or three times the longest pre-treatment interseizure interval [11]. According to this definition, 222 patients in our sample were drug resistant and 288 were responsive. This study was approved by the Ethics Committee of Xiangya Hospital and all participants signed written informed consent.

2.2. Selection of tagging SNPs and genotyping

Information on SNPs of the MVP gene for the Chinese Han population was obtained from the International HapMap Project (HapMap Data Rel 24/Phase II Nov08, on NCBI B36 assembly, dbSNP b126). Using the Haploview software, pairwise tagging of linkage disequilibrium (LD) with minor allele frequency (MAF) ≥0.05 and an r^2 threshold of 0.80 identified three tagging SNPs, rs12149746, rs9938630 and rs4788186, for analysis. The genomic DNA of each participant was extracted from 5 ml of peripheral whole blood using QIAamp DNA Blood Mini Kit (QIAGEN, Germany). Genotyping was conducted on the Sequenom MassARRAY iPLEX (Sequenom, USA) platform by the matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) following the recommended protocols with measurement of charge-to-mass ratio. Genotyping results were then output to the TYPER 4.0 software.

2.3. Statistical analysis

The statistical analysis was performed by SPSS software (version 21.0) and $P < 0.05$ was considered significant. The binary logistic regression was used for the analysis of relationships among genotype distribution, seizure type, epileptic focus, and frequency. The Pearson chi-square test was used for assessing allele distribution, the Hardy–Weinberg equilibrium, and sex distribution. Demographic differences between two groups, such as current age, age of seizure onset, and duration of illness, were evaluated by the independent samples t-test. Linkage disequilibrium (LD) calculation and haplotype analysis was performed on the SHEsis platform [12]. Bonferroni's method was used for multiple testing correction in genotype and allele frequency analysis [13].

3. Results

3.1. Clinical characteristics

Demographic and clinical characteristics of patients are summarized in Table 1. The age and sex between drug resistant and responsive patients were well-matched. Meanwhile, in healthy controls, there were 116 (56.3%) male and 90 (43.7%) female participants with mean age of 42.87 ± 12.31 years old. The AEDs taken by patients in our study included: carbamazepine, oxcarbazepine, phenytoin, phenobarbital, valproate, lamotrigine, topiramate and levetiracetam. Monotherapy was recommended before polytherapy. In general, there were no significant differences in seizure types, epileptic foci and etiologies between the groups. Compared to the drug responsive patients, the drug

Table 1
Demographic and clinical characteristics of patients.

Variables	Drug resistant n = 222	Drug responsive n = 288	OR(95%CI)	*P*-value
Age(years)	27.42 ± 10.63	26.03 ± 12.79	–	0.193
Sex				
male	150(67.6%)	184(63.9%)	1.178(0.813–1.704)	0.386
female	72(32.4%)	104(36.1%)		
Onset age(years)	15.56 ± 9.77	18.30 ± 12.42	–	0.007
Epilepsy duration(years)	11.74 ± 8.20	7.67 ± 7.71	–	<0.001
Seizure types				
SPS	14(6.3%)	26(9.0%)	reference	–
SPS secondarily GS	48(21.6%)	68(23.6%)	1.311(0.621–2.768)	0.478
CPS	88(39.6%)	75(26.0%)	2.179(1.062–4.472)	0.034
CPS secondarily GS	72(32.4%)	119(41.3%)	1.124(0.551–2.291)	0.748
Epileptic foci in EEG				
temporal lobe	105(47.3%)	126(43.8%)	reference	–
frontal lobe	28(12.6%)	42(14.6%)	0.800(0.464–1.378)	0.421
occipital lobe	26(11.7%)	34(11.8%)	0.918(0.518–1.627)	0.769
parietal lobe	13(5.9%)	14(4.9%)	1.114(0.502–2.475)	0.790
above combinations	50(22.5%)	72(25.0%)	0.833(0.534–1.299)	0.421
Etiology				
idiopathic	167(75.2%)	226(78.5%)	1.201(0.793–1.817)	0.387
symptomatic	55(24.8%)	62(21.5%)		
Seizure frequency before treatment				
daily	33(14.9%)	26(9.0%)	reference	–
>once a week	39(17.6%)	16(5.6%)	1.920(0.884–4.174)	0.099
1–4 times per month	93(41.9%)	103(35.8%)	0.711(0.396–1.278)	0.254
<once a month	49(22.0%)	117(40.6%)	0.330(0.179–0.609)	<0.001
<once half a year	8(3.6%)	26(9.0%)	0.242(0.094–0.623)	0.003

OR, odds ratio; 95%CI, 95% confidence interval; SPS/CPS = simple/complex partial seizures; GS = generalized seizures.

Please cite this article as: L. Zhou, et al., Absence of association between major vault protein (MVP) gene polymorphisms and drug resistance in Chinese Han patients with parti..., J Neurol Sci (2015), http://dx.doi.org/10.1016/j.jns.2015.09.363