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Major vault protein (MVP) *gene* polymorphisms and drug resistance in mesial temporal lobe epilepsy with hippocampal sclerosis

Shabeesh Balan [a,b], Sarada lekshmi [b], Koramannil Radha [b], Sanish Sathyan [b], Joseph Vijai [c], Moinak Banerjee [b], Kurupath Radhakrishnan [a,*]

[a] R. Madhavan Nayar Center for Comprehensive Epilepsy Care, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala, India
[b] Human Molecular Genetics Laboratory, Rajiv Gandhi Center for Biotechnology, Trivandrum, Kerala, India
[c] Department of Medicine, and Cancer Biology and Genetics, Memorial-Sloan Kettering Cancer Center, NY, USA

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ABSTRACT

The human major vault protein (MVP) has been implicated in the development of drug resistance in cancer cells. Over expression of MVP has also been reported in brain tissue samples from antiepileptic drug (AED)-resistant human focal epilepsies. To investigate the relationship between single nucleotide polymorphisms (SNPs) involving the *MVP gene* and AED-resistance, we compared the distribution of three SNPs in the *MVP gene*, rs4788187, rs3815824 and rs3815823, among 220 patients with mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) (prototype of AED-resistant epilepsy syndrome), 201 patients with juvenile myoclonic epilepsy (JME) (prototype of AED-responsive epilepsy syndrome) and 213 ethnically matched non-epilepsy controls. All the patients and controls were residents of the South Indian state of Kerala for more than three generations. We did not find any significant difference in allele and genotypic frequencies of the studied SNPs between AED-resistant and AED-responsive cohorts, and between AED-resistant and AED-responsive cohorts independently and pooled together when compared with the controls. We conclude that rs4788187, rs3815824, rs3815823 variants of the *MVP gene* are associated neither with predisposition for epilepsy nor with AED-resistance in the population that we have studied. Our results suggest the need for further research into the link between MVP and AED-resistance.

1. Introduction

Despite optimal antiepileptic drug (AED) therapy, one-third of patients with epilepsy continue to exhibit recurrent seizures (Brodie et al., 2012; Kwan et al., 2010). Underlying mechanisms of AED-resistance are not well understood, but have been attributed to enhanced activity of drug metabolizing enzymes, over expression of the drug efflux protein transporters, and insensitive drug targets (Löscher et al., 2009; Schmidt and Löscher, 2005). A patient, whose seizures are unresponsive to one or two AEDs, is likely to be resistant to a broad range of structurally and functionally unrelated AEDs, termed multi-drug resistance (Löscher et al., 2009; Schmidt and Löscher, 2005; Sisodiya et al., 2002). Until recently, a majority of pharmacogenetic studies on multi-drug resistance in epilepsy have focused on ATP-binding cassette (ABC) transporter proteins at the blood–brain-barrier such as P-glycoprotein (P-gp) and MDR-associated protein 1, and their genes *ABCB1* and *ABCC1*, respectively (Löscher et al., 2009; Schmidt and Löscher, 2005; Sisodiya et al., 2002).

Recent studies have associated an intracellular organelle called vaults (named so because of their resemblance to vaulted ceilings in cathedrals) with multidrug resistance in cancer cells (Gopinath et al., 2005; Kitazono et al., 1999; Mossink et al., 2003; Scheffer et al., 1995). The vault is a 13-megadalton barrel-shaped ribonucleoprotein complex, the predominant mass of which is attributed to 110 kDa major vault protein (MVP) (van Zon et al., 2003). Vaults are localized mainly in the cytoplasm, but a small fraction also resides at the nuclear membrane and the nuclear pore complex (Chugani et al., 1993; Mossink et al., 2003). The remarkable conservation of vaults and their ubiquitous presence in phylogenetically diverse species including vertebrates, echinoderms and protozoa suggest a universal role in cellular activities. Although the exact function of vaults remains unknown, several lines of evidence indicate that they are involved in intracellular vesicular and bidirectional nucleo-cytoplasmic transports (Chugani et al., 1993; Mossink et al., 2003).

Over expression of MVP has been reported in brain tissue samples from rat model of drug resistant temporal lobe epilepsy (TLE) (van Vliert et al., 2004) and from AED-resistant human mesial TLE with hippocampal sclerosis (MTLE-HS) (Sisodiya et al., 2003), frontal lobe

Abbreviations: AED, Anti-epileptic drug; ABC, ATP-binding cassette; MVP, Major vault protein; MTLE-HS, Mesial temporal lobe epilepsy with hippocampal sclerosis; JME, Juvenile myoclonic epilepsy; ATL, Anterior temporal lobectomy; EEG, Electroencephalogram; SNP, Single nucleotide polymorphism; LD, Linkage disequilibrium.

* Corresponding author at: Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum—695 011, Kerala, India. Tel.: +91 471 2446069; fax: +91 471 2446433.
E-mail address: krk@sctimst.ac.in (K. Radhakrishnan).

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epilepsy (Liu et al., 2011), and focal epilepsies due to ganglioglioma (Aronica et al., 2003) and dysembryoplastic neuroepithelial tumor (Sisodiya et al., 2003). The human *MVP gene*, located at chromosome 16p11.2, is 27.6 kb in size and has 15 exons (Lara et al., 2011). We hypothesized that single nucleotide polymorphisms (SNPs) in the *MVP gene* could contribute to AED-resistance. To test this hypothesis, we compared the distribution of three SNPs in the *MVP gene* namely rs4788187, rs3815824 and rs3815823 (Fig. 1) among three cohorts of subjects with South Indian ancestry: MTLE-HS (prototype of AED-resistant epilepsy syndrome), juvenile myoclonic epilepsy (JME) (prototype of AED-responsive epilepsy syndrome) and ethnically matched non-epilepsy controls.

2. Materials and methods

2.1. Study cohorts

2.1.1. AED-resistant cohort

We defined AED-resistant patients as those who were unresponsive to at least two monotherapy and one duotherapy trials, each of ≥6 month duration and had seizure frequency ≥12 per year for ≥2 years. The patients with MTLE-HS are a prototype of AED-resistant focal epilepsy. To conform to this definition, we recruited 220 subjects who had pathologically verified HS after anterior temporal lobectomy (ATL), had no other lesions on magnetic response imaging and were seizure-free for at least one year following ATL. These patients had seizures for a mean duration of 18.7 (range 6 to 50) years, and had failed at least two monotherapy and two polytherapy AED trials prior to ATL. All of them had received at least two of the old AEDs (phenobarbital, phenytoin, carbamazepine and valproate), and half of them, in addition, had received at least one of the new AEDs (clobazam, lamotrigine, oxcarbazepine, topiramate and Levetiracetam). We have described our protocols for presurgical evaluation, selection for ATL and post-ATL follow-up in detail elsewhere (Ramesha et al., 2011; Rathore et al., 2011).

2.1.2. AED-responsive cohort

We defined AED-responsive patients as those who were free of seizures for ≥1 year on AED therapy. The patients with juvenile myoclonic epilepsy (JME) are considered as a prototype of AED-responsive epilepsy. We recruited 201 patients with JME, who conformed to the following diagnostic criteria (Grünewald and Panayiotopulas, 1993; Vijai et al., 2003): 1) bilateral myoclonic seizures involving the upper extremities, with age at onset between 8 and 25 years, occurring after awakening and without loss of conscious with or without additional generalized tonic–clonic seizures and/or absence seizures; 2) otherwise normal neurological status and intelligence; and 3) normal background activity and paroxysmal generalized spike and wave discharges in the electroencephalogram (EEG). Abnormal EEG was utilized to support the diagnosis of JME, but was not mandatory for the diagnosis. The clinical and EEG characteristics of 183 of these JME patients have been published in detail elsewhere (Vijai et al., 2003).

2.1.3. Control subjects

We randomly selected 213 subjects from the general population, who did not have personal or family history of epilepsy or any other neurological disorders as non-epilepsy control cohort. All the MTLE-HS and JME patients and controls were residents of Kerala for more than three generations.

All of the patients and controls gave informed written consent to participate in the study, which was approved by the Institutional Ethics Committee.

Table 1
Clinical characteristics of patients and controls.

Variables	AED-resistant cohort (n = 220)	AED-responsive cohort (n = 201)	Controls (n = 213)
Male, n (%)	122 (55.5)	104 (51.7)	112 (52.6)
Age, mean ± SD, year	28.73 ± 8.8	24.4 ± 7.2	37.4 ± 5.4
Age of first unprovoked seizure, mean ± SD, year	10.3 ± 7.2	14.6 ± 3.6	–
Duration of epilepsy, mean ± SD, year	18.7 ± 9.3	15.1 ± 5.2	–

AED—antiepileptic drug; SD—standard deviation.

2.2. Genotyping

The SNPs for genotyping were selected based on the tagging status that could capture maximum variation. The SNPs rs4788187 and rs3815824 were identified using genotype data obtained from Caucasian individuals in the HapMap project (HapMap Data Rel 24/Phase II Nov08, on NCBI B36 assembly, dbSNP b126) on the basis of pairwise linkage disequilibrium (LD) with a r^2 threshold of 0.8 and minor allele frequency ≥0.05 to capture all the common SNPs (Supplementary Table 1). When compared to other populations, rs4788187 and rs3815824 shows strong LD among Caucasians throughout the gene (Supplementary Figure). We also selected an additional functional SNP rs3815823, with an amino acid change from arginine to glutamine at 766th position, for genotyping. We performed genotyping of the selected SNPs by fluorescence-based competitive allele-specific polymerase chain reaction (PCR) (KASPar) chemistry (KBiosciences, UK). The reaction comprised of 8 μl with 5 ng of DNA, 0.11 μl of assay mix and 4 μl of reaction Mix and the PCR was performed in ABI 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA). The cycling conditions were as follows: 94 °C for 15 min (Hot-start enzyme activation), 94 °C for 20 s, a touchdown step for 10 cycles over 65–57 °C for 60 s (dropping 0.8 °C per cycle), and a final 26 step cycle with 94 °C for 20 s and 57 °C for 60 s. Further, the genotype calling based on the respective allele specific fluorescence was done by allelic discrimination utility of the SDS 7500 v2.0.5 software at an ambient temperature of 25 °C and genotype clusters were plotted.

2.3. Statistical analysis

Genotype and allelic frequencies were computed and were checked for deviation from Hardy–Weinberg equilibrium (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). We performed case–control genetic comparisons using the chi-square test and allelic odds ratios (OR), and 95% confidence intervals (CI) were calculated by Fisher's exact test (two-tailed). All statistical analyses were performed using the

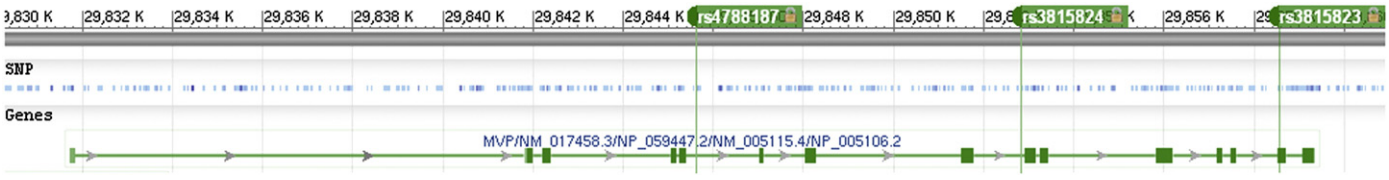

Fig. 1. Schematic diagram showing location of selected SNPs in *MVP gene*.
Source: dbSNP, NCBI Graphical Sequence Viewer version 2.21.