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LETTER TO THE EDITOR

# Novel Susceptibility Loci were Found in Chinese Genetic Generalized Epileptic Patients by Genome-wide Association Study

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Genetic generalized epilepsies (GGEs), also known as idiopathic generalized epilepsies, were characterized by recurring generalized seizures in the absence of detectable brain lesion [1]. Recently, a study using genome-wide association analysis (GWAS) technology identified five new susceptibility loci which may be associated with GGEs [2]. In their study, a two-stage GWAS research was conducted in 3020 GGEs patients and 3954 controls of Europe ancestry. In the end, they got the conclusion that there is significant association for GGEs at 2p16.1 (rs13026414) and 17q21.32 (rs72823592), and suggestive association at 2q24.3 (rs11890028). What's more, they also found significant evidence for association with GAEs at 2q22.3 (rs10496964) and for JME at 1q43 (rs12059546) [2]. High-ranking candidate genes of the above five loci are *CHRM3*, *VRK2*, *ZEB2*, *SCN1A*, and *PNPO*.

The previous studies on the genetics of GGEs mainly concentrate on ion channel genes [3]. However, there are increasing interests in the role of novel nonion channel genes that causing GGEs [4]. The GWAS mentioned before is one of the studies that focusing on novel common susceptibility genes of GGEs; yet, independent replications were needed. Our study is just to verify whether these five loci are associated with GGEs in Chinese population. Furthermore, we aimed to investigate whether these SNPs play a role in the efficacy of antiepileptic drugs (AEDs).

Our study includes 484 Chinese epileptic patients treated with AEDs (298males and 186 female, mean age: 18.3 ± 12.1 years) of Xiangya hospital of Central South University. [2]. In total, 284 age-sex matched healthy controls (184 males and 100 female, mean age: 18.6 ± 12.2 years) were enrolled from volunteers. Diagnosis and classification of epilepsy was made according to the International League against Epilepsy guidelines [5]. Drug resistant is defined as at least four seizures occurred during a year with the treatment of three or more AEDs at the maximum tolerated dosage [6]. While the patients who were free of any type of seizures for a minimum of one year are considered to be drug responsive [7].

Genotyping of the five SNPs was performed using MALDI-TOF-MS. Statistical analysis was conducted using SPSS (Version 13.0 for Windows; SPSS, Chicago, IL, USA). Hardy–Weinberg equilibrium was tested for each SNP in the studied samples. We compared the allele and genotype frequencies between patients and controls using chi-square test, the same as the allele and genotype frequencies between responders and nonresponders. Statistical significance was accepted when $P < 0.05$.

The GGEs subgroups comprised 185(38.4%) childhood absence epilepsy (CAE), 95(19.4%) juvenile absence epilepsy (JAE), 124 (25.6%) juvenile myoclonic epilepsy (JME), and 80(16.5%) generalized tonic-clonic seizures (EGTCS). The study patients taken all kinds of AEDs, among them carbamazepine, valproic acid, and oxcarbazepine were the most widely used drugs. The genotype distributions of the five SNPs were all consistent with Hardy–Weinberg equilibrium both in GGEs patients and controls. There was no significant difference in alleles or genotypes frequencies of the five SNPs between GGEs patients and controls (Table 1). In

**Table 1** Allele and genotype frequencies of 5 SNPs in GGEs patients (n = 484) and healthy controls (n = 284)

| SNP | Genotype/allele | GGEs n (%) | Controls n (%) | Crude *P* | Additive *P* | Additive OR(95%) CI | Dominant *P* | Dominant OR (95%) CI | Recessive *P* | Recessive OR (95%) CI |
|---|---|---|---|---|---|---|---|---|---|---|
| rs13026414 | TT | 7.7 | 5.3 | 0.44 | 0.199 | 1.51 (0.81–2.83) | 0.516 | 1.11 (0.82–1.49) | 0.207 | 1.22 (0.90–1.66) |
| | CT | 32.8 | 32.9 | | | | | | | |
| | CC | 59.5 | 61.8 | | | | | | | |
| | T | 24.1 | 21.7 | 0.286 | | | | | | |
| | C | 75.9 | 78.3 | | | | | | | |
| rs72823592 | AA | 0.4 | 0 | 0.41 | NA | NA | 0.242 | 3.55 (0.43–29.66) | NA | NA |
| | AG | 0.8 | 0.4 | | | | | | | |
| | GG | 98.8 | 99.6 | | | | | | | |
| | A | 0.8 | 0.2 | 0.107 | | | | | | |
| | G | 99.2 | 99.8 | | | | | | | |
| rs10496964 | TT | 1.5 | 2.8 | 0.4 | 0.188 | 0.50 (0.18–1.40) | 0.586 | 0.92 (0.66–1.26) | 0.195 | 0.71 (0.43–1.19) |
| | CT | 27.3 | 27.8 | | | | | | | |
| | CC | 71.2 | 69.4 | | | | | | | |
| | T | 15.1 | 16.7 | 0.402 | | | | | | |
| | C | 84.9 | 83.3 | | | | | | | |
| rs11890028 | GG | 1.7 | 2.1 | 0.77 | 0.624 | 0.77 (0.26–2.24) | 0.506 | 0.89 (0.64–1.25) | 0.655 | 0.89 (0.52–1.51) |
| | GT | 22.2 | 23.9 | | | | | | | |
| | TT | 76.1 | 74 | | | | | | | |
| | G | 12.8 | 14.1 | 0.469 | | | | | | |
| | T | 87.2 | 85.9 | | | | | | | |
| rs12059546 | GG | 11 | 10.9 | 0.41 | 0.781 | 0.93 (0.57–1.53) | 0.176 | 0.82 (0.61–1.09) | 0.944 | 0.99 (0.78–1.25) |
| | AG | 39.6 | 44.4 | | | | | | | |
| | AA | 49.4 | 44.7 | | | | | | | |
| | G | 31 | 33.1 | 0.364 | | | | | | |
| | A | 69 | 66.9 | | | | | | | |

OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism; GGEs, Genetic generalized epilepsies.

addition, we also analyzed allele and genotype frequencies of 5 SNPs in GGEs subtypes (each GGEs subtype and GAEs) and healthy controls, no significant difference was found.

We examined the difference of the 5 SNPs' allele and genotype frequencies between drug-responsive patients and drug-resistant patients, respectively (Table 2). However, there seems to be no significant difference. Interestingly, we found that rs11890028 allele and genotype frequency was significantly different between drug-responsive patients and drug-resistant patients both in JAE group ($P = 0.002$, $P = 0.005$) and JME group ($P = 0.005$, $P = 0.021$).

Genome-wide association analysis is being utilized as a powerful technique to search genetic factors that contribute to the cause of common epilepsy syndromes. To date, there are limited GWAS studies that performed in epilepsy patients, even less in GGEs patients specifically [8]. The first GWAS study of GGEs patients that come from Europe was conducted in 2012, and five susceptible loci were found [2].

In this study, we aimed to test their finding in Chinese GGEs patients, and thus to evaluate whether these five loci are also associate with the vulnerability to GGEs in Chinese population. We genotyped these five SNPs in 484 GGEs patients and 284 healthy

**Table 2** Allele and genotype frequencies of five SNPs in drug responsive GGEs patients (n = 280) and drug resistant GGEs patients (n = 204)

| SNPs | Alleles A/B | Genotypes: Drug responsive (n = 280) AA | AB | BB | Drug resistant (n = 204) AA | AB | BB | *P*-value | Alleles: Drug responsive A:B / Drug resistant A:B | OR (95% CI) | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| rs13026414 | C/T | 164 | 90 | 24 | 122 | 68 | 13 | 0.66 | 418/138;312/94 | 0.91 (0.68–1.23) | 0.55 |
| rs72823592 | A/G | 2 | 1 | 277 | 0 | 3 | 201 | 0.20 | 5/555;3/405 | 1.22 (0.29–5.12) | 0.79 |
| rs10496964 | C/T | 201 | 75 | 3 | 143 | 57 | 4 | 0.69 | 477/81;343/65 | 1.12 (0.78–1.59) | 0.54 |
| rs11890028 | G/T | 5 | 68 | 205 | 3 | 39 | 161 | 0.37 | 78/478;45/361 | 1.31 (0.88–1.94) | 0.18 |
| rs12059546 | A/G | 138 | 112 | 27 | 99 | 78 | 26 | 0.57 | 388/166;276/130 | 1.10 (0.83–1.45) | 0.50 |

controls, and none of these SNPs were found to be related with the occurrence of GGEs. Furthermore, we investigated the association between that five SNPs and AEDs efficacy in 280 drug-responsive patients and 204 drug-resistant patients, but no statistically significant association was found. However, we found that rs11890028 allele and genotype frequency was significantly different between drug-responsive patients and drug-resistant patients both in JAE group and JME group. The polymorphism rs11890028 located nearby the *SCN1A* gene, as *SCN1A* encodes the large alpha subunit of sodium channel which is the target of many AEDs, for example, valproic acid, and carbamazepine. So this polymorphism may influence the efficacy of antiepileptic drugs. However, because of the small sample size of JAE (95 subjects) and JME (124 subjects) subgroup, the clinical significance of this result should be verified later.

Steffen M's GWAS was conducted in European population, and our study was a replicate study performed in Chinese population, because of the ethnic variance, so, we may get the negative results. Epilepsy can be induced by various factors including genetic ones and environmental ones. And a single nucleotide polymorphism can't explain the whole aspects of the cause of epilepsy [9]. Besides, the complexity in the phenotype of epilepsy also accounts [10]. Thus, we were trying to find more accuracy method to define the phenotypes of epilepsy. To elucidate the inner connection between GGEs subtypes and genetic factor, we analyzed the subtypes of GGEs, but no significant association was found between GGEs subtypes susceptibility and the five SNPs. However, as mentioned before our study sample size is relative small, especially for the GGEs subtype patients, this is also a reason why we have not replicated their GWAS results.

To date, the genesis of epilepsy hadn't been elucidated well yet, and it is caused by polygenic-environment interactions. Thus, the real causes of epilepsy in certain patient remain unknown. Moreover, the evaluation criteria of AEDs efficacy is not normalized and unified. And the relative small sample size also accounts for. Besides, the GWAS linkage data do not resolve to individual genes, but rather large genomic regions. All of the above reasons limited the results of most association researches. In conclusion, our data showed that rs13026414, rs72823592, rs11890028, rs10496964, and rs12059546 found by a European population based GWAS study are not associated with the susceptibility of GGEs in Chinese population. However, in considering of the limited sample size of our study, further studies on association identification at large sample size are needed.

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## Conflict of Interest

The authors declare no conflict of interest.

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