Gene-wide tagging study of the effects of common genetic polymorphisms in the α subunits of the $GABA_A$ receptor on epilepsy treatment response

Aim: We aimed to identify the effect of SNPs in the α-subunits of $GABA_A$ receptors on epilepsy treatment outcomes by using a gene-wide tagging method. **Materials & methods:** There were 720 epileptic patients included in the present study. A total of 136 tagging SNPs in *GABRA1*, *GABRA2*, *GABRA3*, *GABRA4*, *GABRA5* and *GABRA6* were genotyped by Illumina® GoldenGate® Genotyping platform. Clinical information, such as prescribed antiepileptic drugs, height, weight, epilepsy syndrome classification, etiology, number of attacks, renal function and liver function were collected. The associations between SNPs and epilepsy treatment outcomes were analyzed using SAS® version 9.1.3. Both multivariate logistic regression and multifactor dimensionality reduction analyses were performed. **Results:** The results of single gene effects did not remain significant after Bonferroni's corrections. Further multivariate logistic regression and multifactor dimensionality reduction analyses of interactions between these genes showed that under adjustment of clinical factors, the epilepsy treatment outcomes were significantly associated with the genotype combinations of *GABRA1* rs6883877, *GABRA2* rs511310 and *GABRA3* rs4828696 ($p < 0.0001$; adjusted $r^2 = 0.149$). **Conclusion:** Our results indicated that genetic variants in the α subunits of $GABA_A$ receptors may interactively affect the treatment responses of antiepileptic drugs. Further replication using an independent sample collection would be essential to confirm our findings.

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KEYWORDS: epilepsy ■ $GABA_A$ receptors ■ gene-wide tagging study ■ treatment outcomes

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Epilepsy, one of the most common neurological disorders, is characterized by highly heterogeneous etiologies, syndromes and treatments [1,2]. Distinct epileptic phenotypes are considered to develop from dysfunctions of various voltage-gated sodium, potassium, calcium and chloride channels, and also of ligand-gated ion channels, nicotinic acetylcholine receptors and GABA receptors [3,4]. GABA is the principal inhibitory neurotransmitter in the CNS, and alteration of GABAergic function can overexcite neurons and induce seizures.

Humans express three types of GABA receptors, the ionotropic $GABA_A$ receptor, the metabotropic $GABA_B$ receptor and the $GABA_C$ receptor, among which the $GABA_A$ receptor has been reported to be relevant to epilepsy [5]. $GABA_A$ receptors form pentameric chloride channels composed of various combinations of eight subunits (α, β, γ, δ, ε, θ, π and ρ) and each of these subunits has several subtypes [6]. There are six subtypes of the $GABA_A$ receptor α subunits, α1–α6, which are encoded by the genes *GABRA1* to *GABRA6*. The α1 subtype is widely expressed in the whole brain, while the α2, α3, α4, α5 and α6 subtypes are expressed in specific brain areas [7]. Most of the $GABA_A$ receptors in the human brain are composed of two α subunits, two β subunits and one γ subunit [7]. The α1, β2 and γ2 subunits are expressed most abundantly in the human brain [8], and variants of the genes encoding these subunits may thus influence ion channel gating, expression and GABA receptor trafficking to the cell surface. The *GABRA1* and *GABRA6* genes are located in chromosome 5, whereas *GABRA2* and *GABRA3* genes are located in chromosome 4. The *GABRA4* and *GABRA5* genes are located in chromosome X and 15, respectively [9]. These genes have been proposed to influence certain drug targets and affect the regulation of neuronal activities in the brain [10]. Several antiepileptic drugs (AEDs), such as phenobarbital, gabapentin and topiramate, bind to $GABA_A$ receptors, and alterations in $GABA_A$ receptor subunits may regulate the responses elicited by the AEDs [11].

AEDs may react with $GABA_A$ receptors composed of distinct α subunits in diverse manners. For example, phenytoin and carbamazepine were reported to increase chloride influx through $GABA_A$ receptors composed of the α1 subunit,

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whereas these AEDs had no effect on $GABA_A$ receptors composed of α3 and α5 subunits [12]. Benzodiazepines, used under status epilepticus, showed extremely low affinity toward $GABA_A$ receptors composed of α4 and α6 subunits [13]. Topiramate also appeared to affect $GABA_A$ receptors in a manner that depended on the composition of the α subunits, enhancing the inhibitory current of $GABA_A$ receptors composed of α1, α2 or α5 subunits, but reducing the inhibitory current of $GABA_A$ receptors composed of α6 subunits [14]. Therefore, the composition and function of α subunits may affect the treatment efficacy of AEDs.

How epilepsy is affected by genetic polymorphisms of *GABRG2*, the gene that encodes the γ2 subunit of the $GABA_A$ receptor, has been studied extensively. Exonic *GABRG2* 588C>T (rs211037), a synonymous SNP, was reported to be associated with febrile seizure in the Taiwanese population [15]. However, a follow-up study did not support such an association in a population of European ancestry [16]. Results from other studies also failed to demonstrate marked effects of certain common SNPs in *GABRG2* on febrile seizures [17], childhood absence seizures [18,19] or infantile myoclonic epilepsy [20]. A recent study using the tagging SNP method to represent all common SNPs across *GABRG2,* also failed to show a strong association of the SNPs with the development of common, complex forms of epilepsy [21].

How SNPs in the α subunits of $GABA_A$ receptors affect epilepsy has also been studied. The intronic *GABRA1* IVS11+15A>G (rs2279020) polymorphism was shown to be associated with a susceptibility to febrile seizures [21,22], and with the development of alcoholism and substance abuse disorders [23,24]. The same polymorphism was also reported to be associated with a drug-resistant form of epilepsy in a North Indian population [25]. Moreover, the *GABRA6* c.1512 T>C (rs3219151) polymorphism was shown to be associated with susceptibility to epilepsy (although not with drug resistance) in North Indian epilepsy patients [26]. These studies focused on individual variants separately and, therefore, their results may not represent variations across the entire gene or in the genomic region.

To elucidate the roles of SNPs in the α subunits of $GABA_A$ receptors in epilepsy, a comprehensive investigation on tagging SNPs, and haplotype and gene–gene interaction analyses in the six subtypes of the α subunits (α1–6) were conducted in the present study.

Materials & methods

Subjects

The study protocol was approved by the Ethics Committee of the National Taiwan University Hospital. Written informed consents were provided and signed by enrolled subjects. EEG and MRI brain scans were performed in all patients. The classifications of epilepsies and epileptic syndromes were identified by experienced neurologists according to the guidelines of International League Against Epilepsy (ILAE) 1989 [27]. In this study, the definitions of 'seizure freedom' and 'drug resistance' were in line with the recent recommendations from ILAE [28]. Seizure freedom was defined as freedom from seizures for a minimum of three-times the longest preintervention interseizure interval (determined from seizures occurring within the past 12 months) or 12 months, whichever was longer [28]. Drug resistance was defined as the failure to achieve sustained seizure freedom with at least two appropriately prescribed AEDs under maximum tolerated doses, according to the ILAE recent recommendations [28]. Clinical information was recorded for each patient, including gender, age, age of onset, weight (kg), epilepsy classification, etiology, epilepsy syndrome, and doses and concentrations of prescribed AEDs. The patient compliance to the medication was checked by counting the remaining pills in the drug bag. All of the recruited patients achieved over 95% compliance. The ethnicity of all patients was Han Chinese according to the patients' self-reports.

Genotyping

Genomic DNA was isolated from a peripheral blood sample using a QIAamp DNA Mini Kit (Qiagen, MD, USA). Genotyping was performed using Illumina® (CA, USA) GoldenGate® Genotyping system at the National Taiwan University Biotechnology Research Center. The whole genes of *GABRA1*, *GABRA2*, *GABRA3*, *GABRA4*, *GABRA5* and *GABRA6* were selected and we also included 10 kb upstream and downstream on the HapMap Genome Browser [101]. Chinese Beijing and Japanese Tokyo SNP genotype data from HapMap Data release #27 (phase 1-, 2- and 3-merged genotypes and frequencies) were downloaded. The tagging SNPs were identified by the Tagger function of the Haploview program [102]. Aggressive tagging was selected given that the minimum minor allele frequency was set at 5% and the r^2 threshold was set at 0.8. The details of the included tagging SNPs are listed in Supplementary Table 1 (see www.futuremedicine.com/doi/suppl/10.2217/pgs.13.158).

Statistical analysis

Pairwise comparisons between the two groups of subjects who were drug-resistant or drug-responsive were conducted using Pearson's χ^2 test, Fisher's exact test and odds ratio for the alleles, genotypes, haplotypes and haplotype combinations. Compliance with Hardy–Weinberg equilibrium was checked before performing pairwise comparisons. Identification of haplotypes was performed using the expectation maximization algorithm [29]. The standardized linkage disequilibrium coefficients were calculated for measurement of the linkage disequilibrium among the investigated loci [30,31]. A p-value less than 0.05 was considered to indicate statistical significance. Multiple comparisons were corrected using Bonferroni's method.

The multivariate logistic regression model was used to identify the overall effect of the potential SNPs on the epilepsy treatment outcome. Only those SNPs that had $p < 0.05$ in the single gene analysis were included in the model. Seven genetic variants (rs6883877, rs1157122, rs6892782, rs511310, rs10068980, rs4828696 and rs1112122), and their interactions were included in the multivariate logistic regression model for assessing whether synergistic effects existed among these genotypes, with the adjustment for liver function tests, gender, height, weight and etiology. Dummy variables were defined for etiological variables and epilepsy syndromes. For the etiological variables, mesial temporal sclerosis (MTS) was divided into two groups, MTS_1 and MTS_2. For MTS_1, coding 1 denoted the etiology was MTS and 0 denoted other etiologies. For MTS_2, coding 1 denoted the etiology was non-MTS and 0 denoted other etiologies. As for epilepsy syndromes, coding 1 denoted temporal lobe epilepsy (TLE) and 0 for other etiologies. The model selection procedure based on Akaike Information Criterion and backward elimination was performed [32]. The analysis was implemented without making any assumption for genetic effects (dummy variables were used by setting TC as 1 and others as 0 for rs6883877_1, and TT as 1 and others as 0 for rs6883877_2). All data analyses were performed using SAS® version 9.1.3 (SAS Inc., NC, USA). The SNP–SNP interactions were examined by multifactor dimensionality reduction software [33].

Results

A total of 720 patients with epilepsy (382 men and 338 women) aged 40.23 ± 10.80 (mean ± standard deviation) were included. Among these patients, 349 subjects (48.2%) were seizure freedom patients and 371 subjects were drug-resistant epilepsies according to the definition of the present study. The etiologies of epilepsy were classified as MTS, non-MTS and unidentified. The proportion of MTS was higher in the drug-resistant epilepsies (15.8%) versus the seizure freedom patients (5.4%), while the proportion of unidentified was slightly higher in the seizure freedom patients (63.9%) versus the drug-resistant epilepsies (48.3%). As for the epilepsy syndromes, the proportion of TLE was higher in the drug-resistant epilepsies (82.6%) versus the seizure freedom patients (66.2%). Among the prescribed AEDs, carbamazepine, phenytoin and valproic acid were the most commonly used drugs in both patient groups (Table 1).

A total of 136 tagging SNPs were successfully genotyped and the genotype distributions of all included tagging SNPs were consistent with

Table 1. Antiepileptic drugs used in the included epilepsy patients.

Drugs	Seizure freedom patients (n = 349)			Drug-resistant patients (n = 371)		
	n	*Dosage, mg (mean ± SD)*	*Concentration, mg/l (mean ± SD)*	*n*	*Dosage, mg (mean ± SD)*	*Concentration, mg/l (mean ± SD)*
Carbamazepine	121	856.2 ± 290.2	8.5 ± 1.5	113	1015.6 ± 250.5	8.9 ± 2.3
Phenytoin	133	384.4 ± 70.4	14.9 ± 3.1	153	405.5 ± 55.5	15.6 ± 3.5
Valproate	88	1050.6 ± 420.6	70.6 ± 15.9	117	1550.8 ± 350.5	78.4 ± 15.5
Lamotrigine	40	310.6 ± 125.2	–	115	335.7 ± 105.7	–
Topiramate	26	225.2 ± 60.1	–	113	255.8 ± 85.5	–
Gabapentin	24	1885.4 ± 650.9	–	81	2205.5 ± 527.3	–
Oxcarbazepine	44	880.5 ± 325.0	–	103	1328.3 ± 326.2	–

For seizure freedom patients, the number of currently used drugs are: only one drug = 234; two combined drugs = 98; three combined drugs = 17; four combined drugs = 0.
For drug-resistant patients, the number of currently used drugs are: only one drug = 0; two combined drugs = 149; three combined drugs = 168; four combined drugs = 54.
–: Concentration not measured.

the Hardy–Weinberg equilibrium in both seizure freedom and drug-resistant patients. The details of genotype frequencies of all included tagging SNPs are listed in the Supplementary Table 2. In the single gene analysis, only rs6883877, rs1157122, rs6892782 and rs10068980 in *GABRA1*, rs511310 in *GABRA2,* and rs4828696 and rs1112122 in *GABRA3* demonstrated a significant difference in genotype distribution between seizure freedom and drug-resistant patients (unadjusted p-value <0.05). However, these associations did not remain significant after Bonferroni corrections (Table 2). Further haplotype analysis showed that haplotypes composed of rs6883877, rs1157122, rs6892782 and rs10068980 in *GABRA1*, and rs4828696 and rs1112122 in *GABRA3* demonstrated a significant distribution difference between seizure freedom and drug-resistant patients (unadjusted p-value <0.05). However, further *post hoc* analysis of haplotypes did not remain significant after Bonferroni corrections (Table 3).

Logistic regression analysis was applied to evaluate the combined effect of rs6883877, rs1157122, rs6892782 and rs10068980 in *GABRA1*, rs511310 in *GABRA2,* and rs4828696

Table 2. Genotype frequencies of tagging SNPs in seizure freedom and drug-resistant patients (only listed SNPs of p < 0.05).

Genotypes	Seizure freedom patients (n = 349)		Drug-resistant patients (n = 371)		p-value
	n	%	*n*	%	
GABRA1 *rs6883877*†					
CC	262	75.3	312	83.9	0.01
TC	81	23.3	57	15.3	
TT	5	1.4	3	0.8	
GABRA1 *rs1157122*†					
CC	38	10.9	22	5.9	0.02
TC	147	42.1	147	39.4	
TT	164	47.0	204	54.7	
GABRA1 *rs6892782*†					
CC	45	12.9	28	7.5	0.01
TC	154	44.3	156	41.8	
TT	149	42.8	189	50.7	
GABRA2 *rs511310*†					
AA	19	5.5	8	2.2	0.01
AG	115	33.1	103	27.8	
GG	213	61.4	259	70.0	
GABRA1 *rs10068980*†					
AA	58	16.7	37	10.0	0.02
AG	159	45.7	186	50.0	
GG	131	37.6	149	40.0	
GABRA3 *rs4828696*†					
CC	65	18.6	54	14.6	0.01
TC	71	20.4	51	13.8	
TT	213	61.0	264	71.6	
GABRA3 *rs1112122*†					
GG	78	22.5	69	18.7	0.04
TG	82	23.7	68	18.4	
TT	186	53.8	232	62.9	

†*p < 0.05 as compared between seizure freedom and drug-resistant patients.*

Table 3. Frequencies of haplotypes of *GABRA1* and *GABRA3* genes in seizure freedom and drug-resistant patients.

Haplotypes	Seizure freedom patients (n = 349)		Drug-resistant patients (n = 371)		p-value
	n	%	*n*	%	
GABRA1 *rs6883877 C>T–rs1157122 C>T–rs6892782 C>T–rs10068980 A>G*					
CCCA	130	18.62	127	17.02	0.0230†
CTTA	53	7.59	69	9.25	
CTTG	391	56.02	458	61.39	
TCCA	84	12.03	57	7.64	
Other‡	40	5.73	35	4.69	
GABRA3 *rs4828696 C>T–rs1112122 G>T*					
CG	183	26.22	151	20.35	0.0108†
CT	18	2.58	11	1.48	
TG	59	8.45	56	7.55	
TT	438	62.75	524	70.62	

†Overall p-value of ANOVA test between seizure freedom and drug-resistant patients; none of the haplotypes showed significance after post hoc *analysis.*
‡The other nine haplotypes with frequencies less than 3% were combined in this group.

and rs1112122 in *GABRA3* on epilepsy treatment outcome under adjustment of cofactors, such as epilepsy syndromes, etiologies, weight and age. Among the included genetic variants, rs6883877 in *GABRA1*, rs511310 in *GABRA2* and rs4828696 in *GABRA3* demonstrated significant effect on epilepsy treatment outcome. The model of best fit indicated that age, MTS, TLE, *GABRA1* rs6883877, *GABRA2* rs511310, and *GABRA3* rs4828696 worked synergistically on epilepsy treatment outcome ($p < 0.0001$; adjusted $r^2 = 0.149$; Table 4). The results of the multifactor dimensionality reduction analyses demonstrated that significant SNP–SNP interaction was detected between *GABRA1* rs6883877 and *GABRA3* rs4828696 (Table 5).

Discussion

This study has demonstrated the combined effect of SNPs in *GABRA1*, *GABRA2* and *GABRA3* on the outcome of epilepsy treatments. Although the analysis revealed that the single-gene effect was not significant after Bonferroni correction, further analysis of interactions between these genes showed that, under adjustment for clinical factors, the epilepsy treatment outcomes were significantly associated with the genotypic combinations of *GABRA1* rs6883877, *GABRA2* rs511310 and *GABRA3* rs4828696.

The α subunits of $GABA_A$ receptors are the targets of many AEDs [11–14,34]. Alterations in the expression, function or conformation of α subunits may lead to insufficient binding of AEDs to their target α subunits and thus result in unfavorable treatment outcome. How epilepsy is affected by several SNPs in genes encoding α subunits, such as *GABRA1* and *GABRA6*, has been examined previously [25,26]. Recently, rs2279020 in *GABRA1* was reported to affect the treatment response to AEDs and patients carrying the variant G allele were considered more likely to suffer from drug-resistant epilepsy, but this association was not significant after correction for multiple comparisons [25]. Another study examining SNPs in *GABRA6* failed to demonstrate significant association between genetic polymorphisms in this gene and the

Table 4. Logistic regression analysis of factors related to epilepsy treatment outcomes.

Parameter	Estimate values	Standard error	p-value
Intercept	-1.7026	0.5856	0.0036
Age	-0.0139	0.0063	0.0263
MTS (MTS vs unidentified)	1.2671	0.2994	<0.0001
MTS (non-MTS vs unidentified)	0.6533	0.1844	0.0004
TLE	0.9418	0.1946	<0.0001
GABRA1 rs6883877 C>T (TC vs CC)	-0.6226	0.2070	0.0026
GABRA2 rs511310 A>G (GG vs AA)	1.2583	0.4898	0.0102
GABRA3 rs4828696 C>T (TT vs CC)	0.5024	0.2212	0.0231

MTS: Mesial temporal sclerosis; TLE: Temporal lobe epilepsy.

Table 5. Gene–gene interactions analyzed by multifactor dimensionality reduction.

Number of loci	SNP combinations	Testing balanced accuracy values	Crossvalidation consistency	p-value
2	*GABRA1* rs6883877 *GABRA3* rs4828696	0.5781	10/10	0.008
3	*GABRA1* rs6883877 *GABRA2* rs511310 *GABRA3* rs4828696	0.5345	6/10	0.36
4	*GABRA1* rs6892782 *GABRA2* rs511310 *GABRA1* rs10068980 *GABRA3* rs1112122	0.4952	4/10	0.86

response to epilepsy treatment [26]. In this study, which used the gene-wide tagging method, the combined effect of *GABRA1* rs6883877, *GABRA2* rs511310 and *GABRA3* rs4828696 on epilepsy treatment response was identified under adjustment for clinical factors. The heterozygote genotype of *GABRA1* rs6883877 was demonstrated to be associated with possible protection from resistance epilepsy; on the other hand, the homozygote variant genotypes of *GABRA2* rs511310 and *GABRA3* rs4828696 were associated with drug-resistant epilepsy. To be clinically relevant, pharmacogenomic studies must analyze clinical factors. Since treatment responses to AEDs reflect the complex effects of multiple contributors, such as etiology, epilepsy syndromes and genetics, the best-fitting model in this study probably reveals more useful information than other models. The causative SNPs identified in this study are located in introns and in the downstream 3′-UTR, but the functional effects of the SNPs are not clear. Intronic polymorphism has been proposed to potentially alter the conformation of mature proteins or affect RNA stability, and polymorphisms in the UTR may affect transcription and even alter mRNA folding [35]. There are several transcription factor binding sites near *GABRA2* rs511310 and *GABRA3* rs4828696 (Supplementary Figure 1 & 2), which may thus influence the expression level of genes. These complex processes may, in turn, affect the affinities of protein–DNA interactions to regulate gene expression [35].

In addition to the α subunits, the γ2 subunit of $GABA_A$ receptors (encoded by *GABRG2*) has been studied widely. *GABRG2* rs211037 was reported to be associated with susceptibility to febrile seizure or idiopathic generalized epilepsy [10,15]. However, inconsistent results were reported by distinct research groups and thus the finding remains to be demonstrated conclusively [10,15,21]. The current gene-wide tagging SNP study on *GABRG2* was conducted to address the discrepancy in the effect of the SNP, focusing on whether common genetic variations in *GABRG2* contribute to the development of common, complex forms of epilepsy; however, the results of this study also failed to demonstrate any significant association [21]. Using a systematic review and meta-analysis, another study evaluated the association between *GABRG2* rs211037 and susceptibility to epilepsy [36]. The meta-analysis, which included eight studies, revealed significant association between *GABRG2* rs211037 and febrile seizures [36]. However, the authors concluded that this correlation may have risen from the strong association results presented by two of the studies that had small sample sizes and, therefore, they proposed that the results should be confirmed by studies with larger sample sizes [36].

The sample size in the current study had a maximal power of 80% to detect the association between a specific genetic variant and drug-resistant epilepsy, with a minor allele frequency of 0.1 and an odds ratio of 2 at $p < 0.05$. A major limitation of this study was the possible bias introduced into analysis results by the mixed treatment outcomes obtained with distinct AEDs; however, patients with drug-resistant epilepsy are commonly prescribed combinations of AEDs that have various mechanisms of action. The distributions of allele frequencies among diverse ethnic groups may limit the applicability of the results of this study to other populations. According to the HapMap database, the minor allele frequencies of *GABRA1* rs6883877 C>T and *GABRA2* rs511310 A>G are similar in European and Asian populations, whereas *GABRA3* rs4828696 C>T has a higher minor-allele frequency in the Asian population (T allele frequency ~0.7) than in the European population (T allele frequency ~0.35). Therefore, the European population may have distinct risk alleles for drug-resistant epilepsy.

Conclusion

In conclusion, using the gene-wide tagging SNP method, the current study demonstrated that the interaction effect of *GABRA1* rs6883877 C>T, *GABRA2* rs511310 A>G and *GABRA3* rs4828696 C>T combined with age, etiology and the epilepsy syndrome, strongly influences outcomes of epilepsy treatment in Taiwanese epilepsy patients. Further studies are required to evaluate the functional effects of the identified SNPs and to ascertain whether the genetic associations described here can be applied to other populations.

Financial & competing interests disclosure

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Aim

- The aim of the present study was to identify the association between SNPs in the α subunits of $GABA_A$ receptors and epilepsy treatment outcomes using a gene-wide tagging method.

Results & conclusion

- The multifactor dimensionality reduction analyses showed a significant gene–gene interaction between *GABRA1* rs6883877 and *GABRA3* rs4828696.
- The multivariate logistic regression showed that, under adjustment of clinical factors, the epilepsy treatment outcomes were significantly associated with the genotype combinations of *GABRA1* rs6883877, *GABRA2* rs511310 and *GABRA3* rs4828696.
- The proposed model may be used to estimate epilepsy treatment outcomes of individual patients.

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- ▪ of interest
- ▪▪ of considerable interest

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