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Association of alpha subunit of $GABA_A$ receptor subtype gene polymorphisms with epilepsy susceptibility and drug resistance in north Indian population

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ABSTRACT

GABA (γ-amino butyric acid) receptors have always been an inviting target in the etiology and treatment of epilepsy because of its role as a major inhibitory neurotransmitter in the brain. The aim of our study was to find out the possible role of single nucleotide polymorphisms (SNPs) present in *GABRA1* IVS11 + 15 A > G (rs2279020) and *GABRG2* 588C > T (rs211037) genes in seizure susceptibility and pharmaco-resistance in northern Indian patients with epilepsy. A total of 395 epilepsy patients and 199 control subjects were enrolled for present study. The genotyping was done by PCR-RFLP methods. The *GABRA1* IVS11 + 15 A > G polymorphism conferred high risk for epilepsy susceptibility at genotype 'AG' ($P = 0.004$, OR = 1.77, 95% CI = 1.20–2.63), 'GG' ($P = 0.01$, OR = 1.80, 95% CI = 1.15–2.80) and G allele level ($P = 0.001$, OR = 1.50, 95% CI = 1.16–1.92). Moreover this polymorphism was also associated with multiple drug resistance in patients with epilepsy for homozygous variant 'GG' genotype ($P = 0.031$, OR = 1.84, 95% CI = 1.05–3.23) and G allele ($P = 0.020$, OR = 1.43, 95% CI = 1.05–1.95). However *GABRG2* 588C > T polymorphism was not found to be associated either with epilepsy susceptibility or with drug resistance. Overall results indicate differential role of different subunits of $GABA_A$ receptor subtypes in epilepsy susceptibility and pharmacotherapy.

1. Introduction

Epilepsy is most common paroxysmal and heterogeneous neurological disorder affecting an estimated 42 million people worldwide with distinct symptoms, etiology, prognosis and treatments.[1] Overall prevalence of epilepsy roughly lies in the range of 5–10 per 1000 people, which is usually higher in developing countries.[2,3] Majority of epilepsy phenotypes result from interaction between genes and environmental factors. It is just over a decade since the discovery of the first human epilepsy associated ion channel gene mutation, at least 25 different genes have been described till now, although the strength of the evidences for these genes having a pathogenic role in epilepsy varies. Only 1–2% idiopathic epilepsies seem to be monogenic; whereas most of them are believed to be polygenic.[4] These gene and their variants influence seizures, epileptogenesis and epilepsy at multiple levels.[5] Therefore, genes encoding voltage gated Na^+, K^+, Ca^{++}, Cl^- and HCN,[6,7] and ligand-gated (nicotinic acetylcholine and GABA receptors) ion channels are considered to be major class of genes associated with various epilepsy phenotypes.

In the central nervous system, GABA is the major inhibitory neurotransmitter that controls neuronal excitability and network interactions in the cerebral cortex of the brain. It acts through three receptor classes: the ionotropic $GABA_A$, $GABA_C$ receptors and the metabotropic $GABA_B$ receptors. Among the three receptors, recent findings highlight the significance of $GABA_A$ receptor heterogeneity for the concept of E/I (excitation/inhibition) balance and its relevance for epilepsy.[8] Structurally $GABA_A$ receptors are pentameric chloride ion channels formed from various combinations of proteins encoded by α (α1–α6), β (β1–β3), γ (γ1–γ3); δ, ε, π, θ, and ρ (ρ1–ρ3) subunit gene families. The α1β2γ2 subunit combination of $GABA_A$ receptor is most abundant in almost all regions of the brain.[3] Dysfunction of genes coding these subunits affects ion channel gating, expression, and trafficking of the GABA receptor to the cell surface. These genes are also believed to influence important drug targets necessary for the regulation of neuronal activity in the brain.[9] Antiepileptic drugs (AEDs) such as benzodiazepines, phenobarbital, gabapentin and topiramate are important targets of $GABA_A$ receptor.[10] Recently it has been reported that AED resistant rats differ from drug responsive rats in $GABA_A$ receptor subunit expression in rat model of temporal lobe epilepsy. It also suggests that alterations in $GABA_A$ receptor subunits may be involved in resistance to AEDs.[11]

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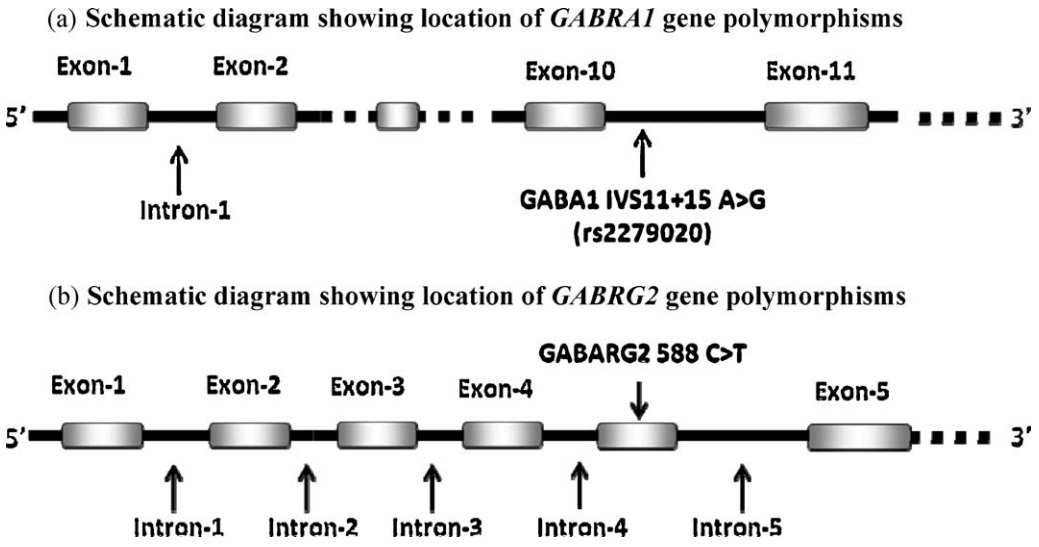

Fig. 1. (a) Schematic diagram showing location of *GABRA1* gene polymorphisms. (b) Schematic diagram showing location of *GABRG2* gene polymorphisms.

Several SNPs in the $GABA_A$ receptor subtypes have been described so far but only few including intronic *GABRA1* IVS11 + 15 A > G and an exonic *GABRG2* 588C > T gene polymorphisms are found to have functional significance in different neurological disorders. These gene variants have been attributed as one of the several susceptibility factors for febrile seizures[9,12]; with the development of alcoholism and substance abuse disorders affecting neuronal channels.[13,14]

Thus, genes encoding $GABA_A$ receptor subunits represent high ranking candidates for epilepsy susceptibility and targets for pharmacotherapeutic agents in epilepsy treatment. Therefore, on the basis of functional significance, previous observations and current knowledge we investigated the possible role of these genetic polymorphisms *GABRA1* IVS11 + 15 A > G (rs2279020) and *GABRG2* 588C > T (rs211037) [Fig. 1(a) and (b)] in epilepsy susceptibility and antiepileptic drug (AED) response in northern Indian patients with epilepsy.

2. Materials and methods

2.1. Patients and controls

Epilepsy patients were enrolled from the outpatients department (OPD) of neurology attending the clinics of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, India. The patients were diagnosed and classified by an experienced neurologist. The clinical profile of drug responsive and drug resistant epilepsy patients were based on hospital investigations. Exclusion criteria included severe adverse drug reactions; poor compliance with AEDs, unreliable record of seizure frequency, history of pseudo seizures, alcohol or drug abuse, or any other malignant diseases such as brain tumor, secondary metastasis, hepatic failure or renal failure. An informed consent was signed by each participant or responsible adult and they were personally interviewed for information on ethnicity, seizure frequency, and duration of seizure, compliance and other habits. After screening of more than 500 patients a total of 395 patients were included rest were excluded. 259 patients were diagnosed as drug responsive and 122 are nonresponsive. We found that in responders group, 123 (45.1%) patients were on monotherapy and 150 (54.9%) were on polytherapy, i.e. on more than two drugs. In case of nonresponsive epilepsy patients, all were undergoing polytherapy. It was observed that patients who respond early to treatment are less likely to become drug resistant. There appears to be no significant differences in response when compared on the basis of monotherapy and polytherapy. Fourteen patients showed only partial response and therefore excluded from the study analysis involving drug response.

A total of 199 healthy controls were recruited from staff of SGPGIMS and unrelated persons from north India visiting the hospital for minor medical or surgical problems, reported no history of epileptic seizures, and other brain abnormalities. All controls, drug resistance and drug responsive patients were of same ethnic origin. The study was approved by local ethics committee of the institute at SGPGIMS, Lucknow, India.

2.2. Definition of drug resistance and responsiveness

The main criterion for drug resistance was the occurrence of at least four seizures over a period of one year with three appropriate antiepileptic drugs (AEDs) at maximum tolerated doses.[15,16] Patients who had under-gone surgeries for seizure control were considered refractory irrespective of their outcome after surgery. The epilepsy patients who had complete freedom from seizures for at least one year from last follow up visit were considered drug responsive.

In order to ascertain drug compliance, antiepileptic drug levels in plasma were measured using HPLC (Perkin Elmer) in 20% of patients to confirm compliance and all patients enrolled in the study showed drug compliance. Mean carbamazepine, phenytoin and valproate levels were 8.26 ± 5.25 μg/ml, 11.27 ± 8.12 μg/ml and 68.0 ± 36.22 μg/ml respectively in epileptic patients; and were in therapeutic range. The maximum tolerated doses were different for different individuals in our epilepsy patients. These were 20 mg/kg/day for carbamazepine, 20 mg/kg for phenytoin and 10 mg/kg for valproate.

2.3. Laboratory protocols

2.3.1. Genotyping of GABRA1 (rs2279020) and GABRG2 (rs211037)

The genomic DNA was extracted from peripheral blood leucocytes pellet using the standard salting out method with slight modifications.[17] The plasma was separated and stored at −20 °C for drug level assay. We genotyped total 395 epilepsy patients and 199 healthy controls. Genotyping was performed using PCR-RFLP method as reported previously (Table 1). Twenty percent of samples from patients including samples of each genotype were re-genotyped by different laboratory personnel and