Neuroscience Letters 506 (2012) 170–174

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Influence of *GRIA1*, *GRIA2* and *GRIA4* polymorphisms on diagnosis and response to antipsychotic treatment in patients with schizophrenia

Concetta Crisafulli[a], Alberto Chiesa[b], Diana De Ronchi[b], Changsu Han[c], Soo-Jung Lee[d], Moon Ho Park[e], Ashwin A. Patkar[f], Chi-Un Pae[d,f,*], Alessandro Serretti[b]

[a] Department of Biomorphology and Biotechnologies, Division of Biology and Genetics, University of Messina, Italy
[b] Institute of Psychiatry, University of Bologna, Bologna, Italy
[c] Department of Psychiatry, College of Medicine, Korea University, Ansan, Kyounggi-Do, Republic of Korea
[d] Department of Psychiatry, Bucheon St. Mary's Hospital, The Catholic University of Korea College of Medicine, Bucheon, Kyounggi-Do, Republic of Korea
[e] Department of Neurology, College of Medicine, Korea University, Ansan, Kyounggi-Do, Republic of Korea
[f] Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC, USA

ARTICLE INFO

Article history:
Received 16 March 2011
Received in revised form
21 September 2011
Accepted 29 October 2011

Keywords:
Glutamate receptors
GRIA1
GRIA2
GRIA4
Schizophrenia
Antipsychotics

ABSTRACT

The present study is aimed at exploring whether some single nucleotide polymorphisms (SNPs) within *GRIA1*, *GRIA2* and *GRIA4* could be associated with schizophrenia and whether they could predict clinical outcomes in Korean in-patients treated with antipsychotics. One hundred forty five patients with MD, 221 in-patients with schizophrenia and 170 psychiatrically healthy controls were genotyped for 17 SNPs within *GRIA1*, *GRIA2* and *GRIA4*. Baseline and final clinical measures, including the Positive and Negative Symptoms Scale (PANSS), were recorded. No significant association was found with the diagnosis of schizophrenia. We observed an association between rs3813296 genotype and improvement on PANSS negative scores. Our findings provide no evidence for an association between SNPs within *GRIA1*, *GRIA2* and *GRIA4* under investigation and schizophrenia susceptibility, although rs3813296 (*GRIA2*) could be associated with improvement on PANSS negative scores. However, taking into account the several limitations of our study, further research is needed to draw more definitive conclusions.

1. Introduction

Schizophrenia (SKZ) is a severe psychiatric disorder that affects approximately one percent of the population worldwide [13]. Although both genetic and environmental factors are thought to play a significant role into the development of SKZ [27], genetic factors seem to play a major role [32,36]. In particular, evidence from twin, family and adoption studies points to a strong genetic component, with an estimated heritability as high as 70% [32].

In recent years, several post-mortem and in vivo receptor studies provided evidence for a significant disruption of the glutamatergic system in subjects with SKZ [4,18,21,24,44,45,47] leading to the development of the "glutamatergic dysfunction hypothesis of SKZ". Such hypothesis focuses on the abnormalities in genes involved in the glutamatergic system [15], including those coding for glutamate receptors (AMPA, NMDA, kainite and metabotropic receptors) [20] and is supported by a growing amount of empirical research suggesting a link between this neurotransmitter and SKZ [7].

More in detail, several studies showed abnormal levels of AMPA receptor transcripts and/or proteins in brains of SKZ patients [14]. AMPA receptors mediate fast excitatory synaptic transmission in the CNS and play a key role in hippocampal synaptic long-term potentiation (LTP) and depression (LTD) [8]. Of note, abnormalities of AMPA receptors may be well reconciled with recent hypotheses of SKZ as a disorder of the CNS plasticity and/or development [38]. AMPA receptors are composed of four types of subunits, referred to as GluR1 (*GRIA1*), GluR2 (*GRIA2*), GluR3 (*GRIA3*), and GluR4, alternatively called GluRA-D2 (*GRIA4*), which are combined to form tetramers [35]. In particular, in this paper, we focused our attention on *GRIA1*, *GRIA2* and *GRIA4*.

GRIA1 is located on chromosome 5q33 and encodes for the GluR1 subunit. *GRIA1* is primarily found in the forebrain and hippocampus, brain areas that are particularly involved in memory formation and retention of spatial memory tasks [39]. *GRIA1* itself was found to influence cognitive functions, such as working memory and reward learning [39]. Furthermore, increasing evidence points to the involvement of this receptor in psychiatric disorders such as psychotic bipolar disorder (BD) [23]. Also, polymorphisms

* Corresponding author at: Department of Psychiatry, Bucheon St. Mary's Hospital, The Catholic University of Korea College of Medicine, 2 Sosa-Dong, Wonmi-Gu, Bucheon, Kyeonggi-Do 420-717, Republic of Korea. Tel.: +82 32 340 7067; fax: +82 32 340 2255.
E-mail address: pae@catholic.ac.kr (C.-U. Pae).

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.10.074

within this gene have been associated with SKZ [28] and regional specific abnormalities in gene expression have been reported in postmortem brains of individuals with SKZ [2].

GRIA2 is located on chromosome 4q32–33 and encodes for GluR2 subunits. This chromosomal region was found to be in strong association with some psychiatric disorders such as BD and SKZ [11,33]. As an example, Hovatta and colleagues reported the 4q31 region to be linked to SKZ [19]. Also, *GRIA2* is one of the genes down-regulated by chronic lithium treatment [40]. Furthermore, chronic lithium or valproate treatment causes decreased synaptic expression of GluR2 in hippocampal neurons [10].

GRIA4 maps on 11q22–23 and encodes for GluR4 subunits. *GRIA4* is localized in a chromosomal region where a higher rate of induced fragile sites has been observed in SKZ patients [46]. In addition, an abnormal expression of *GRIA4* is observed in the brains of SKZ patients [34]. As an example, Makino and colleagues reported that a rs609239, rs641574 and rs659840 haplotype (G-G-A) within *GRIA4* was positively associated with SKZ in the Japanese population [30]. However, the same polymorphisms were not associated with SKZ in a following study in a Chinese sample [17].Taking into account the dearth of studies focusing on such SNPs and the substantial risk for Type I (false positive) errors in genetic association studies [25], the aim of this study is to investigate the existence of possible associations between a set of *GRIA1* (rs707176 and rs6875572), *GRIA2* (rs6536221, rs4260586, rs4302506, rs4441804, rs3813296 and rs4403097) and *GRIA4* (rs11226805, rs2166318, rs11822168, rs1938956, rs10736648, rs528205, rs11226867, rs667174 and rs641574) SNPs and SKZ. In addition, in the present study we investigate, to the best of our knowledge for the first time, the effects of the same SNPs on clinical improvement in a sample of SKZ in-patients treated with antipsychotics.

2. Methods

2.1. Sample

The sample under investigation in the present study included 221 SKZ in-patients who were consecutively recruited at the Department of Psychiatry of the Catholic University of Korea College of Medicine, Seoul, Korea. Patients were eligible for inclusion if they had a documented clinical diagnosis of SKZ according to the DSM-IV criteria, as assessed by the Mini-International Neuropsychiatric Interview (M.I.N.I.) [42]. The same sample has been previously investigated by our group regarding other gene variants [41].

No particular restriction was employed with regard to treatments, duration of illness and first vs. following episodes of disease. However, patients were excluded if they had current severe or unstable medical and neurological conditions, current treatment with a long-acting antipsychotic, concomitant alcohol and substance abuse disorders and if they were not of Korean ethnicity. A further sample of 170 Korean psychiatrically healthy subjects, who underwent the same assessment of psychiatric patients to exclude possible psychiatric disorders, deriving from the same location of the psychiatric patients included in the present study, was also included to compare genotype and allelic frequencies between SKZ patients and psychiatrically healthy controls.

All patients admitted to the hospital were assessed for the severity of illness at baseline and at discharge by means of the Positive and Negative Symptoms Scale (PANSS) [22]. Scorers were trained with the specific instruments with good inter-rater reliability ($k > 0.8$). Additionally, the following clinical and demographic variables were recorded: gender, age, age at onset, familiar history of psychiatric disorders (based on subjects' reports after direct questioning by clinicians), lifetime suicide attempts, duration of admission, drugs at discharge and concomitant anxiolytics. The study protocol was approved by the institutional review board (approval number HC10TISI0031). All patients (18–65 years old) provided written informed consent before participating into the study.

2.2. Outcome measures

The main outcome measures of the present study were: (1) differences between genotypic and allelic frequencies in patients with SKZ as compared with healthy control subjects and (2) possible influences of the 17 SNPs within *GRIA1*, *GRIA2* and *GRIA4* under investigation on clinical improvement as measured with the PANSS in SKZ patients. Further outcomes of interests included baseline and endpoint PANSS scores, baseline and endpoint CGI scores and response rates. Both continuous and categorical analyses were performed. In accordance with previous studies, response was *a priori* defined as a $\geq$50% symptoms' reduction from baseline to discharge [26].

2.3. DNA analysis

Genomic DNA was extracted from blood by standard methods and quantified. The high-throughput genotyping method using pyrosequencer (Biotage AB, Sweden) was used for genotyping the SNPs mentioned above (Table 1). Genetic SNPs were chosen among those (1) previously investigated in association with schizophrenia (e.g. [28,29]), (2) with a reported prevalence of at least 5% for the variant allele among Asian samples (data from http://hapmap.ncbi.nlm.nih.gov/, $R^2 = 0.08$ and MAF = 0.05) or (3) with availability of a validated assay in our laboratory. PCR primers (Bioneer, Daejeon, Korea) and sequencing primers (Bioneer, Daejeon, Korea) used for the pyrosequencing assay were designed by using the Pyrosequencing Assay Design Software v1 (Biotage AB, Sweden) and one primer of each primer set was biotinylated.

2.4. Statistical analysis

Statistical analyses were performed using 'Statistica' package [43]. Differences in the allelic and genetic frequencies between healthy subjects and patients with SKZ as well as effects of such variants on response rates and further categorical outcomes were calculated using the χ^2 statistics. The influence of the SNPs under investigation and continuous outcomes were calculated using the ANOVA. Clinical improvement on PANSS total scores was calculated according to the following formula:

$$\left(\frac{\text{PANSS}_{\text{final}} - \text{PANSS}_{\text{baseline}}}{\text{PANSS}_{\text{baseline}}}\right) \times 100$$

In the case of positive findings, clinical variables correlated with the outcome measures under investigation were added as covariates. Haploview 3.2 was used to generate a linkage disequilibrium (LD) map and to test for Hardy–Weinberg equilibrium (HWE) [1]. Tests for associations using multi-marker haplotypes were performed using the statistics environment "R" (http://www.R-project.org), package "haplo.score", to compare clinical and socio-demographic outcomes among different haplotypes.

All *p*-values were 2-tailed, and statistical significance was conservatively set at the 0.005 level (corresponding to the Bonferroni correction for the 10 blocks of SNPs under investigation, see below for further information) in order to reduce the likelihood of false positive results. With these parameters we had a sufficient power (0.80) to detect a small-medium effect size ($\omega = 0.18$) that, as an example, corresponded to an odds ratio (OR) of 2.1 between the schizophrenic patients and the group of controls and to detect a