Investigation of possible epistatic interactions between *GRIA2* and *GRIA4* variants on clinical outcomes in patients with major depressive disorder

Alberto Chiesa[1,2], Loredana Lia[1], Claudia Lia[1], Soo-Jung Lee[3], Changsu Han[4], Ashwin A Patkar[5], Chi-Un Pae[3,5] and Alessandro Serretti[1]

Abstract

Objectives: To investigate the effects of glutamate receptor, ionotropic, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) 2 (*GRIA2*) rs4260586 and glutamate receptor, ionotropic, AMPA 4 (*GRIA4*) rs10736648 single nucleotide polymorphisms (SNPs) on response to antidepressants in Korean patients with major depressive disorder (MDD), and to ascertain whether epistatic interactions might exist between these SNPs.

Methods: In this retrospective analysis, patients were assessed at hospital admission and discharge using the Montgomery–Åsberg depression rating scale (MADRS). A multiple regression model was employed to investigate the effects of the two SNP variants on clinical/sociodemographic outcomes relating to MDD.

Results: Out of 145 Korean patients, the presence of both *GRIA2* rs4260586 and *GRIA4* rs10736648 polymorphisms had no significant association with MADRS improvement scores or other clinical/sociodemographic variables.

Conclusions: These data potentially suggest a lack of epistatic interaction between *GRIA2* and *GRIA4* variants, regarding clinical outcomes in patients with MDD. The study was limited by small sample size, use of different antidepressants and incomplete coverage of genes under investigation.

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
[2]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy
[3]Department of Psychiatry, Bucheon St Mary's Hospital, The Catholic University of Korea College of Medicine, Bucheon, Kyounggi-Do, Republic of Korea
[4]Department of Psychiatry, Korea University, Seoul, Republic of Korea

[5]Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC, USA

Corresponding author:
Dr Chi-Un Pae, 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.
Email: pae@catholic.ac.kr

Future research should include larger patient samples treated with different antidepressants, analysis of different SNPs and/or investigation of different gene–gene interactions within the glutamatergic system.

Introduction

Major depressive disorder (MDD) is a common mental disorder, with a lifetime US incidence of 12% in men and 20% in women.[1] Evidence suggests that MDD has a strong genetic aetiology.[2] Several genetic variants – including single nucleotide polymorphisms (SNPs) within genes encoding the serotonin transporter and several serotonin receptors – significantly modulate the response to antidepressants.[3]

Pharmacogenetic studies of psychotropic drug response that focus on one only SNP have shown inconsistent results or relatively small effects.[4] Consequently, there is increasing emphasis on investigating SNPs within the genes encoding transporters and receptors that are unrelated to the serotonergic system. These include investigations of the glutamatergic system,[5] or of epistatic interactions between genetic polymorphisms in candidate genes that may influence treatment outcomes in complex diseases, such as psychiatric disorders.[6,7]

Research investigating possible associations between the glutamatergic system and antidepressant response has gained attention, with Maeng and Zarate hypothesizing that the efficacy of antidepressant drugs might be explained (at least in part) by direct or indirect actions of these drugs on the glutamatergic system.[5] Consistent with this hypothesis, there is evidence that antidepressants can modulate glutamatergic transmission,[8] and that variations within some glutamatergic genes can modulate antidepressant response.[9]

Epistasis is defined as a functional interaction between genes, encompassing various events including promoter activity control, epigenetic control, chromatin remodelling, and many other molecular reactions.[10] These events can impact cell life-cycles and complex traits, and are orchestrated through genetically driven complex, yet flexible, activities.[10] Thus, research into epistatic interactions between different candidate genes could be relevant in understanding the biological diversity that could influence antidepressant response.

Interactions between trace amine-associated receptor 6 and heat shock protein-70 gene polymorphisms have been found to affect the development of schizophrenia and mood disorders.[11,12] The present retrospective analysis of a published dataset[13] investigated the relationship between genetic variants of glutamate receptor, ionotropic, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) 2 (*GRIA2*) and glutamate receptor, ionotropic, AMPA 4 (*GRIA4*), in a sample of 145 Korean MDD patients treated with different antidepressants; such variants may influence antidepressant response. These two genes, which code for two different subunits of the glutamatergic AMPA-sensitive receptors, have been investigated individually;[13] no association between the *GRIA2* and *GRIA4* polymorphisms investigated and clinical outcomes was found.

Patients and methods

Study population

The present retrospective study was conducted at Bucheon St Mary's Hospital, The Catholic University of Korea College of Medicine, Bucheon, Kyounggi-Do, Republic of Korea, in October 2012. Details of the study population have been reported.[13] Briefly, consecutive inpatients diagnosed with MDD according to the Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) criteria[14] were included and underwent clinical interview using the Mini International Neuropsychiatric Interview (MINI).[15] There was no restriction regarding the type of antidepressant treatment administered. Patients were excluded if they had: current severe (or unstable) medical or neurological conditions; current treatment with long-acting antipsychotic drugs; concomitant alcohol and substance abuse disorders. Patients were also excluded if they were not of Korean ethnicity.[13] All patients admitted to the hospital – including those in the present study – are routinely assessed for severity of illness at admission and at discharge, by means of the Montgomery–Åsberg Depression Rating Scale (MADRS).[16] Response was defined a priori as a ≥50% reduction in symptoms between admission and discharge; remission was defined as a MADRS score ≤7 at discharge, both as described previously.[17] The following additional clinical and sociodemographic variables were recorded: total MADRS scores at admission and discharge; MDD subtypes; age; sex; age at MDD onset; family history of MDD; past history of suicide attempt; type of antidepressant and concomitant medication used.

The study protocol was approved by the Institutional Review Board of Bucheon St Mary's Hospital, The Catholic University of Korea College of Medicine (approval number, HC10TISI0031).

All patients included in the study provided written informed consent.

Outcome measures

The main outcome measure was the relationship between *GRIA2* rs4260586 and *GRIA4* rs10736648 SNPs and improvements in depressive symptoms, as measured by the change in total MADRS scores between admission and discharge. Secondary outcome measures included: presence of both *GRIA2* rs4260586 and *GRIA4* rs10736648 polymorphisms and total MADRS scores at admission and discharge; any response to treatment; remission; MDD subtypes; age; sex; age at MDD onset; family history of MDD; past history of suicide attempt; duration of admission; type of antidepressant used; concomitant anxiolytic therapy.

Genotyping of GRIA2 *rs4260586* and GRIA4 *rs10736648* SNPs

The protocol used to genotype the patient population has been described previously.[13] To cover the highest possible proportion of the gene, the *GRIA2* rs4260586 and *GRIA4* rs10736648 SNPs were selected from SNPs within the original dataset.[13] One SNP was selected for each gene that tagged the largest number of SNPs within the same gene. This approach ensured that ~14% of *GRIA2* variations and ~1% of *GRIA4* variations were covered.

Statistical analyses

Statistical analyses were performed using the STATISTICA software package, version 5 (StatSoft Italia, Vigonza, Padua, Italy) for Windows®. A multiple regression model was used to investigate the relationship between the two genotypes and clinical and sociodemographic variables. All *P*-values were 2-tailed and statistical significance was set at the $P \leq 0.004$ level,

after correction of multiple comparisons. These parameters gave sufficient power (0.80) to detect a medium–large (d = 0.29) effect size for patients carrying the TT rs4260586 genotype, compared with those carrying the AT genotype.[18] Such an effect size corresponded with the possibility of detecting four-point differences in final MADRS scores.

Results

A total of 145 Korean inpatients with MDD, diagnosed according to DSM-IV criteria, were included. No significant correlation was observed between *GRIA2* rs4260586 and *GRIA4* rs10736648 and improvement of depressive symptoms, as measured by the change in total MADRS scores from admission to discharge (β = 0.17). In addition, no significant correlation was observed between rs4260586 within *GRIA2* and rs10736648 within *GRIA4,* and secondary outcome measures (Table 1).

Discussion

In an attempt to understand whether potential epistatic interactions exist, the present study explored whether the presence of both the *GRIA2* rs4260586 and *GRIA4* rs10736648 SNPs was associated with improved MADRS scores and other clinical variables, in a sample of patients with MDD who were being treated with various antidepressants. There was no significant correlation between the presence of the two SNPs and improvement in MADRS scores, which potentially suggests that there is no epistatic interaction between the *GRIA2* rs4260586 and *GRIA4* rs10736648 polymorphisms, in terms of MDD. A possible explanation for this negative finding could be that the sample size was too small to detect subtle differences that are usually associated with single genes or gene–gene interactions, in complex disorders.[19]

Another possibility is that the results reflect a lack of epistatic interaction between the two SNPs investigated and clinical improvement, in terms of MADRS scores. In another study that focused on single SNPs within *GRIA2* and *GRIA4*, no significant association with MADRS scores was observed.[13] There is, however, some evidence to suggest that epistatic interactions can be observed, even when single SNPs considered on an individual basis do not lead to positive findings.[20,21] Such a possibility raises substantial computational and theoretical difficulties, when considering all possible gene–gene interactions and other forms of gene-expression control.[22]

No statistically significant effects were observed regarding any of the secondary outcome measures in the presence of both *GRIA2* rs4260586 and *GRIA4* rs10736648 polymorphisms, further suggesting the possibility that no relevant epistatic interactions exist between the two SNPs under investigation. These data support results from a study that investigated the presence of these polymorphisms on an individual basis.[13]

Several limitations of the present study should be considered. First, the lack of associations observed could be simply due to the lack of statistical power that, in turn, could obscure small effects exerted by single SNPs. This issue is of particular concern if one considers that, even among pharmacogenetic studies with large sample sizes, results tend to remain conflicting.[23,24] A further limitation is the use of antidepressants with different mechanisms of action; this prevents conclusions from being drawn, with regard to the influence of these SNPs on specific drugs or classes of drugs. The decision to include patients who were being treated with different drugs could, however, have the advantage of being closer to 'real-world' clinical practice. The duration of hospitalization in the present study may be considered insufficient to ascertain a lack of response and remission; however, this time

Table 1. Influence of *GRIA2* rs4260586 and *GRIA4* rs10736648 single nucleotide polymorphisms on clinical and sociodemographic outcomes, in Korean patients with major depressive disorders (MDD).

Clinical or demographic characteristic	Patients *n* = 14	Influence of rs4260586 β	Influence of rs10736648 β	Influence of both rs4260586 and rs10736648 β
Male sex	75 (52)	0.05	0.07	0.03
Age, years	41.37 ± 14.07	0.13	−0.02	0.05
Total MADRS score				
Baseline	34.35 ± 8.95	−0.36	−0.43	0.48
Discharge	17.12 ± 9.88	−0.05	0.18	−0.23
Positive responders	78 (54)	−0.07	0.13	−0.13
Remitters	22 (15)	0.13	0.18	−0.23
Clinical subtypes[a]				
MDD without PF	105 (72)	0.14	0.28	−0.32
MDD with PF	11 (8)			
Dysthymia	4 (3)			
MDD NOS	5 (3)			
Age at onset, years	38.08 ± 13.29	−0.04	−0.23	0.40
Family history of psychiatric disorders, yes	30 (21)	−0.07	0.11	−0.13
Suicide attempts, yes	36 (25)	0.01	−0.02	0.17
Duration of admission, days	32.31 ± 20.55	−0.37	0.02	0.18
Antipsychotic drug[b]				
Paroxetine	40 (28)	−0.02	0.20	−0.95
Venlafaxine	35 (24)			
Fluoxetine	23 (16)			
Mirtazapine	21 (14)			
Other	3 (2)			
Concomitant anxiolytics				
Alprazolam	30 (21)	0.08	0.16	−0.24
Lorazepam	73 (50)			
Clonazepam	3 (2)			
Buspirone	4 (3)			
None	35 (24)			

Data presented as *n* (%) of patients or mean ± SD.
[a]20 patients were excluded from this part of the analysis due to insufficient information; [b]23 patients were excluded from this part of the analysis due to insufficient information.
A multiple regression model was used to investigate the existence of possible interactions between the two genotypes, and clinical and sociodemographic variables.
No statistically significant differences were observed ($P \geq 0.004$).
MADRS, Montgomery–Asberg Depression Rating Scale; PF, psychotic feature; NOS, not otherwise specified.

frame is consistent with common clinical practice.[25,26] Finally, this study was limited by the incomplete coverage of the genes under investigation, due to the tagging approach used.

The analysis of epistasis between different genes is particularly relevant to genetic-association studies, as it increases knowledge of the mechanism of action of the so-called reactome.[11,12] The analysis of both

gene epistasis and the impact of single genotypes and haplotypes should be standard practice in future association studies.[11,12]

The preliminary findings that are reported as a consequence of the present study suggest that it is likely that no epistatic interactions exist between *GRIA2* and *GRIA4,* in terms of clinical outcomes, in patients with MDD treated with different antidepressants. Further research should include a larger sample size of patients with MDD and treated with different antidepressants, or include different SNPs and/or different gene–gene interactions within other genes that are known to be involved in the glutamatergic system.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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