Research paper

Influence of GRIK4 genetic variants on the electroconvulsive therapy response

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HIGHLIGHTS

• GRIK4 variants modulate ECT outcome.
• Kainate receptor modulation in ECT.
• Genetic factors & ECT.

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ABSTRACT

Several lines of evidence have shown the involvement of the glutamatergic system in the function of electroconvulsive therapy (ECT). In particular, patients with treatment resistant depression (TRD) and chronic depression have lower levels of glutamate/glutamine than controls, and ECT can reverse this deficit. Genetic factors might contribute to modulating the mechanisms underlying ECT.

This study aimed to evaluate the relationship between three polymorphisms (rs1954787, rs4936554 and rs11218030) of the glutamate receptor ionotropic kainate 4 (GRIK4) gene and responsiveness to ECT treatment in a sample of one hundred individuals, TRD or depressive Bipolar Disorder patients resistant to pharmacological treatments.

The results revealed that GRIK4 variants were significantly associated with the response to ECT. In particular, we found that patients carrying the G allele of the GRIK4 rs11218030 had a significantly poorer response to ECT (p = 2.71 × 10^{-4}), showing five times the risk of relapse after ECT compared to the AA homozygotes. Analogously, patients carrying the GG rs1954787 genotype and rs4936554 A allele carriers presented a double risk of lack of response after ECT (p = 0.013 and p = 0.040, respectively).

In conclusion, the current study provides new evidence, indicating that some GRIK4 variants modulate the response to ECT in patients with depression resistant to treatment, suggesting a role for kainate receptor modulation.

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1. Introduction

Electroconvulsive therapy (ECT) is a safe and well-established effective treatment option for severe depression that was introduced in 1938 by Bini and Cerletti. Despite controversial issues, ECT remains one of the most eligible therapies among Treatment Resistant Depression (TRD) patients or those intolerant to antidepressant medications or when a rapid and definitive response is required (e.g., because of psychosis or a risk of suicide) [1–3]. Furthermore, ECT is the most powerful antidepressant treatment strategy available today with success rates higher than pharmacological treatment in patients affected by refractory unipolar or bipolar depression [4–8].

Although the exact mechanism of the action of ECT is not entirely known, changes in neuroplasticity and in the activity of certain neurotransmitter systems have been reported [9–11]. More and
more studies show evidence that neurotrophic factors are related to ECT function and/or to its effectiveness [12–17]. Converging data obtained from biological and imaging studies support findings related to the enhancement of serotonergic neurotransmission and the activation of the mesocorticolimbic dopamine system after ECT [18].

Increasing evidence indicates the relevant involvement of the glutamate system in the neurobiology and treatment of Major Depressive Disorder (MDD) with imbalances in glutamate and GABA (γ-aminobutyric acid) metabolism and region-specific alterations of these neurotransmitters [19–21].

Several glutamate receptor genes have been investigated in antidepressant-treatment outcome. One of the most studied is the Glutamate Receptor Ionotropic Kainate 4 (GRIK4) gene, which encodes the kainate receptor subunit KA1 and is predominantly expressed in the hippocampus [22], exerting a modulatory effect on synaptic plasticity [23,24]. In particular, several GRIK4 single nucleotide polymorphisms (SNPs) were initially found to be associated with a non-response to antidepressant therapy in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study [25]. However, these results were partly or none at all replicated in further studies [26–30]. In our previous study, we found that two GRIK4 variants were associated with the risk of developing psychotic symptomatology during a depressive episode [30]. This is of particular importance because the presence of psychotic symp-
toms is one of the strongest negative predictive factors of response to treatment in MDD [31,32].

ECT results in a normalisation of glutamate deficits [33,34] by modifying the inhibitory neurotransmitter systems and by affecting neurogenesis through the increase of neurotrophic factors [9,13,14,17,35,36].

The genetic factors associated with the ECT response are poorly known and, to date, few studies have been conducted [37–43] and none have investigated the glutamergic system.

The data concerning the GRIK4 gene and treatment outcome in depression combined with the evidence that ECT affects the glutamatergic pathway provide the grounds for hypothesising a possible association between the GRIK4 gene and the ECT response. In particular, we focused on the three most significant polymorphisms (rs1954787, rs4936554 and rs11218030) found to be associated with treatment outcome in the STAR-D cohort [25].

2. Methods

2.1. Sample

One hundred individuals were voluntarily enrolled in the study, which was approved by the local ethics committee (Ethics Committee of the province of Verona N: 4997/09.11.01), and written informed consent was obtained. The group was made of 92 MDD and 8 Bipolar Disorder (BD) patients, in accordance with the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) classification system criteria. All of the BD patients were in a severe depressive state. The diagnoses were confirmed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) diagnostic scale. The exclusion criteria were as follows: (a) mental retardation and cognitive disorders; (b) a lifetime history of schizophrenic or schizoaffective disorder; (c) personality disorders, obsessive compulsive disorder, post-traumatic stress disorder, substance abuse or dependency as a primary diagnosis; and (d) comorbidity with eating disorders.

All of the patients were referred to the Psychiatric Hospital “Villa Santa Chiara”, Verona, Italy, and they were scheduled to undergo ECT because they had been evaluated as treatment-resistant patients. TRD was defined as at least the failure of the patient to respond to two or more adequate trials of two or more different classes of antidepressants and to an adequate trial of a tricyclic (TCA) drug referred to as the Stage III of Thase and Rush Staging Method [44]. Treatment nonresponsiveness in the patients with bipolar depression was defined as the failure to respond to at least three mood disorder treatments, comprising an adequate trial with a TCA, and/or a combination with a mood stabilizer(s) [45].

Illness severity and the outcome of ECT were assessed using the Montgomery and Asberg Depression Rating Scale (MADRS) before the treatment (T0) and about one month after the end of ECT (T1). In the month after the end of ECT, the pharmacological treatment was maintained the same with only a possible light reduction in the dosage. All of the socio-demographical, clinical and pharma-

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Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TRD patients (N = 100)</th>
<th>ECT responders (N = 69)</th>
<th>ECT non-responders (N = 31)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (SD)</td>
<td>56.3 (13.5)</td>
<td>55.7 (14.2)</td>
<td>57.8 (11.9)</td>
<td>0.47</td>
</tr>
<tr>
<td>% Gender (F)</td>
<td>70.0</td>
<td>72.5</td>
<td>64.5</td>
<td>0.42</td>
</tr>
<tr>
<td>Education (years), mean (SD)</td>
<td>8.5 (3.6)</td>
<td>8.2 (3.6)</td>
<td>9.1 (4.8)</td>
<td>0.27</td>
</tr>
<tr>
<td>% Smokers</td>
<td>31.6</td>
<td>31.3</td>
<td>32.3</td>
<td>0.93</td>
</tr>
<tr>
<td>MADRS at T0, mean (SD)</td>
<td>33.3 (6.3)</td>
<td>33.0 (6.3)</td>
<td>33.9 (6.3)</td>
<td>0.48</td>
</tr>
<tr>
<td>% of ΔMADRS, mean (SD)</td>
<td>60.2 (34.7)</td>
<td>80.8 (14.8)</td>
<td>14.4 (18.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age of onset (years), mean (SD)</td>
<td>36.4 (14.8)</td>
<td>36.8 (15.5)</td>
<td>35.9 (13.3)</td>
<td>0.83</td>
</tr>
<tr>
<td>% psychotic symptoms</td>
<td>69.0</td>
<td>71.0</td>
<td>64.5</td>
<td>0.52</td>
</tr>
<tr>
<td>% comorbidity with personality disorders</td>
<td>25.0</td>
<td>20.3</td>
<td>35.5</td>
<td>0.11</td>
</tr>
<tr>
<td>% comorbidity with anxiety disorders</td>
<td>32.0</td>
<td>29.0</td>
<td>38.7</td>
<td>0.34</td>
</tr>
<tr>
<td>% comorbidity with alcohol abuse</td>
<td>2.0</td>
<td>1.4</td>
<td>3.2</td>
<td>0.56</td>
</tr>
<tr>
<td>Number of treatments, mean (SD)</td>
<td>7.6 (2.5)</td>
<td>7.2 (1.9)</td>
<td>8.3 (3.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>% administration of typical antipsychotics i</td>
<td>55.1</td>
<td>60.9</td>
<td>40.0</td>
<td>0.07</td>
</tr>
<tr>
<td>% administration of atypical antipsychotics i</td>
<td>58.4</td>
<td>57.8</td>
<td>60.0</td>
<td>0.85</td>
</tr>
<tr>
<td>% administration of SSRIs i</td>
<td>61.8</td>
<td>67.2</td>
<td>48.0</td>
<td>0.09</td>
</tr>
<tr>
<td>% administration of SNRI s</td>
<td>29.2</td>
<td>28.1</td>
<td>32.0</td>
<td>0.72</td>
</tr>
<tr>
<td>% administration of TCAs i</td>
<td>41.6</td>
<td>40.6</td>
<td>44.0</td>
<td>0.77</td>
</tr>
<tr>
<td>% administration of NaSSAs i</td>
<td>27.0</td>
<td>29.7</td>
<td>20.0</td>
<td>0.36</td>
</tr>
<tr>
<td>% administration of benzodiazepines s</td>
<td>93.3</td>
<td>93.8</td>
<td>92.0</td>
<td>0.77</td>
</tr>
<tr>
<td>% administration of mood stabilizers i</td>
<td>16.9</td>
<td>15.6</td>
<td>20.0</td>
<td>0.62</td>
</tr>
<tr>
<td>% administration of anxiolytics i</td>
<td>11.2</td>
<td>7.8</td>
<td>20.0</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Bold numbers indicate significant p-values (<0.05).

* i The total number could exceed the number of subjects due to the presence of multiple drugs administration.
Table 2: Allele and genotype distributions in responder and non-responder patients to ECT treatment for rs11218030, rs1954787, rs4936554, and rs11218030

<table>
<thead>
<tr>
<th>SNP</th>
<th>G/C</th>
<th>p</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11218030</td>
<td>A</td>
<td>0.08</td>
<td>3.28 × 10⁻³</td>
<td>0.0009</td>
</tr>
<tr>
<td>rs1954787</td>
<td>A</td>
<td>0.40</td>
<td>0.007</td>
<td>0.0017</td>
</tr>
<tr>
<td>rs4936554</td>
<td>G</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

+ OR = Odd Ratios were computed only for the most significant model.

collogical treatment characteristics of the patients are shown in Table 1.

2.2. ECT treatment

A medical history and a physical examination along with routine blood and urine examinations, an electrocardiogram, a cerebral computed tomography scan and a chest film were requested to screen for general medical conditions. Anaesthesia for ECT was routinely induced by intravenous thiopental sodium (3.0 mg/kg for males and 2.5 mg/kg for females). Muscle relaxation was achieved with intravenous succinylcholine (0.7 mg/kg). In addition, the patients were premedicated with atropine sulphate (0.5 mg intravenously).

ECT was performed between 7:00 and 9:00 a.m. 3 times per week using a Thymatron DG (Somatics, Inc., Lake Bluff, IL, USA) with standard settings [46,47] with a bipolar brief pulse square wave and bilateral electrode placement [1]. The maximum ECT stimulus was a charge of 504 mC, with a current of 0.9 A, frequency 30–70 Hz, pulse width of 1 ms, duration maximum of 8 s. Stimulus intensity was selected using the age-related method. The ECT treatment was complete based on the clinical judgement of the treating physicians.

2.3. Genotyping

The intronic GRIK4 polymorphisms (rs1954787, rs4936554, and rs11218030) were genotyped using the BeadXpress System and the VeraCode Assay according to the manufacturer’s protocols (http://www.illumina.com). The raw BeadXpress data were processed using the Illumina BeadStudio software suite (genotyping module 3.3.7), producing report files containing the normalised intensity data and SNP genotypes.

The genotypes of all of the samples were confirmed performing SNaPshot assay (Life Technologies). The SNaPshot details have been described elsewhere [48].

2.4. Statistical analysis

Chi-square (χ²) tests were conducted to evaluate the association between the categorical variables, while an analysis of variance (ANOVA) was used to compute possible differences for continuous variables. We used a χ² test to investigate the allelic and genotype association with the ECT response status by comparing the allele and frequencies between the responders and non-responders (the patients were defined as a responder if the percentage MADRS reduction at T1 was >50%); we used Fisher’s exact test when one genotype was rare. In order to quantify the association between the genotype and the response to ECT, the odd ratios (OR) and the corresponding 95% confidence intervals (CI) were used.

All of the analyses were conducted using SPSS Version 17.0 statistical software (SPSS Inc. Chicago, IL).

3. Results

The ECT treatment reduced depression symptomatology as measured with MADRS (T0 = 33.27 ± 6.27; T1 = 13.08 ± 11.84; F₁,99 = 259.00; p < 0.0001), and 69.0% of the patients were considered responders. The socio-demographical and clinical features of the TRD patients and the responder and non-responder to ECT subgroups are shown in Table 1. The mean number of treatment sessions received was 7.6 ± 2.5 (range 5–15). Non-responder patients group received a greater number of trials (p = 0.001).
The rs11218030 analysis revealed differences in the genotype and allele distributions between the responder and non-responder groups ($p = 1.81 \times 10^{-4}$ and $p = 3.28 \times 10^{-5}$, respectively). In particular, the analyses were carried out to test the hypothesis that rs11218030 constitutes a risk factor for a lack of response to ECT. Under the assumption of a codominant, recessive, and dominant model, the dominant model best fit the data ($p = 2.71 \times 10^{-4}$). According to this model, G allele carriers presented an OR of 5.62 (95% CI: 2.17–14.61) for a non-response to ECT compared to the AA homozygotes (see Table 2). The risk effect of the rs11218030 G allele remained significant after adjusting for multiple comparisons in terms of the number of SNPs analysed ($p_{\text{corrected}} = 0.0008$).

With respect to rs1954787, the analysis showed differences in the genotype and allele distributions between the responders and non-responders to ECT ($p = 0.045$ and $p = 0.009$, respectively). The GG homozygous patients had a significant higher risk for a non-response to treatment with ECT ($p = 0.013$; OR $= 2.70$, 95% CI, $= 1.24$–7.13) compared to the A allele carriers (see Table 2). This effect remained significant even after an adjustment for multiple comparisons in terms of the number of SNPs analysed ($p_{\text{corrected}} = 0.039$).

Finally, significant effects were found even for rs4936554, showing differences in the genotype and allele distributions between the two groups ($p = 0.024$ and $p = 0.017$, respectively). In particular, the A allele carriers were more frequent in the non-responder patients ($p = 0.040$; OR $= 2.52$, 95% CI, $= 1.03$–6.16) compared to the GG homozygotes (see Table 2). However, this effect did not remain significant after adjusting for multiple comparisons in terms of the number of SNPs analysed ($p_{\text{corrected}} = 0.12$).

### 4. Discussion

Our study showed that GRIK4 variants were significantly associated with the response to ECT in a sample of TRD and depressive BD patients resistant to pharmacological treatments. In particular, our findings indicate that patients possessing the G allele or the GG genotype of the GRIK4 polymorphism rs11218030 had a significantly poorer response to ECT compared to the patients carrying the A allele and the AA genotype. Interestingly, the rs11218030 G allele carriers showed five times the risk of non-response after ECT treatment compared to the AA homozygotes as soon as a month from the end of the treatment. Analogously, the patients carrying the GG rs1954787 genotype and the rs4936554A allele carriers presented a double risk of non-response after ECT, but the differences found for the rs4936554 polymorphism did not hold up after Bonferroni correction.

In our previous study [30], we showed that the G allele carriers of rs11218030 and the rs1954787 GG homozygotes had a higher risk of developing a psychotic symptomatology during a depressive episode.

The presence of psychotic symptoms is one of the strongest negative predictive factors of MDD treatment [31,32], and because most of treatments fail, ECT is considered an elective therapy for patients with psychotic subtype MDD [49]. Taken together, all of these data could indicate that some of the GRIK4 variants might contribute to a more severe and resistant subtype of MDD.

The particular localisation of GRIK4 in specific brain areas makes it critical for learning and memory along with complex cognitive behaviours, mood and personality [50]. Moreover, it is involved in the rapid transmission of an excitatory synaptic and plays an important role in excitotoxicity neural death [50,51]. All of these peculiar functions make GRIK4 intriguing to study as it is related to ECT activity mechanisms.

Indeed, several lines of evidence showed that both ECT in humans and electroconvulsive shocks (ECS, an animal model of ECT) in rodents impact glutamatergic as well as GABAergic neuro- transmission [33,34,36,52–56]. In particular, it has been reported that ECT results in a normalisation of glutamate deficits in different brain areas [34,53,54,56], and this result is consistent with observations arising from preclinical studies showing an increased glutamate level in rodents brain shortly after ECS [33,55]. Finally because glutamate and GABA exert their functional effects in conjunction, some studies focused on this issue showing that ECS regulates the glutamate to GABA ratio in the hippocampus of the animal models used [52,57]. These neurochemical changes might be an important mechanism of ECT action, mediating the neuroplasticity induced by treatment and producing neuronal remodelling.

Some limitations in our study must be conceded: (1) our samples are comprised of MDD and BD patients resistant to treatment. This could represent a confounding factor; however, several studies have shown that the remission and response rates and numbers of ECT both for unipolar and bipolar patients in a depressive state were equivalent, and consequently, polarity seems not to affect ECT treatment outcome [58]; (2) the data on the functional properties of the SNPs studied are insufficient. Even if there are previous studies showing significant associations between the polymorphisms studied here and pharmacological treatment outcome, there is a very limited number of studies related to the functionality of these polymorphisms useful to explain the biological mechanisms of this involvement; (3) the GRIK4 gene has largely been studied in relation to antidepressant response, while our results showed its involvement in modulating the response to ECT; however, the mechanisms linking the pharmacogenetic data and ECT outcome are not known.

In conclusion, the current study provides new evidence indicating that some GRIK4 variants modulate the response to ECT in patients with depression resistant to treatment, suggesting a role for kainate receptor modulation. Genetic marker information might improve the already demonstrated cost-effectiveness of ECT treatment [59], by contributing to the development of algorithm tools for the previson of the efficacy that takes into consideration several factors, such as clinical, pharmacological, technical and biological variables.

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### References


