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Genetic variables of the glutamatergic system associated with treatment-resistant depression: A review of the literature

Genetics of the glutamatergic system associated with treatment-resistant depression

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Abstract

Depression is a common, recurrent mental disorder and one of the leading causes of disability and global burden of disease worldwide. Up to 15-40% of cases do not respond to diverse pharmacological treatments and, thus, can be defined as treatment-resistant depression (TRD). The development of biomarkers predictive of drug response could guide us towards personalized and earlier treatment. Growing evidence points to the involvement of the glutamatergic system in the pathogenesis of TRD. Specifically, the N-methyl-D-aspartic acid receptor (NMDAR) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), which are targeted by ketamine and esketamine, are proposed as promising pathways. A literature search was performed to identify studies on the genetics of the glutamatergic system in depression, focused on variables related to NMDARs and AMPARs. Our review highlights *GRIN2B*, which encodes the NR2B subunit of NMDAR, as a candidate gene in the pathogenesis of TRD. In addition, several studies have associated genes encoding AMPAR subunits with symptomatic severity and suicidal ideation. These genes encoding glutamatergic receptors could, therefore, be candidate genes for understanding the etiopathogenesis of TRD, as well as for understanding the pharmacodynamic mechanisms and response to ketamine and esketamine treatment.

Key Words: Genetics; NMDA receptor; AMPA receptor; Treatment-resistant depression; Ketamine; Esketamine

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Core Tip: Depression is a common mental disorder and one of the leading causes of disability worldwide. Up to 15-40% of cases are considered treatment-resistant depression, which seems to be conditioned by environmental and genetic factors. The

glutamatergic system and, specifically, N-methyl-D-aspartic acid receptor (NMDAR) dysfunction, has been proposed to be involved in the pathogenesis of TRD. A literature search was performed to identify studies on the genetics of the glutamatergic system in depression, focused on NMDAR and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. Our review highlights *GRIN2B*, which encodes the NR2B subunit of NMDAR, as a candidate gene in the pathogenesis of TRD.

INTRODUCTION

DEPRESSION: AN OVERVIEW.

Depression is characterized by sustained low mood, anhedonia, psychomotor inhibition and, frequently, somatic alterations that significantly affect an individual's functioning and, as such, poses a social and economic problem, as well as a health problem. It is, according to the World Health Organization, a common, recurrent mental disorder and one of the leading causes of disability and global burden of disease worldwide [1]. Thus, it has been highlighted as one of the priority conditions covered by the Mental Health Gap Action Programme. The 12-month prevalence of major depressive disorder (MDD) is estimated to be approximately 6% [2], whereas the lifetime risk of depression is between 15 and 18% [3]. Thus, MDD is common, with almost one in five people experiencing one episode at some point in their lifetime. Between 2005 and 2015, the incidence of depression increased by 18.4% worldwide [4]. Regarding gender differences, the lifetime-incidence of a major depressive episode in females has been reported to be twice that of males [5].

Depression significantly affects family, social and occupational functioning and is, therefore, a health, social and economic problem. A recent review calculated that the direct costs of depression, due to the higher use of healthcare services, may be up to 24,069€ per patient/year, depending on the jurisdiction wherein the analyses were performed [6]. Productivity losses, for their part, were estimated to be between 1963€ and 27,364€ per person per year [6]. It is among the leading causes of loss of disability-adjusted life years, mainly in the age range between 10 and 49 years [7], and is the most

frequently identified diagnosis in people who have died by suicide [8]. Thus, in recent years, depression has become a major target of public health policies [9,10] due to the consequences that both depression itself, as well as associated events such as suicide, have on society.

If depression is untreated or inadequately treated, it is associated with higher rates of medical morbidity, lower productivity, decreased life expectancy, higher rates of suicide and higher rates of functional disability. However, sometimes, despite evidence-based treatment, the patient may not respond favorably to treatment. Even though we have a growing number of therapeutic alternatives available to treat depression, approximately half of patients do not respond, and up to two-thirds do not achieve remission after first-line treatment [11]. In this context, the development of biomarkers predictive of drug response, which could guide us towards personalized treatment for each patient, is a challenge for the future.

TREATMENT-RESISTANT DEPRESSION

Although there is no consensus on the definition of treatment-resistant depression (TRD), it is a useful concept to characterize a group of patients with MDD that do not respond to traditional monoaminergic antidepressants. The European Medicines Agency considers TRD to be that which has not responded to two antidepressants of different classes, prescribed at adequate doses (within a therapeutic range), for the appropriate time (>6 wk) and ensuring correct adherence to the protocol [12][13]. Some authors add that potentiation strategies, such as lithium, neuroleptic drugs, or electroconvulsive therapy, also need to have been used.

According to the well-known Sequenced Treatment Alternatives to Relieve Depression study, one-third of patients with depression could be classified as TRD, as they do not respond to two different antidepressant treatments [11]. Along the same lines, other works have described that 15-40% of patients with MDD do not respond to multiple pharmacological treatments [14].

Patients with MDD are more likely to make attempts and/or complete suicide, as well as to experience more frequent relapses and hospitalizations, and to have a worse overall prognosis. In other words, they form a subgroup of depressive patients characterized by clinical severity and higher health and social costs [15].

Resistance to treatment seems to be conditioned by genetic and environmental factors [16]. The underlying genetic factors of individuals cannot be modified, but genetic information could be used to predict response and tailor treatments to the idiosyncrasies of each patient. Emerging evidence has shown that genetic variations associated with antidepressant responses appear to cluster in families, supporting the importance of these variations in the underlying mechanism of depression, especially in TRD [17].

Identifying biomarkers that can predict the antidepressant response could be helpful in designing the initial treatment, decreasing the need for trial-and-error testing, also avoiding suffering and possible chronicity. Single nucleotide polymorphisms (SNPs) have been suggested to be a decisive factor in the antidepressant response; numerous genetic polymorphisms have been described as possible risk factors for MDD and TRD [18–21].

THE GENETICS OF DEPRESSION

Albeit a clinically heterogeneous pathology, there is consistent evidence, based on twin and adoption studies, that there is a heritability of 29-49% in MDD (reviewed in [22]). Research has also been performed to identify more genetically homogeneous groups of MDD, indicating that clinical severity, the need for certain therapeutic strategies, recurrent episodes and postpartum depression show differences in heritability [23,24].

It is a polygenic disease caused by the combined effect of polymorphisms, common to the general population, in different genes [25,26]. The genetics of depression has been studied for years *via* a candidate gene approach, mainly focusing the study on genes involved in the serotonergic, noradrenergic and dopaminergic pathways, targets

of the usual treatments [27–29]. A recent literature review of 18 candidate genes showed that most of the studies performed lacked sufficient statistical power and, thus, questioned previous depression candidate gene findings [30]. More recent work has begun to focus on the glutamatergic pathway as a candidate in the study of genetic factors involved in depression [31].

In recent years, genome-wide association studies (GWAS) have proliferated in an attempt to identify genes involved in various pathologies, including depression. A recent GWAS identified 102 independent variants, 269 genes, and 15 gene-sets associated with depression, including both genes and gene pathways associated with synaptic structure and neurotransmission, providing further evidence of the importance of prefrontal brain regions. A previous GWAS implicated voltage-gated calcium channels, the D2 dopamine receptor and, interestingly, glutamate receptors [32]. The authors stated that all humans carry a lesser or greater number of genetic risk factors for MDD.

Along this line, many authors have investigated the interaction between genetics and environment in the pathogenesis of depression. Recent reviews concluded that various genetic polymorphisms in the serotonergic system moderate the association between adverse childhood experiences and depression [33], and that early-life stress produces transcriptomic changes that are moderated by the female sex [34].

Finally, postmortem studies have also been conducted to investigate differential gene expression in human brains. *GluR* gene expression in the dorsolateral prefrontal cortex has been studied in small postmortem cohorts of MDD subjects and controls, with inconclusive results to date [35,36]. Nonetheless, the data seemed to indicate a fundamental dysfunction of the glutamatergic system in the frontal cortex in MDD [37].

THE GLUTAMATERGIC SYSTEM AND TRD

The neurotransmitter systems most studied in the etiopathogenesis of depression have been the serotonergic, noradrenergic and dopaminergic systems, which are targeted by the most commonly used antidepressant drugs. However, another system

involved is the glutamatergic system. Glutamate exerts its action *via* ionotropic receptors (N-methyl-D-aspartic acid receptor [NMDAR], α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor [AMPAR], 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine kainate receptors [KAR]) and metabotropic receptors. In the last two decades, the glutamatergic system, and specifically NMDAR dysfunction, has been shown to be involved in the pathogenesis of TRD [38]. In particular, NMDAR antagonism has been highlighted, marking it as a target of numerous drugs indicated for TRD [39], such as ketamine and esketamine [40–42].

Intravenous ketamine and intranasal esketamine, rather than inhibiting, activate glutamate release [43], resulting in a rapid antidepressant effect, a prompt disappearance of suicidal ideation [44,45] and a reduction of anhedonic symptoms [46]. This emerging hypothesis suggests that NMDAR antagonism in GABAergic interneurons (the mechanism of action of ketamine and esketamine) leads to glutamate release [47]. Regarding this gamma-amino butyric acid (GABA)-glutamate neurotransmitter system, animal and human studies have described that intravenous ketamine administration reduces GABA concentration in several brain areas, such as the frontal cortex [47–49].

Treatment with ketamine and esketamine has proven particularly useful in cases of TRD [50], thus their mechanism of action in glutamatergic pathways, being the major difference with respect to usual antidepressant treatments, is an interesting starting point for understanding the etiopathogenesis of TRD.

Central glutamatergic activity is measured at the peripheral level *via* plasma levels of glutamate (pGlu) and GABA (pGABA). pGlu and pGABA levels have been described to significantly correlate with cerebrospinal fluid glutamate levels [51,52], indicating that, although the plasma levels assessed derive from both the brain and the periphery, the plasma levels of these amino acids reflect brain concentrations [53,54]. Previous studies reported altered levels of pGlu in blood, cerebrospinal fluid, and prefrontal, frontal, and occipital cortical areas of patients with depression compared with healthy volunteers [55–58].

In relation to GABA, as a neurotransmitter system closely related to the glutamatergic, a recent meta-analysis indicated a decrease in pGABA levels in patients with depression compared with healthy controls, although the heterogeneity was significant [59].

All these findings indicate that alterations in the glutamatergic system may play a key role in the development of TRD. Therefore, it has been proposed that genes involved in glutamatergic transmission could be candidate genes to explain the neurobiological basis of TRD, i.e., genetic risk factors for the development of depression, especially TRD [58,60].

GENETIC VARIABLES OF THE GLUTAMATERGIC SYSTEM ASSOCIATED WITH TRD

Methods

A literature search was performed to identify studies regarding the genetics of the glutamatergic system in depression. A total of 118 articles, published up to October 15, 2021, were retrieved from the PubMed database using broad search terms in order to identify as many potentially eligible studies as possible: [(NMDA receptor OR AMPA receptor) AND gene* AND depression]. An age filter was added: "Adults: 19+ years". Studies were included according to three criteria: 1) they investigated the influence of genetics/epigenetics on glutamate receptors in depression; 2) they were systematic reviews, meta-analyses, narrative reviews or original research studies; 3) they were written in English or Spanish. The reference lists of the selected studies and reviews were also checked to identify additional relevant articles using a snowballing approach. Finally, 46 papers were included in the review.

This is not a systematic review, but a narrative one; it summarizes the findings described in the selected reports and, in this way, provides an overview of the subject. The main results are summarized in Table 1.

The NMDA receptor

NMDAR, indicated as a therapeutic target in TRD [61], consists of four subunits (Figure 1). Two of them must be the NR1 subunit, mandatory for the receptor to be functional, while the other two subunits can be any of the four NR2 subunits (NR2A-D), or two NR3 [62]. The NR2A-D subunits bind glutamate [63]. These subunits are encoded by the *GRIN1, GRIN2A-D* and *GRIN3* genes [64,65].

NR2B subunit: the GRIN2B gene

Associations between the functionality of these subunits and depression or response to antidepressant molecules were mostly found with the NR2B subunit. This is encoded by the *GRIN2B* gene, located on chromosome 12p12, and consists of 13 exons. Three potentially functional SNPs have been identified in this gene, all located in the 3'-UTR region that governs gene expression: rs1805502 (A to G), rs1806201 (T to C) and rs890 (A to C). They may contribute to the regulation of *GRIN2B* gene expression and influence glutamate release activity in the brain.

Ketamine users have also been reported to have a higher frequency of the TT-rs1806201 genotype and a lower frequency of the CC-genotype than controls, suggesting that this polymorphism may play a role in ketamine abuse [66]. Clinical trials report the superior therapeutic efficacy of NMDAR NR2B subunit antagonists over conventional antidepressants in patients with TRD [67,68].

Different GWAS have revealed a relationship between SNPs in the *GRIN2B* gene and depression [39,69,70]. *In vivo* studies performed evaluating glutamatergic activity at the brain level have shown that carriers of the G-rs1805502, T-rs1806201 or C-rs890 allele have decreased glutamate concentrations in the anterior cingulate cortex. These alleles have been related to several psychiatric disorders [71–73], suggesting that they be a risk factor (genetic predictor) for TRD [72] in MDD patients. Recently, in a preclinical study in transgenic mice with selective mutations in the NR2B subunit in GABAergic interneurons, deletion of NR2B was found to block the antidepressant action of ketamine [74].

These *GRIN2B* gene polymorphisms have been described both as risk variables for MDD and predictors of TRD. An association was reported between the GT haplotype (rs1805502-rs890) and increased TRD risk compared with controls, as well as between the G -rs1805502 allele and TRD (compared with non-resistant depression) [72].

A GWAS-based study reported a significant association between *GRIN2B* and suicide attempts, as well as a gene-environment relationship with a history of physical abuse in childhood and adolescence, which also increases the risk of suicide [75]. Indeed, they found that *GRIN2B* and *ODC1* (encoding ornithine decarboxylase, a rate-limiting enzyme of the polyamine synthesis pathway) seem to be associated with severe suicide attempts, as well as with serious physical assault in childhood and adolescence [76,77], which in turn increases the risk of suicide attempts, thereby configuring a gene-by-environment interaction.

Finally, human postmortem studies found *GRIN2B* expression to be higher in suicidal MDD patients, compared with non-suicidal MDD patients [37], and in the locus coeruleus of depressed individuals [78,79]. It is therefore postulated that *GRIN2B* mRNA levels may be biomarkers of suicide; indeed, *GRIN2B* genetic polymorphisms in MDD have been reported to predict treatment resistance, suicide attempts, and reasoning ability [75].

Based on the data described, *GRIN2B* is considered a promising candidate gene for MDD susceptibility, and more specifically for TRD, supporting the contention that TRD can be classified as a specific subtype of MDD [72].

Other NMDAR subunits: GRIN1, GRIN2A, C, D and GRIN3

Regarding other NMDAR subunits, postmortem studies in rodents using depression models have observed that chronic stress, besides increasing NR2B subunit mRNA, also increases *NR1* and *NR2A* in several brain regions [80,81]. Postmortem studies in humans reported higher expression levels of *GRIN1* and *GRIN2A* in the brains of depressed patients than in controls, and of *GRIN2B* in suicidal compared with non-suicidal MDD patients [37,82]. Likewise, the *GRIN2A* rs16966731 polymorphism (T to C

intron area) has been associated with the rapid and persistent antidepressant effect of ketamine [83]. Chandler *et al*., also reported altered expression of the *GRIN2C* gene at the locus ceruleus in depressed patients [78]. Finally, one paper reported that women with MDD had higher expression levels of all the NMDAR subunit genes; the only one not reaching statistical significance was *GRIN3A* [37].

From a gene-environment interaction perspective, an epigenetic study showed that *GRIN1* methylation was a significant predictor of depression in a sample of abused children [84]. In one study, *GRIN2A* hypermethylation in the hippocampus and prefrontal cortex in postmortem studies was related to overexpression of the GluN2A subunit [85,86]. Interestingly, maternal separation increases the expression of this subunit in the hippocampus of adult rats, but not of subunit 2B. Numerous rat stress models have evaluated *GRIN1A, GRIN2B* and *GRIN2A* with results similar to those described above [81,87,88].

The AMPA receptor: the GRIA2 and GRIA3 genes

AMPARs are transmembrane ionotropic glutamatergic receptors and the main receptors mediating rapid synaptic neurotransmission in the brain. They consist of four subunits (GluR1-4) encoded by four genes (*GRIA1-4*) [89]. Evidence also suggests that the antidepressant mechanism of action of ketamine and esketamine involves the activation of AMPARs, with a subsequent increase in brain-derived neurotrophic factor levels (usually reduced in patients with depression) [47], as has been observed in rodent models [90]. Therefore, AMPARs have been proposed to play a key role in the antidepressant effect associated with ketamine [91].

Ketamine and its enantiomer, esketamine, lead to the disinhibition of glutamatergic neurons that modulate AMPARs by antagonizing the NMDAR of GABAergic interneurons [47,92]. In addition, ketamine metabolites such as hydroxynorketamine seem to exert their antidepressant effect *via* AMPAR activation [88,93].

In mice models reproducing depression and stress, an increased expression of AMPARs has been observed [80,94]. Postmortem studies described increased *GRIA2-4* expression in the prefrontal cortex of MDD patients *vs* controls [37], and of *GRIA3* in suicidal *vs* non-suicidal patients with MDD. As regards the *GRIA2* gene, several authors have observed an association between carriers of the C-allele (rs4302506; C to T, located in the coding exon) and carriers of the T-allele (rs4400397; C to T, 3'-UTR), and a lower age of MDD onset [95]. Also, the G-allele (rs4825476; G to A, intron 3) of the *GRI3A* gene has been associated with suicidal ideation in patients with major depression treated with monoaminergic antidepressants [17,96]. The AMPAR subunits GluA2-4 had a significantly higher expression in female MDD patients [37].

Other glutamatergic receptors associated with treatment-response

Animal studies have suggested that ionotropic glutamate receptors play a role in the action of antidepressant drugs [97,98]. Another widely investigated gene is *GRIK4*, which encodes subunit 4 of the ionotropic glutamate KAR. Here, an association was observed between the rs1954787 polymorphism and antidepressant response [99]; however, the *GRIK4* polymorphism with the highest predictive value for treatment outcome was rs12800734. Nonetheless, these findings have not been replicated in other studies, probably due to design differences [100]. Existing data also revealed increased expression of the KAR subunits, GluK1, and GluK2. In addition, the strongest predictor of suicide was *GRIK3* (GluK3) expression in both sexes [37]. KARs appear to regulate L-glutamate release by functioning as facilitatory or inhibitory autoreceptors during repetitive synaptic activation. The KAR activity may contribute to excitotoxic cell death; however, the role of these receptors in the dorsolateral prefrontal cortex of MDD subjects remains to be elucidated. Genetic variation in *GRIK3* has been associated with recurrent MDD [37].

In addition to ionotropic glutamatergic receptors, metabotropic receptors have also been involved in the genesis of MDD. An increased expression of *GRIN1, GRIN2A-D, GRIA2-4, GRIK1-2, GRM1, GRM4, GRM5* and *GRM7* has been observed in female MDD

patients. In contrast, *GRM5* expression was lower in male MDD patients relative to male controls. When suicidal MDD patients were compared with non-suicidal patients, *GRIN2B, GRIK3* and *GRM2* were expressed at higher levels in suicidal patients [37]. Recent studies show that mGluR4 regulation is altered in male suicidal individuals, leading to a relatively higher expression of mGluR. Higher expression levels of the *mGluR2* gene, *GRM2*, were also detected; *GRM2* has been proposed as a biomarker of suicide [37].

Repeated stress in male rats has been reported to be associated with a lower expression of AMPARs and NMDARs, and, also, with a lower activity of these receptors. In contrast, in female rats exposed to stress, the expression of AMPARs and NMDARs was normalized *via* the activation of estrogen receptors, resulting in a neuroprotective and procognitive effect [101]. The authors propose that, in female patients, estrogenic activity may lead to a differential response to ketamine; it should be noted that two-thirds of MDD patients are women.

Finally, there is downregulation of metabotropic receptors in mice reproducing models of depression, especially in the mGlu2 subunit, which is completely restored by ketamine administration [102].

LIMITATIONS AND STRENGTHS

The main limitation of this review is the scarcity and heterogeneity of the literature available on the topic. Few studies have employed similar methodology and, thus, there is limited replication of the described findings. Due to the small number of studies, all research conducted in humans and animals has been included in the review, although the extrapolation of the results, in this case, is limited. As we have noted, this was a narrative review, and limitations inherent to this type of review should also be mentioned: study selection, data extraction and synthesis are not protocol-based and, thus, could be prone to bias.

Nonetheless, it should also be noted that this is the first review, to our knowledge, of this specific topic, making it possible to summarize the current state-of-the-art, highlighting the need to advance research in this field.

CONCLUSION

Patients with TRD often experience long periods of therapeutic trials with different antidepressant medications, resulting in a worse outcome, a delay in symptomatic remission and an increased risk of fatal events, such as suicide. Therefore, the management of TRD with appropriate therapy could be facilitated by the identification of biological markers of TRD, which could guide treatment choice from the outset.

Although the serotonergic, noradrenergic and dopaminergic pathways were those historically studied, more recent work indicates the involvement of the glutamatergic pathway. This proposal is consistent with new therapeutic strategies in TRD, such as ketamine and esketamine, which act mainly on glutamatergic receptors.

Our review highlights *GRIN2B*, which encodes the NR2B subunit of NMDAR, as a candidate gene in the pathogenesis of TRD. In addition, several studies have associated genes encoding AMPAR subunits with symptomatic severity and suicidal ideation. These genes encoding glutamatergic receptors could, therefore, be candidate genes for understanding the etiopathogenesis of TRD, as well as for understanding the pharmacodynamic mechanisms and response to ketamine and esketamine treatment. However, further empirical work is required to replicate the observed associations and to confirm the involvement of these genes in the pathogenesis of TRD.

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