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## Early and delayed treatment of bipolar disorder

Using Danish registry data, Kessing *et al* examined the relationship between lithium response and the timing of treatment (early *v.* delayed).[1] Early treatment was associated with an increased probability of lithium response. This is a clinically important finding, given the increasing emphasis on early intervention in bipolar disorder. The results of the Kessing *et al* study are sobering. Only few patients, particularly among those for whom treatment was delayed, responded to lithium. Several factors may have contributed to the reported results.

The study did not – and possibly could not – control for the cycle shortening that is observed after successive episodes of bipolar disorder. Although the interpretation of such cycle shortening has been debated,[2] it is well established that early cycles are significantly longer than those occurring later; consequently, early in the course of illness one would expect longer spontaneous remissions regardless of treatment. This effect may be partially responsible for the greater treatment response in patients receiving early intervention in the Kessing *et al* study.

Naturalistic studies typically demonstrate full response in about 30% of participants[3] (that is, no recurrences, or the Kessing *et al* criterion, in treatment-adherent patients), which is markedly greater than the response rate observed by Kessing *et al*. This discrepancy could be related to age at first contact. The average age of participants whom Kessing *et al* reported as having received early and late treatment was 46.7 years and 49.1 years, respectively. The natural history of bipolar disorder includes an average age at onset in the second or third decade of life. The trajectory of the illness, where mania typically develops as the last stage, delays the diagnosis of bipolar disorder. Also, there is often a substantial delay in starting treatment even following the diagnosis of bipolar disorder.[4,5] These reports, in conjunction with the advanced age at index presentation, and high rates of antidepressant, antipsychotic and anticonvulsant use in the Kessing *et al* study suggest that participants may have been afflicted with bipolar disorder for some time before 'first contact'. In a sample of 450 participants, Baldessarini *et al* reported a negative relationship between treatment latency and effect of treatment on time spent ill.[5] If the aforementioned findings are generalisable to the Danish sample, the reduced overall treatment responses may be interpreted as a consequence of relatively advanced participant age.

Finally, Kessing *et al* analysed data collected since 1995. Is it possible that participants had received lithium during the years prior? This would further complicate the interpretations of sample responsiveness to lithium, regardless of early or late initiation. In conclusion, we suggest that the findings presented by Kessing *et al* are limited by the lack of control for inter-participant differences in the manifestation of the natural history of bipolar disorder. Such control may be difficult, or in some cases impossible, to achieve using registry-based observational data, but is nevertheless imperative to understanding the effects of early *v.* late treatment prophylaxis in relapsing–remitting illnesses such as bipolar disorder.

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**Authors' reply:** We are confident that the relatively low response rates to lithium in our study relate to the narrow definition of lithium response, rather than to characteristics of the included patients.[1] Thus, we intended to characterise patients who had an excellent response to lithium monotherapy; that is, patients who were 'cured' from further affective episodes following a start-up period of lithium as in a prior study.[2] We used two robust clinical indicators to define excellent lithium response: (a) lithium prescribed in monotherapy; and (b) no need for psychiatric hospital admission. By doing this, we defined lithium response in a rather rigorous way, resulting in relatively low rates of response. We do not find that our definition of lithium response hampered the finding of the study that early treatment with lithium was associated with increased probability of excellent lithium response compared with delayed treatment, or hampered the generalisability of this finding. Although cycle acceleration occurs on average in bipolar disorder[3,4] the results of our study may suggest that early treatment with lithium might prevent progression of bipolar disorder.

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## 'Reasonable adjustments' for vulnerable patients

We support the views of Tuffrey-Wijne & Hollins[1] and their argument for the NHS to take an organisational approach to embed documentation and provision of reasonable adjustments for those with protected characteristics under the Equalities Act 2010. Lord Darzi defined quality for the NHS as comprising three dimensions: safety, effectiveness and patient experience.[2] The provision of reasonable adjustments is central to each of these.

Safety – Tuffrey Wijne & Hollins rightly identify the lack of provision of reasonable adjustments as being a patient safety issue. The Confidential Inquiry into Premature Deaths of People with

Learning Disabilities (CIPOLD)[3] demonstrated an underlying culture in which people with intellectual disabilities were disadvantaged in accessing equitable healthcare and at risk of premature death because equality for disabled people was assumed to mean treating them the same as others. It does not. Alternative methods of making services available have to be found in order to achieve equality of outcomes. Mizen *et al*, for example, demonstrated that clinical guidelines can actually increase health inequalities for people with intellectual disabilities if reasonable adjustments are not made.[4] If the lack of reasonable adjustments threatens to compromise safety as, in very many cases, it does for people with intellectual disabilities, this needs to be reported and reviewed as a patient safety issue.

Effectiveness – evidence put forward by Tuffrey-Wijne *et al* suggests that ward culture, staff attitudes and staff knowledge are crucial in ensuring that hospital services are accessible to vulnerable patients.[5] Effective care is that which is tailored to the needs of the patient, and this must involve an understanding of the adjustments they need in order to be able to receive appropriate medical and nursing care. In our view, we should go further than Tuffrey-Wijne & Hollins' requirement for Care Quality Commission inspections in England and Wales to oversee patient-specific recording of reasonable adjustments. We also need to be confident that such adjustments are being delivered, and for evidence to be provided of adequate arrangements being in place.

Patient experience – Turner & Robinson note that it is difficult for people with intellectual disabilities and their families to influence policy and practice in healthcare systems if they are not visible within them and if involvement mechanisms such as surveys and focus groups are not accessible to them.[6] Both the Death by Indifference[7] and CIPOLD reports highlighted the lack of attention paid to the views of patients and their families, preventing them from becoming active partners in their care; the CIPOLD report additionally noted the devastating impact on future care that a poor experience of healthcare can have for some people with intellectual disabilities. The provision of reasonable adjustments needs to extend to the ways in which we garner the views of people with intellectual disabilities, communicate with them, and place them at the centre of their care.

The CIPOLD report made 18 recommendations, which included (a) clear identification of people with intellectual disabilities on the NHS central registration system and in all health care records, and (b) reasonable adjustments required by, and provided to, individuals, to be audited annually and examples of best practice shared across agencies and organisations.[3]

It is now 4 years since the Equalities Act 2010 came into force. Our adherence to the Act must be sharpened in the light of the health inequalities faced by people with protected characteristics, including those with intellectual disabilities, so clearly demonstrated in successive reports. We all have a responsibility, and we all have a role to play, in ensuring equal outcomes for vulnerable people through the provision of reasonable adjustments, but strong leadership is central to making it happen.


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**Authors' reply:** We welcome the detailed response from Heslop *et al* giving more evidence in support of our recommendation for the effective use of reasonable adjustments during in-patient care. They also draw attention to the need for these to be properly audited by staff who understand the Equality Act 2010, which in our view would require an extensive educational programme, as there is no evidence that current audits are much more than a box-ticking exercise.

They repeat an earlier and often made recommendation that people with intellectual disabilities should be identified on a national NHS database. NHS England has already decided to set up a national learning-disability mortality review function, which will require a national database. Regrettably, this cannot commence until data linkages have been enabled by the NHS and the Health and Social Care Information Centre and it seems unlikely that this will be achieved until next summer.[1] Strong advocacy is needed to ensure there are no further delays in giving priority to this work.


1 Hansard. HL Deb 30 July 2014 vol 755 col 1583.



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## Corrections

Aripiprazole once-monthly for treatment of schizophrenia: double-blind, randomised, non-inferiority study. *BJPsych*, **205**, 135–144. Figure 3(a), p. 141: x-axis label should be 'Days from randomisation'. The online version of this paper has been corrected post-publication, in deviation from print and in accordance with this correction.

Cost-effectiveness of injectable opioid treatment *v.* oral methadone for chronic heroin addiction. *BJPsych*, **203**, 341–349. In the abstract, the second sentence of the Results should read: 'Costs overall were highest for oral methadone (mean £15 805 *v.* £13 410 injectable heroin and £10 945 injectable methadone; $P$ = n.s.) due to higher costs of criminal activity'. These data were reported correctly in the body of the paper (Table 2, p. 344). The online version of this paper has been corrected post-publication, in deviation from print and in accordance with this correction.


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LETTER TO THE EDITOR

# Brain-derived neurotrophic factor (BDNF) and its precursor proBDNF as diagnostic biomarkers for major depressive disorder and bipolar disorder

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## Letter to the editors

Major depressive disorder (MDD) and bipolar disorder (BD) are the most common adult-onset mood disorders. They are two etiologically related, but clinically distinct psychiatric illnesses. Their shared clinical features result in high rates of misdiagnosis, due to lack of differentiating biomarkers between the disorders. More frequently, BD is misdiagnosed as MDD, because of their overlapping symptomology, the often later onset of mania, and the frequent occurrence of depressive episodes in BD patients. Misdiagnosis is also high when BD patients present symptoms indicative of a clinically significant depressive episode, but are premorbid for manic symptoms, or previous manic states have not been recognized. Incorrect treatment of BD with antidepressant monotherapy increases the risk of antidepressant-induced mania and "cycle acceleration" (an increased frequency of episodes), both of which can have detrimental effects on disease prognosis in these patients. Therefore, the development of specific biomarkers for these disorders would be invaluable for establishing the correct diagnosis and treatment of MDD and BD [1].

Brain-derived neurotrophic factor (BDNF), a major neurotrophic factor in the brain, is integral to the pathophysiology of mood disorders, as well as the therapeutic mechanisms of antidepressants and mood stabilizers [2–6]. BDNF (mature BDNF) is a 13 kDa polypeptide, which is initially synthesized as a precursor protein, preproBDNF, in the endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF (~32 kDa) is converted to mature BDNF by extracellular proteases. It was initially thought that only secreted, mature BDNF was biologically active, and that proBDNF, which localizes intracellularly, served as an inactive precursor. However, accumulating evidence shows that both proBDNF and mature BDNF are active, eliciting opposing effects via the $p75^{NTR}$ and TrkB receptors, respectively, and that both forms are important in several physiological functions [2–6].

In 2003, we reported that serum levels of BDNF in drug-naive patients with MDD were significantly lower than those of healthy controls and that serum BDNF levels increased after antidepressant treatment [7]. A recent meta-analysis of 179 associations ($N = 9{,}484$ subjects) showed that serum levels of total BDNF (mature BDNF and proBDNF) in antidepressant-free patients with MDD were significantly (Cohen's $d = -0.71$, $p < 0.0000001$) lower than those of healthy controls [8], supporting our previous report [7]. The BDNF enzyme-linked immunosorbent assay (ELISA) kits, used in the previous reports, recognized both proBDNF and mature BDNF, due to the limited specificity of this particular BDNF antibody [9]. Using new commercially available human BDNF ELISA kits which differentiate between proBDNF and mature BDNF, we reported high concentrations of both proBDNF and mature BDNF in human serum [9]. Subsequently, we reported that in medicated patients with MDD, serum levels of mature BDNF, but not proBDNF, were significantly lower than those of healthy controls [10]. In contrast, we recently reported that serum levels of mature BDNF and the ratio of mature BDNF to proBDNF in mood-stabilized BD patients were significantly higher than in healthy controls [11]. Interestingly, serum levels of proBDNF in mood-stabilized patients with BD were significantly lower than those in healthy controls [11]. These findings in BD were confirmed in two

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independent cohorts (Sahlgrenska set and Karolinska set in Sweden) [11]. It is therefore possible that measuring the blood levels of mature BDNF and proBDNF could provide a method for distinguishing between MDD and BD, thus reducing the high rates of misdiagnosis between these diseases. Further studies using larger sample sizes are needed to confirm this theory.

In contrast, Tunca et al. [12] reported that BD patients showed significantly lower serum levels of total BDNF (proBDNF and mature BDNF) during mania and depression, compared with euthymic patients and healthy controls. They also found that serum lithium levels showed significant positive correlation with BDNF levels. This paper suggests that lithium may exert its therapeutic action by up-regulating BDNF to achieve euthymia [12]. Epigenetic regulation of the *BDNF* gene has been implicated in the pathophysiology of both MDD and BD. A recent study showed higher levels of DNA methylation at the *BDNF* gene promoter in MDD and BD II, compared with BD I patients, as well as higher methylation levels in depressed patients compared with manic/mixed patients [13]. A recent meta-analysis study showed that the *BDNF* (Val66Met) gene polymorphism is not associated with serum BDNF levels [14] although previous reports showed mixed evidence for this association. Therefore, the effects of medication, epigenetics and *BDNF* gene polymorphisms (e.g., Val66Met) on serum levels of proBDNF and mature BDNF need be taken into account.

In conclusion, considering the differential expression of proBDNF and mature BDNF in human blood, their ease of detection and putative opposing functions, measurements of proBDNF and mature BDNF could represent diagnostic biomarkers for MDD and BD.

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**Conflict of interest** Dr. Hashimoto is a holder of the patents "Diagnostic and examination method for eating disorder" (US 7,754,434 B2) and "Diagnostic agent for ischemic heart disease risk group" (US 2013/0310321A1); which pertain to the measurement of BDNF as a biomarker. In addition, Dr. Hashimoto has served as a scientific consultant to Astellas, Dainippon-Sumitomo and Taisho, and he has also received research support from Abbvie, Dainippon-Sumitomo, Otsuka and Taisho.

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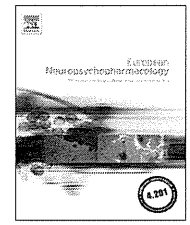

# Markers of glutamate signaling in cerebrospinal fluid and serum from patients with bipolar disorder and healthy controls

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N-methyl-D-aspartate

**Abstract**
Glutamate is the major excitatory neurotransmitter in the brain. Aberrations in glutamate signaling have been linked to the pathophysiology of mood disorders. Increased plasma levels of glutamate as well as higher glutamine+glutamate levels in the brain have been demonstrated in patients with bipolar disorder as compared to healthy controls. In this study, we explored the glutamate hypothesis of bipolar disorder by examining peripheral and central levels of amino acids related to glutamate signaling. A total of 215 patients with bipolar disorder and 112 healthy controls from the Swedish St. Göran bipolar project were included in this study. Glutamate, glutamine, glycine, L-serine and D-serine levels were determined in serum and in cerebrospinal fluid using high performance liquid chromatography with fluorescence detection. Serum levels of glutamine, glycine and D-serine were significantly higher whereas L-serine levels were lower in patients with bipolar disorder as compared to controls. No differences between the patient and control group in amino acid levels were observed in cerebrospinal fluid. The observed differences in serum amino acid levels may be interpreted as a systemic aberration in amino acid metabolism that affects several amino acids related to glutamate signaling.


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## 1. Introduction

Glutamate is the major excitatory neurotransmitter in the brain. Aberrations in glutamate signaling have been linked to psychiatric disorders such as schizophrenia (Javitt and Zukin, 1991), alcohol dependence (Lovinger et al., 1989), and obsessive-compulsive disorder (Rothbaum, 2008). Aberrant glutamate signaling has also been implicated in the pathophysiology of mood disorders. Specifically, increased plasma glutamate levels have been observed in patients with mood disorders compared to healthy controls (Altamura et al., 1993; Mauri et al., 1998), and a recent meta-analysis concluded that patients with bipolar disorder have higher glutamine+glutamate levels in the brain compared to healthy controls, as measured by magnetic resonance spectroscopy (Gigante et al., 2012). Further, a postmortem study showed higher glutamate levels in the frontal cortex of patients with bipolar disorder (Hashimoto et al., 2007). A role of the glutamatergic system in the pathophysiology of bipolar disorder is also supported by findings of altered glutamate receptor expression and glutamate receptor polymorphisms as well as significant effects of mood stabilizers on glutamatergic transmission (Sanacora et al., 2008).

Several of the currently used mood stabilizers have affinity for glutamate receptors (Machado-Vieira et al., 2009). The N-methyl-D-aspartic acid receptor (NMDAR) subtype of glutamate receptors is of particular interest since the non-competitive NMDAR antagonist ketamine has shown antidepressant effects in humans (Berman et al., 2000) as well as in animal models of depression (Duman and Aghajanian, 2012). Moreover, administration of synthetic NMDAR antagonists can also cause psychotic symptoms (Krystal et al., 1994), which is a common feature during manic episodes in bipolar disorder. In line with this, we have shown that kynurenic acid, which is an endogenous antagonist of NMDAR, is increased in cerebrospinal fluid (CSF) of patients with bipolar disorder who have had psychotic episodes (Olsson et al., 2012).

Measurements of peripheral levels of amino acids related to glutamate signaling may improve the understanding of glutamatergic function in psychiatric disorders and how the glutamatergic system is modified by psychoactive medications. However, the biochemical composition in blood does not necessarily translate to the brain biochemistry influencing neural operation. Analyses of peripheral biomarkers should therefore preferably be paralleled by analyses of neurochemicals in CSF.

The primary aim of this study was to test if serum concentrations of amino acids correlate with CSF concentrations and thus provide information on brain glutamatergic signaling. A secondary aim was to test if amino acid sampling might serve as a marker of pharmacological treatment effects related to changes in glutamatergic signaling. To these ends, we determined serum levels of glutamate, glutamine, glycine, L-serine, and D-serine from mood stabilized bipolar disorder patients and sex- and age-matched healthy controls. In a subset of these patients and controls, we also determined these amino acids in CSF.

## 2. Experimental procedures

The study population was recruited from the St. Göran bipolar project, which provides assessment, treatment, and follow-up of patients with bipolar disorder within the Northern Stockholm Mental Health Service and serves as a basis for research into bipolar disorder. The methodology has previously been outlined in detail (Ekman et al., 2010; Jakobsson et al., 2012; Ryden et al., 2009). A total of 215 patients with bipolar disorder and 112 healthy controls were included in this study. All patients met the DSM-IV-TR criteria for bipolar disorder. Healthy controls matched by sex and birth date were selected randomly from the national population register by Statistics Sweden (www.scb.se). These control subjects were living in the same catchment area as the patients. Exclusion criteria for controls were (1) any on-going psychiatric or neurological disorder; (2) current treatment with any psychotropic drugs; (3) past bipolar disorder, schizophrenia, recurrent depression, or other psychiatric disorder leading to functional disability; (4) a first-degree relative with schizophrenia or bipolar disorder; (5) subjects presenting conditions that precluded magnetic resonance imaging of the brain (e.g., metal implants, shrapnel and certain heart operations).

Prior to the study, all subjects were provided with verbal and written information about the study and about potential risks and benefits of study participation. All subjects consented orally and in writing to participate in the study. The Regional Research Ethics Board in Stockholm, Sweden approved the study.

### 2.1. Assessment of clinical variables

Patients were assessed by a psychiatrist or resident in psychiatry using the Affective Disorders Evaluation (ADE), which is a standardized protocol adapted from the Systematic Treatment Enhancement Program of Bipolar Disorder (STEP-BD) (Sachs et al., 2003). The ADE guides the interviewer through a systematic assessment of the patient's current and past mental state and provides a diagnosis according to DSM-IV criteria. The number of lifetime affective episodes and their characteristics are documented. Other modules assess alcohol and drug misuse, violent behavior, childhood history, family history, treatment history, reproductive history and somatic illnesses. Suicide attempt was defined as a deliberate and serious self-injury, including intoxication with medication. The ADE was complemented with the M.I.N.I Neuropsychiatric interview (Sheehan et al., 1998). The final diagnosis was established using LEAD (Longitudinal observation by Experts using All Data) (Spitzer, 1983) and confirmed by a consensus panel of 2-4 experienced clinicians. Inclusion criteria for this sub-study were bipolar I or II diagnosis. Psychosocial functioning was assessed using the clinician rated Global Assessment of Function (GAF) scale (Guy, 1976; Luborsky, 1962). Mood stability was determined by the treating physician's overall diagnostic judgment, but was complemented with the Montgomery-Åsberg Depression Rating Scale (MADRS) and the Young Mania Rating Scale (YMRS) to assess depressive and manic symptom severity, respectively.

### 2.2. Measurement of serum and CSF levels of mature glutamine, glycine, glutamate, L-serine and D-serine

Serum samples were collected from fasting subjects between 8:00 and 9:00 am. The samples were centrifuged on site and stored at −80 °C until delivered by courier mail, frozen on dry ice, to Chiba University, Japan, for analysis. CSF sampling was performed by lumbar puncture between 9:00 and 10:00 am on the same day as the serum sampling. The spinal needle was inserted into the L3/L4 or L4/L5 interspace, and a total volume of 12 mL of the CSF was collected, gently inverted to avoid gradient effects, and divided into 1.0-1.6 mL aliquots that were stored at −80 °C pending analysis.

Measurement of total, D- and L-serine levels in serum and CSF were carried out using a column-switching high performance liquid chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan) as previously reported (Fukushima et al., 2004; Hashimoto

et al., 2005b). Measurement of glycine, glutamine and glutamate was carried out using a HPLC system with fluorescence detection, as previously reported (Hashimoto et al., 2005a).

## 3. Statistical analyses

SPSS version 20.0 (IBM Corp.) was used to analyze data. Amino acid concentration variables were inspected for skewness and analyzed using the Kolmogorov-Smirnov test, and 10log transformations were used when needed to normalize distributions. Ratios were arctan-transformed prior to analysis. A linear regression model was computed for each amino acid concentration, where the amino acid served as dependent variable, and patient/control status, sex, age and BMI served as independent variables. Spearman correlations were used to study bivariate associations between amino acid concentration and other continuous variables. Multivariate linear regression modeling was used to evaluate the effect of psychotropic medication on amino acid levels. The assumptions of the models (linearity, homoscedasticity and lack of multicollinearity) were checked. False discovery rate was used to control for multiple comparisons as indicated in the results (Benjamini and Hochberg, 1995) and $p<0.05$ was considered statistically significant.

## 4. Results

### 4.1. Patient-control comparisons

Serum samples from 215 patients with bipolar disorder and 112 healthy controls (Table 1) were analyzed. In this group, CSF samples were available from 132 patients and 87 controls that were analyzed. Demographics and clinical characteristics of the patient population are presented in Table 1. We determined the serum and CSF concentrations of five amino acids: glutamine, glycine, glutamate, D-serine and L-serine. In addition, the D-serine/L-serine, L-serine/glycine and glutamine/glutamate ratios were calculated. The Kolmogorov-Smirnov test was used to determine that data was normally distributed. The test indicated non-normal distribution for serum glycine, glutamate, D-serine and L-serine, as well as CSF glycine, glutamate and D-serine. Hence, these variables were log-transformed prior to further analyses.

As shown in Table 2, serum levels (adjusted for age, sex and BMI) of glutamine, glycine and D-serine were significantly higher in patients with bipolar disorder as compared to controls. Serum L-serine levels were lower in the patient group. These differences remained significant when adjusting for multiple testing. Hence, the patient group had higher D/L-serine ratio ($t=5.0$, $p<5.2\times10^{-7}$) but lower L-serine/glycine ratio ($t=-6.61$, $p<2.3\times10^{-10}$) than the control group. The glutamine/glutamate ratio did not differ between the groups.

In CSF, the mean concentration of glutamine (adjusted for age, sex, BMI and height) was significantly higher in bipolar disorder patients than in healthy controls ($p=0.026$, Table 3). However, this difference did not survive correcting for multiple testing. We found no other significant differences between patients and controls with respect to the mean CSF amino acid concentrations or ratios.

Smoking status has previously been associated with both serum and CSF amino acid levels (Luykx et al., 2013) and smoking was more common in the patient group. However, adding smoking status as a co-variate to the regression models did not explain the observed differences between the patient and control group.

The patients included in the study were mood stabilized but not consistently euthymic as defined by a MADRS score below 14. Some patients suffered from lingering depressive symptoms with MADRS>13. Previous work has shown associations between acute depression and altered plasma levels of glutamate, glutamine, glycine and L-serine (Mitani et al., 2006). We therefore tested the association between MADRS scores and amino acid levels using Spearman correlations. Only serum L-serine was significantly associated with MADRS score ($r=-0.15$, $p=0.039$) and this result did not survive correcting for multiple testing.

### 4.2. Amino acid associations with medications

In order to identify possible glutamatergic markers for mood stabilizing medication, we conducted a post-hoc analysis where we analyzed the association of current treatment with lithium, valproate, or lamotrigine with serum or CSF amino acid levels using multiple linear regression models. We also included sex (female as reference), age, height (CSF only), BMI, GAF score, total number of episodes, and current treatment with antidepressants and antipsychotics as co-variates in the model to control for potential confounders.

As shown in Table 4, regression models that included statistically significant effects for at least one of the mood stabilizers were found for serum glycine and L-serine, as well as CSF glutamine, D-serine and L-serine. Briefly, serum glycine and L-serine, as well as CSF glutamine and D-serine levels were positively associated with lithium and valproate treatment. CSF L-serine levels were positively associated with all three mood stabilizers included in the model. These associations remained significant when correcting for potential confounders. Correction for multiple comparisons was not performed for these exploratory post-hoc analyses.

All patients included in the study were in a stable mood at sampling but some patients experienced residual symptoms. Thus, YMRS and MADRS scores were added to all models but this did not explain the observed associations of mood stabilizer treatment and amino acid levels.

## 5. Discussion

This is the first study to investigate the glutamatergic system in bipolar disorder by assessing both serum and CSF concentrations of glutamine, glutamate, glycine, L-serine and D-serine in mood-stabilized bipolar patients and healthy controls. We found that the serum levels of glutamine, glycine and D-serine were significantly higher in clinically stable patients with bipolar disorder as compared to healthy controls, while L-serine serum levels were significantly lower in the patient group. This pattern was not mirrored in CSF where no statistically significant differences were found between the patient and control group.

**Table 1** Characteristics of the study sample.

| | Controls | | Bipolar disorder | |
|---|---|---|---|---|
| Sex (male/female) | 50/62 | | Sex (male/female) | 82/133 |
| | Median | IQR | Median | IQR |
| Age (years) | 35 | 27-45 | 36 | 28-48 |
| BMI | 23.3 | 21.6-25.4 | 24.7 | 22.1-27.8 |
| | *N* | % | *N* | % |
| Smoker[a] | 16 | 14 | 67 | 34 |
| Diagnosis | | | *N* | % |
| Bipolar disorder type I | | | 108 | 50 |
| Bipolar disorder type II | | | 82 | 38 |
| Not otherwise specified | | | 25 | 12 |
| Previous psychosis | | | 106 | 49 |
| Alcohol or substance use disorder[b] | | | 69 | 32 |
| Anxiety disorder[c] | | | 91 | 42 |
| ADD[d] | | | 28 | 13 |
| History of suicide attempt | | | 81 | 38 |
| Clinical data | | | Median | IQR |
| Age first symptoms[e] | | | 16 | 13-24 |
| Depressive episodes[f] | | | 10 | 5-20 |
| Hypomanic episodes | | | 2 | 0-6 |
| Manic episodes | | | 1 | 0-2 |
| Mixed episodes | | | 0 | 0 |
| GAF | | | 67 | 60-75 |
| MADRS[g] | | | 4 | 0-11 |
| YMRS[h] | | | 0 | 0-2 |
| Medication | | | *N* | % |
| Lithium | | | 129 | 60 |
| Valproate | | | 28 | 13 |
| Lamotrigine | | | 47 | 22 |
| Antidepressants | | | 81 | 38 |
| Antipsychotics | | | 56 | 26 |

[a]Missing data for 15 individuals in the patient group.
[b]Missing data for 3 individuals in the patient group.
[c]Missing data for 1 individuals in the patient group.
[d]Missing data for 4 individuals in the patient group.
[e]Missing data for 2 individuals in the patient group.
[f]Missing data for 3 individuals in the patient group.
[g]Missing data for 31 individuals in the patient group.
[h]Missing data for 31 individuals in the patient group.

Previous work using magnetic resonance spectroscopy has shown an association between bipolar disorder and higher glutamine+glutamate levels in the brain (Gigante et al., 2012). Our finding of higher glutamine levels in serum in patients as compared to controls thus partly concur with this observation, though we did not observe increased glutamine or glutamate in CSF. Further, it is not clear if the observed differences in amino acid levels are specific to bipolar disorder. Other studies have shown decreased D-serine levels in both serum and CSF from patients with schizophrenia (Hashimoto et al., 2003, 2005b). However, increased serum levels of D-serine (Ohnuma et al., 2008) and total serine (Sumiyoshi et al., 2004) have also been observed. With respect to bipolar disorder, one previous study using postmortem brain samples found no differences in L-serine or D-serine as compared to a control group (Hashimoto et al., 2007). The present findings of increased peripheral D-serine and decreased L-serine levels suggest an aberration in serine metabolism in patients with bipolar disorder. Previous studies in unipolar depression have shown both decreased (Mitani et al., 2006) and increased (Sumiyoshi et al., 2004) peripheral serine levels.

There are no previous reports on glycine levels in bipolar disorder, but decreased CSF glycine levels were found in a mixed unipolar and bipolar cohort (Frye et al., 2007). Further, associations between serum glycine levels and negative symptoms (Hons et al., 2010) and sensory gating (Heresco-Levy et al., 2007) have been reported in schizophrenia. These studies suggest that serum levels of glycine provide some information on brain function. When it comes

**Table 2** Serum amino acid concentrations.

| Amino acid | Controls | | Bipolar disorder | | Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Beta | *t* | df | *p* | $q^a$ |
| Glutamine (μM) [b,c] | 507 | 65.5 | 523 | 75.9 | 0.14 | 2.61 | 1 | 0.010 | 0.025 |
| Glycine [d] (μM) [e,b] | 236 | 62.8 | 256 | 78.3 | 0.12 | 2.23 | 1 | 0.027 | 0.034 |
| Glutamate [d] (μM) [e,b,c] | 54 | 19.1 | 58.4 | 24.9 | 0.02 | 0.46 | 1 | 0.65 | 0.65 |
| D-serine[d] (μM) [c] | 1.21 | 0.27 | 1.30 | 0.38 | 0.13 | 2.26 | 1 | 0.024 | 0.034 |
| L-serine [d] (μM) [b] | 130 | 28.1 | 115 | 22.9 | −0.29 | −5.37 | 1 | $1.5 \times 10^{-7}$ | $7.5 \times 10^{-7}$ |

Serum amino acid concentrations in 215 bipolar disorder patients and 112 sex and age matched healthy controls. Differences between patients and controls were analyzed with linear regression using age, sex and BMI as covariates.

[a] *p*-Value adjusted for false discovery rate.

[b] Significant effect of sex.

[c] Significant effect of BMI.

[d] Log transformed prior to statistical analysis.

[e] Significant effect of age.

**Table 3** Cerebrospinal fluid amino acid concentrations.

| Amino acid | Controls | | Bipolar disorder | | Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Beta | *t* | *df* | *p* | $q^a$ |
| Glutamine (μM) [b,c,d] | 499 | 65.6 | 516 | 82.5 | 0.13 | 2.24 | 1 | 0.026 | 0.13 |
| Glycine [e] (μM)[b,f] | 7.6 | 3.5 | 8.2 | 4.4 | 0.06 | 0.92 | 1 | 0.36 | 0.53 |
| Glutamate [e] (μM) [b] | 0.68 | 0.19 | 0.75 | 0.38 | 0.09 | 1.44 | 1 | 0.15 | 0.38 |
| D-serine[e] (μM) | 1.77 | 0.38 | 1.84 | 0.42 | 0.06 | 0.81 | 1 | 0.42 | 0.53 |
| L-serine (μM) | 24.6 | 4.4 | 24.6 | 5.2 | −0.02 | −0.24 | 1 | 0.81 | 0.81 |

Cerebrospinal fluid amino acid concentrations in 135 bipolar disorder patients and 87 sex and age matched healthy controls. Differences between patients and controls were analyzed with linear regression using age, sex, BMI, and height as covariates.

[a] *p*-Value adjusted for false discovery rate.

[b] Significant effect of age.

[c] Significant effect of sex.

[d] Significant effect of BMI.

[e] Log transformed prior to statistical analysis.

[f] Significant effect of height.

**Table 4** Mood stabilizer treatment and amino levels.

| Regression model | Lithium | | | Valproate | | | Lamotrigine | | |
|---|---|---|---|---|---|---|---|---|---|
| **Serum** | *β* | $p^a$ | $p^b$ | *β* | $p^a$ | $p^b$ | *β* | $p^a$ | $p^b$ |
| Glycine[c] | 0.17 | 0.019 | 0.005 | 0.39 | $1.2 \times 10^{-8}$ | $3.4 \times 10^{-8}$ | 0.09 | 0.16 | 0.10 |
| L-serine[c] | 0.20 | 0.007 | 0.005 | 0.19 | 0.010 | 0.002 | 0.06 | 0.41 | 0.45 |
| **CSF** | | | | | | | | | |
| Glutamine | 0.26 | 0.004 | 0.005 | 0.51 | $4.2 \times 10^{-8}$ | $2.8 \times 10^{-8}$ | 0.11 | 0.18 | 0.098 |
| D-serine[c] | 0.32 | $4.9 \times 10^{-4}$ | $4.3 \times 10^{-4}$ | 0.45 | $1.0 \times 10^{-6}$ | $2.0 \times 10^{-6}$ | 0.15 | 0.083 | 0.067 |
| L-serine | 0.27 | 0.005 | 0.006 | 0.35 | $2.0 \times 10^{-4}$ | $1.0 \times 10^{-4}$ | 0.21 | 0.017 | 0.003 |

Multiple linear regression analysis for each amino acid using lithium, valproate and lamotrigine as explanatory variables.

[a] Unadjusted *p*-values.

[b] *p*-Values adjusted for sex (female as reference), age, BMI, height (for CSF measures), GAF, number of episodes, use of antidepressants and antipsychotics.

[c] Log transformed prior to statistical analysis.

to unipolar depression, there have been reports of both increased (Mitani et al., 2006) and unaltered (Maes et al., 1998) blood glycine levels.

It is not clear whether changes in amino acid levels are a trait marker or dependent on mood state at sampling. A number of patients in our study cohort had lingering depressive symptoms. However, we found no significant associations between amino acid concentrations and MADRS scores, which suggests that altered amino acid levels can be found in euthymic as well as depressed or manic patients.

With the exception of L-serine, the observed differences in amino acid levels between bipolar disorder patients and healthy controls were relatively small. Further, none of these observations were mirrored in CSF. Interestingly, a recent meta-analysis of studies measuring NMDA-receptor co-agonists in schizophrenia found consistently increased serine levels in blood across studies, but could not verify any significant findings in CSF (Brouwer et al., 2013). Taken together, the present study and previous work do not present a clear picture on changes in blood or CSF amino acid concentrations, with relevance for glutamatergic signaling, in bipolar disorder, schizophrenia or major depression.

There are several possible explanations for the discrepancy between serum and CSF measures of amino acid levels. CSF concentrations of glycine and L-serine are normally around 10% of the blood levels (Hawkins et al., 2006). For glutamate, the CSF proportion is even lower whereas glutamine is present in almost the same concentration as in blood. The reason is thought to be an active transport of amino acids from CSF to blood via the blood-brain barrier (Hawkins et al., 2006). This adds complexity when extrapolating from CSF amino acid levels to brain tissue levels.

It should also be noted that glutamine, glutamate, L-serine and glycine perform numerous roles in metabolic and synthesis pathways both centrally and peripherally. Thus, the observed differences in amino acid levels could be related to changes in cellular metabolism rather than glutamatergic signaling. Indeed, mitochondrial dysfunction and impaired cellular metabolism have been implicated in bipolar disorder by findings from, e.g., magnetic resonance spectroscopy studies (Stork and Renshaw, 2005). Changes in D-serine on the other hand may be more directly linked to altered glutamatergic neurotransmission (Pollegioni and Sacchi, 2010) as its main role appears to be as a signaling molecule.

In this study, potential confounding effects of psychotropic medication should be considered. Indeed, serum glycine levels were positively correlated with both lithium and valproate treatment. Thus the observed difference between patients and controls may be explained by current treatment with mood stabilizers. Valproate has previously been associated with increased serum levels of glycine in children (Castro-Gago et al., 1990). However, negative findings on plasma glycine and valproate treatment in adults have also been published (Verity et al., 1983). Treatment with valproate has previously been demonstrated to increase the CSF levels of glutamine (Akiyama et al., 2012; Perry et al., 1976; Scholl-Burgi et al., 2008). This finding was confirmed in the current study, where valproate treatment was positively correlated with CSF levels of glutamine. It has also been described previously that valproate treatment increases the CSF levels of serine, which was also observed in this study (Akiyama et al., 2012). Although, the effect sizes of lithium treatment in relation to amino acid levels were smaller than valproate treatment, lithium treatment was also associated with increased CSF levels of glutamine, D-serine and L-serine. The effect of lithium on glutamate levels has previously been tested in experimental animal models, where lithium treatment increased glutamate release in monkey and mouse cerebral cortex slice preparations (Dixon et al., 1994) and also increased glutamine levels on a trend level (Vargas et al., 1998).

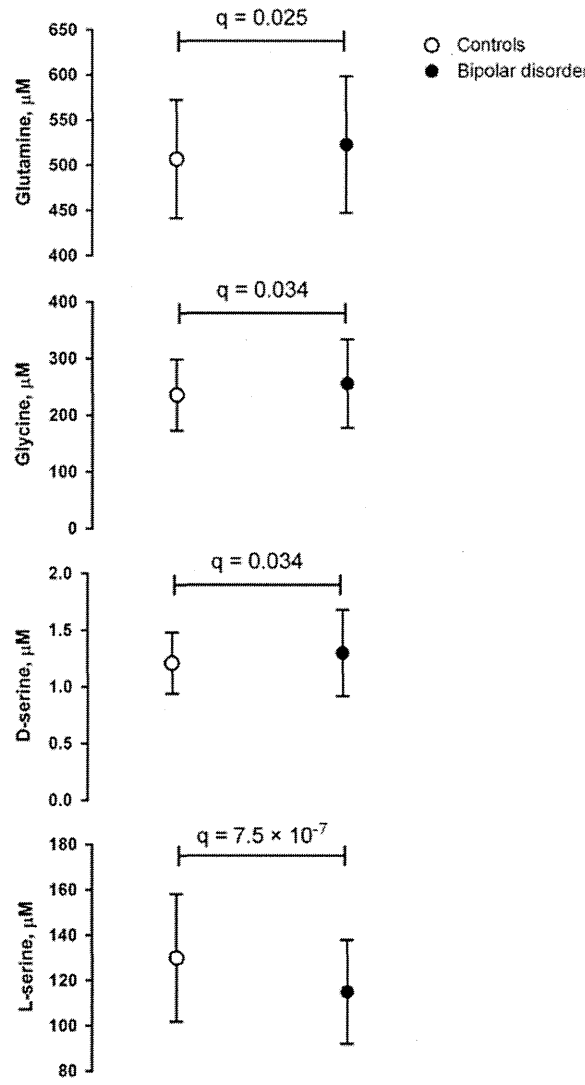


**Figure 1** Serum amino acid levels in persons with bipolar disorder and healthy controls. Serum concentrations (mean±SD) of glutamine, glycine, D-serine and L-serine in healthy controls ($n$=112) and bipolar disorder patients ($n$=215). Linear regression was used for comparisons between patient and controls while controlling for age, sex and BMI. $Q$-values adjusted for multiple comparisons using the false discovery rate method.

The present study includes data from a relatively large group of patients with bipolar disorder in a stable mood. Serum and CSF samples were handled similarly and analyzed in parallel, indicating that differences between the samples are unlikely to be caused by methodological factors. Further, we used a population-based control group that was matched for age and sex in order to minimize bias caused by a non-representative control group. However, the cross-sectional design of the present study does limit the conclusions that can be drawn on medication effects on amino acid levels. The associations between current medication and amino acid levels need to be corroborated in longitudinal studies. In addition, the naturalistic setting of the study means that many patients are treated with more than one drug, which makes it more difficult to draw firm conclusions on the specific effects of different types of medications. The lack of an un-medicated patient group also limits the conclusions that can be drawn with respect to medication effects. In addition, the naturalistic design of the present study means that a significant proportion of the patients were comorbid for other psychiatric disorders such as anxiety disorders or attention deficit disorder.

### 5.1. Conclusions

Serum measures of glutamine, glycine, L-serine and D-serine were associated with a diagnosis of bipolar disorder. In CSF, no statistically significant differences that survived correction for multiple testing between patients and controls were observed. Taken together, the results support the notion of a systemic aberration in amino acid metabolism that affects several amino acids related to glutamate signaling. Further, treatment with mood stabilizers was associated with amino acid levels, particularly in CSF. This supports previous work where interaction between, e.g., lithium and brain glutamate signaling has been demonstrated. Further work is needed to determine the mechanism of the observed differences in amino acid levels, both in relationship to bipolar disorder and the effects of psychotropic medication Figure 1.

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## Contributors

E. Pålsson, K. Södersten, J. Jakobsson, C. Sellgren, H. Ågren and M. Landén designed the study. C.-J. Ekman, C. Sellgren and M. Landén acquired the data. K. Hashimoto and Y. Fujita performed the amino acid measurements. E. Pålsson and J. Jakobsson undertook the statistical analysis of the data. Authors Pålsson E. and Södersten K. managed the literature searches and author E. Pålsson wrote the first draft of the manuscript. All authors contributed to the final draft of the article and approved its publication.

## Conflict of interest

The authors declare no conflict of interest.

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Research report

# Presence of psychological distress symptoms associated with onset-related life events in patients with treatment-refractory depression

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ABSTRACT

*Background:* Previous studies have reported that various non-life-threatening life events could cause psychological distress symptoms like posttraumatic stress disorder in adults and adolescents. We examined whether patients with treatment-refractory depression (TRD) perceive their experiences of life events, of which they think as triggering the onset of depression, as more serious psychological distress symptoms than remitted or mildly symptomatic patients with major depressive disorder (MDD).
*Methods:* This study employed a cross-sectional design. We recruited 78 outpatients consisting of 31 TRD patients, 31 remitted MDD patients, and 16 mildly symptomatic MDD patients. We adopted the Impact of Event Scale-Revised (IES-R) to assess the severity of psychological distress symptoms associated with the events that patients thought as triggering the onset of depression. We also evaluated clinical features and variables including the Hamilton Depression Rating Scale (HDRS).
*Results:* The mean [ ± SD] score of the IES-R in patients with TRD (46.7 [15.1]) was significantly higher than in remitted (10.3 [9.9], $p < 0.001$) or mildly symptomatic (31.3 [7.7], $p < 0.001$) patients with MDD. The HDRS scores showed significant correlations with those of the IES-R among all patients ($r = 0.811$).
*Limitations:* This study was not able to exclude the possibility that the severity of psychological distress symptoms associated with onset-related events could influence the difficult therapeutic course in patients with TRD due to the cross-sectional design.
*Conclusions:* This study demonstrated that patients with TRD perceive their onset-related life events as serious psychological distress symptoms. This result contributes to understanding the pathophysiology of TRD.


## 1. Introduction

Major depressive disorder (MDD) is a common mental illness with a high social burden. Although pharmacotherapy such as antidepressants plays a pivotal role in the treatment of MDD, approximately 20–30% of antidepressant-treated patients with MDD are classified as having treatment-refractory depression (TRD) (Fava and Davidson, 1996; Keller et al., 1992). Previous studies suggest that the concept of TRD is advocated as a failure to achieve sufficient remission after at least two adequate antidepressant treatment trials during a current depressive episode (Schlaepfer et al., 2012; Schosser et al., 2012; Souery et al., 2006). Therefore, it is necessary to further investigate the clinical features of TRD, to understand the pathophysiology of TRD, and to develop better management of patients with TRD.

Accumulating evidence has reported that physical and psychological stresses are closely linked with depression (Flynn and Himle, 2011). Some reports show that various stressful life events (e.g., divorce, unemployment, and public humiliation), which by themselves do not lead to fatal outcomes, could trigger depressive disorders (Brown et al., 1995; Honkalampi et al., 2005; Hosang et al., 2010; Kendler et al., 1998; Tennant, 2001). Moreover, it was reported that stressful life events cause symptoms such as intrusion, avoidance, and hyperarousal similar to posttraumatic stress disorder (PTSD) in adults and adolescents (Meiser-Stedman et al., 2012; Mol et al., 2005). A recent study suggests that childhood trauma (e.g., emotional neglect, psychological abuse) is associated with chronic and refractory depression in adults (Hovens et al., 2012). However, it is unknown whether patients with TRD perceive psychological distress symptoms as being related to adulthood life events, of which they think as triggering the onset of depression (here called "onset-related events"). Therefore, we developed the hypothesis that patients with TRD perceive symptoms of psychological distress including intrusion, avoidance, and hyperarousal as being associated with the life events, of which they thought as triggering the onset of

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depression (here called "onset-related psychological distress symptoms") similar to patients with PTSD.

The purpose of this study was to determine whether patients with TRD perceive their experiences of onset-related events as psychological distress symptoms. We conducted a cross-sectional study to assess onset-related psychological distress symptoms in patients with TRD, and compare them with remitted MDD patients or mildly symptomatic patients with MDD. In addition, we explored the factors of onset-related psychological distress symptoms in terms of severity of depression, clinical features such as bipolarity, childhood experiences of abuse or stressful events, strength of onset-related events, and duration of illness or treatment.

## 2. Methods

### 2.1. Study design

Our study employed a cross-sectional design and was approved by the ethics committee of Chiba University Graduate School of Medicine, Sodegaura Satsukidai Hospital, and Fujita Hospital. All subjects provided written informed consent for their participation in this study after the procedure had been fully explained to them.

### 2.2. Participants and procedure

We surveyed potential candidates from available outpatient charts at Chiba University Hospital, Sodegaura Satsukidai Hospital, and Fujita Hospital. This study was conducted from November 2012 to November 2013. All subjects were outpatients with ages ranging from 20 to 79 years, and were diagnosed with MDD according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, text revision (DSM-IV-TR) criteria (American Psychiatric Association, 2000) using the Japanese version of the Mini International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998). We excluded patients with PTSD, schizophrenia, bipolar disorders, comorbid dementia, organic mental disorder, alcohol or drug dependence, mental retardation, or impending suicide attempt. We also excluded patients who were hospitalized, under 20 years old, showing poor compliance with medication, and uncertain about their life events related to the onset of depression. A total of 247 outpatients underwent eligibility screening for the study, 158 patients did not meet the criteria for eligibility, and 89 patients were eligible to be included in the study. Nine patients declined an interview, and 2 patients answered that they experienced no life events related to the onset of depression. Finally, 78 patients participated in this study.

### 2.3. Assessment of depression and definition of TRD

We assessed the severity of depression using the Structured Interview Guide for the 17-item Hamilton Depression Rating Scale (HDRS-17) (Hamilton, 1967, Williams, 1988). We applied the criteria for TRD and non-TRD (i.e., remitted or mildly symptomatic depression) using the study protocol of Schosser et al. (2012) as a reference. In this study, TRD was defined as not reaching an HDRS-17 score of 17 or fewer points after two or more antidepressant treatment trials with adequate dosage and sufficient duration (i.e., longer than four weeks in the current depressive episode). Non-TRD was defined as achieving 17 or fewer point of the HDRS-17 after a single antidepressant treatment or after a second course after initial treatment failure. Moreover, within non-TRD, remission of MDD was defined as 7 or fewer points of the HDRS-17 score (Frank et al., 1991). The patients who met neither TRD nor remitted MDD criteria with an HDRS-17 score between 8 and 17 points, were categorized as intermediate MDD.

### 2.4. Assessments of clinical characteristics

We assessed demographic data such as age, gender, comorbidity, physical diseases, family history of psychiatric disorders in first-degree relatives, years of education, current employment, present medication, disease and therapy duration, childhood stressful life events and abuse before reaching an age of 15 years, and clinical features of atypical depression according to the DSM-IV-TR definition. In the current study, physical diseases included patients under treatment for hypertension, diabetes, hyperlipidemia, lumber disc hernia, and ulcerative colitis. Childhood life events and abuse consisted of any of the following: the experience of parental divorce, bereavement after the loss of a parent, the experience of strong violence or violent language from parent or other people, sexual harassment or abuse, neglect, the intervention by the child consultation center, or other stressful experiences.

### 2.5. Measures

#### 2.5.1. The Impact of Event Scale-Revised

The Impact of Event Scale-Revised (IES-R) is a self-report measure for assessing the severity of symptomatic responses to stressful life events in the past seven days and consists of 22 items and three subcategories, including intrusion (8 items: intrusive thoughts, nightmares, intrusive feelings and imagery, re-experiencing), avoidance (8 items: numbing of responsiveness and avoidance of feelings, situations, and ideas), and hyperarousal (6 items: sleep difficulties, anger outbursts, irritability, hyper-vigilance, difficulty concentrating, and increased startle) (Weiss and Marmar, 1997). Each item is rated on a 5-point scale (0=not at all, 1=a little, 2=moderately, 3=a lot, 4=enormously). The total sum of points ranges from 0 to 88. The internal consistency and concurrent validity of the IES-R were confirmed (Baumert et al., 2004). The Japanese version of the IES-R has already been standardized (Asukai et al., 2002). The IES-R was developed and has been widely used to evaluate traumatic symptoms in patients with PTSD (Weiss and Marmar, 1997), and covers all aspects of PTSD symptoms such as intrusion, avoidance, and hyperarousal. Because we hypothesized that patients with TRD could perceive onset-related psychological distress symptoms similar to patients with PTSD, as described in the introduction, we adopted the IES-R to evaluate onset-related psychological distress symptoms in this study. We instructed patients to write their onset-related event into the blank space of the introduction document of the IES-R, and to answer each item of the IES-R regarding their onset-related event.

#### 2.5.2. Life Change Units Value

To evaluate stress level of life events objectively, we used the social readjustment rating scale and scored by the means of Life Change Units Value (LCU) (Holmes and Rahe, 1967). The LCU is a rating scale to measure the stress of life events proposed by Holmes and Rahe and is based on their multifaceted and extensive investigation. The stress of important life events is quantified from 11 to 100 points. For example, 100 points for the spouse's death, 73 for divorce, 53 for one's disease or impairment, 50 for marriage, and 20 for change of address.

#### 2.5.3. Assessment of bipolarity

We further defined the patients as having bipolarity if they satisfied the criteria of either "bipolar spectrum disorder" (Ghaemi et al., 2002) or "bipolarity specifier" (Angst et al., 2003a, 2003b). We examined bipolarity in all participants, because several studies have demonstrated that there could be a high prevalence of bipolarity in patients with TRD (Correa et al., 2010; Dudek et al., 2010; Parker et al., 2005). The concept of bipolar spectrum and bipolarity specifier, which suggests that depressive patients with

subthreshold hypomanic episodes or characteristics of bipolar disorder should be considered closer to bipolar depression rather than unipolar depression, has been of importance in terms of assessing the clinical condition of mood disorders (Angst and Marneros, 2001).

#### 2.5.4. Assessment of patient's impression for onset-related events

We also examined the perceived causal relationship between incidence of depression and onset-related event among the patients, using our original questionnaire form, which asked each participant to simply answer how much they rated the causal relationship between incidence of depression and onset-related event from 1 to 100 percent.

### 2.6. Primary endpoint

The score of the IES-R for onset-related events for the three groups was set as the primary endpoint in this study.

### 2.7. Statistical analysis

We analyzed the data separately for the three groups including TRD, intermediate, and remission group. We performed all analyses using SPSS for Windows, Version 19. We employed Chi-square or Fisher's exact test for categorical variables and Student's *t*-test or one-way ANOVA for the other variables. We performed one-way ANOVA for total and sub-category scores of the IES-R and HDRS-17, followed by Games–Howell test for multiple comparisons. We also tested the correlation between HDRS and IES-R by means of Pearson's product moment correlation. The level of significance was set at $p < 0.05$ and the level for power at 0.80.

## 3. Results

### 3.1. Characteristics

The characteristics of participants included in the analysis are presented in Table 1. The 78 patients with MDD consisted of three groups: the TRD group ($n=31$), remission group ($n=31$), and intermediate group ($n=16$). There were no significant differences in age, gender, and years of education between the three groups. Furthermore, there were no significant differences in the proportion of current employment, physical diseases, psychiatric comorbidity, and first-degree relatives with psychiatric disorders between the three groups. As for the breakdown of psychiatric comorbidities, there were patients with dysthymic disorder ($n=3$), social anxiety disorder ($n=3$), panic disorder ($n=1$), generalized anxiety disorder ($n=1$), pain disorder ($n=1$), and bulimia nervosa ($n=1$) in the TRD group, and two patients of this group suffered from double psychiatric comorbidities. Psychiatric comorbidities in the intermediate group consisted of social anxiety disorder ($n=1$), panic disorder ($n=2$), generalized anxiety disorder ($n=1$), and obsessive-compulsive disorder ($n=1$), and one patient of this group suffered from double comorbidity. In the remission group, psychiatric comorbidities included social anxiety disorder ($n=1$), panic disorder ($n=1$), generalized anxiety disorder ($n=1$), and obsessive-compulsive disorder ($n=2$), and one patient suffered from double comorbidity.

Table 2 shows the breakdown of onset-related life events among all participants. Many patients thought there was greater than 80% in percentage of patients' impression of causal relationship between their stressful life events around the time of onset and depression (70.9% in TRD, 62.5% in intermediate, and 74.2% in remission group).

Table 3 shows the medication profiles for all participants. The combination of antidepressant and benzodiazepine was the predominant profile for each group (38.7% in TRD, 43.8% in intermediate, and 51.6% in remission group). The proportion of patients treated with a single antidepressant was highest in the remission group (29.0%). On the other hand, almost all patients with TRD had been treated with multiple-drug combination therapy.

### 3.2. The scores of the IES-R for onset-related events

Fig. 1 shows the IES-R for onset-related events for all groups. The total score of the IES-R showed a significant difference between the three groups ($F(2)=72.81$, $p < 0.001$). Post-hoc analysis showed significant differences between each group; the IES-R total score was significantly higher in the TRD group (mean=46.7, standard deviation (SD)=15.1) than intermediate (mean=31.3, SD=7.7; $t(45)=4.63$, 95% CI 7.34–23.45, $p < 0.001$), and remission MDD group (mean=10.3, SD=9.9; $t(60)=11.25$, 95%CI 28.61–44.23, $p < 0.001$). The total score of the IES-R was also significantly higher in the intermediate than remission group ($t(45)=7.40$, 95%CI 14.62–27.42, $p < 0.001$).

As shown in Fig. 1, for each sub-category of the IES-R, there were significant differences between the three groups: intrusion ($F(2)=42.65$, $p < 0.001$), avoidance ($F(2)=28.92$, $p < 0.001$), and hyperarousal ($F(2)=81.48$, $p < 0.001$). Each sub-category score of the TRD group was significantly higher compared to the intermediate and remission groups. For intrusion, the mean score of

**Table 1**
Characteristics of the patient group with treatment-refractory depression (TRD), intermediate group, and remission group.

| | TRD ($n=31$) | Intermediate ($n=16$) | Remission ($n=31$) | *p*-Value |
|---|---|---|---|---|
| Age, years (SD) [Age range] | 47.3 (12.6) [24–64] | 48.6 (12.7) [28–67] | 53.5 (13.8) [25–77] | NS |
| Gender, male/female | 13/18 | 5/11 | 12/19 | NS |
| Education, years (SD) | 13.2 (2.5) | 13.0 (2.4) | 13.9 (1.8) | NS |
| Current employment (%) | 10 (32.3) | 9 (56.3) | 15 (48.4) | NS |
| Psychiatric comorbidity (%) | 8 (25.8) | 4 (25.0) | 4 (12.9) | NS |
| Physical disease (%) | 19 (61.3) | 8 (50.0) | 20 (64.5) | NS |
| Family psychiatric history (%) | 13 (41.9) | 7 (43.8) | 8 (25.8) | NS |
| Childhood life events and abuse (%) | 10 (32.3) | 7 (43.8) | 7 (22.6) | NS |
| LCU of life events, points (SD) | 48.1 (30.0) | 50.4 (22.8) | 44.2 (24.7) | NS |
| HDRS-17 items, points (SD) | 23.2 (3.4) | 11.5 (3.0) | 3.6 (2.0) | < 0.001[a] |
| Atypical depression (%) | 5 (16.1) | 1 (6.3) | 1 (3.2) | NS |
| Disease duration, months | 87.5 (52.6) | 85.4 (70.6) | 94.3 (69.9) | NS |
| Therapy duration, months | 70.8 (41.9) | 73.9 (61.2) | 77.1 (71.7) | NS |

*Note:* Variables represent mean (standard deviation: SD)
Abbreviations: LCU, Life Change Units Value; HDRS, Hamilton Depression Rating Scale; NS, not significant.
[a] The data for three groups were analyzed with one-way ANOVA, followed by Games–Howell test for multiple comparisons.

**Table 2**
The breakdown of onset-related life events and the patient's impression about causal relationship between incidence of depression and onset-related event.

| | TRD ($n=31$) | Intermediate ($n=16$) | Remission ($n=31$) |
|---|---|---|---|
| Job-related events | 15 | 4 | 20 |
| Family-related events | 14 | 6 | 6 |
| Health-related events | 5 | 2 | 7 |
| Money-related events | 3 | 1 | 1 |
| Other events | 1 | 3 | 0 |
| Overlapping events | 7 | 0 | 1 |
| Patients' impression of causal relationship between their events and depression[a] | *n* | *n* | *n* |
| 100% | 10 | 3 | 9 |
| 81–99% | 12 | 7 | 14 |
| 61–80% | 6 | 2 | 4 |
| 41–60% | 2 | 3 | 4 |
| 21–40% | 1 | 1 | 0 |
| 1–20% | 0 | 0 | 0 |

Abbreviation: TRD, treatment-refractory depression

[a] Degree in percentage of patients' impression of causal relationship between their stressful life events around the time of onset and depression.

**Table 3**
Medication profiles of the three patient groups.

| Class of medication | TRD ($n=31$) | Intermediate ($n=16$) | Remission ($n=31$) |
|---|---|---|---|
| Antidepressants (AD) | | | |
| SSRI | 17 | 7 | 16 |
| SNRI | 8 | 5 | 4 |
| NaSSA | 4 | 3 | 5 |
| Trazodone | 2 | 2 | 2 |
| Other | 4 | 3 | 6 |
| Total | 35 | 20 | 33 |
| Benzodiazepine (BZ) | 22 | 9 | 19 |
| Mood stabilizers (MS) | | | |
| Lithium | 2 | 0 | 1 |
| Valproic acid | 2 | 1 | 0 |
| Lamotrigine | 5 | 0 | 0 |
| Total (MS) | 9 | 1 | 1 |
| Antipsychotics (AP) | | | |
| Olanzapine | 1 | 2 | 0 |
| Quetiapine | 3 | 0 | 0 |
| Aripiprazole | 1 | 0 | 1 |
| Other | 1 | 0 | 0 |
| Total (AP) | 6 | 2 | 1 |
| Medication combination | | | |
| Single drug (AD) | 1 | 4 | 9 |
| Two AD | 1 | 1 | 0 |
| AD+BZ | 12 | 7 | 16 |
| AD+MS | 3 | 0 | 1 |
| AD+AP | 1 | 0 | 1 |
| Three or more classes | 8 | 2 | 0 |
| Other | 5 | 2 | 4 |

Abbreviation: TRD, treatment-refractory depression

the TRD group was 16.4 (SD=7.6), while it was lower for the intermediate (mean=11.6, SD=4.8) and remission groups (mean=3.2, SD=3.5). For avoidance, the mean score of the TRD group was 16.0 (SD=5.6), while it was lower for the intermediate (mean=12.4, SD=6.5) and remission group (mean=5.2, SD=5.3). For hyperarousal, the mean score of the TRD group was 14.3 (SD=5.0), while it was lower for the intermediate (mean=7.3, SD=3.1) and remission group (mean=2.0, SD=2.4).

### 3.3. The relationship between the IES-R and the HDRS

There was a significant positive correlation between the score of the IES-R for onset-related events and the HDRS for all three groups combined ($r=0.82$, $p<0.001$), although there were no correlations between the two measures within each group. There were no

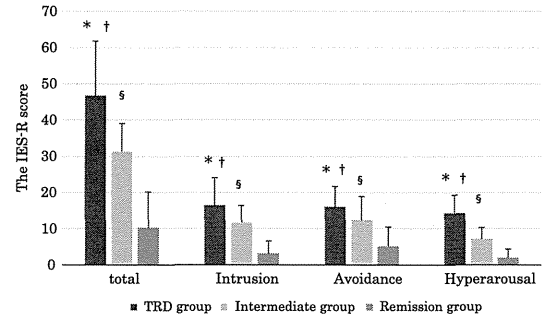


**Fig. 1.** IES-R scores (total and sub-categories: intrusion, avoidance, and hyperarousal) for treatment-refractory depression (TRD), remission, and intermediate group. *Comparison between TRD and remission group ($p<0.001$); †comparison between TRD and intermediate group ($p<0.001$); §comparison between intermediate and remission groups ($p<0.001$).

significant correlations between the IES-R score and the duration of disease, nor were there significant correlations between the IES-R score and duration of treatment. Furthermore, there was no correlation between the IES-R score and the LCU. There were no significant differences between the scores of the IES-R for patients with or without childhood life events or abuse, neither in all patients nor in the patients with TRD.

### 3.4. Relationship between TRD and bipolarity

The number of patients with bipolarity were calculated for the TRD ($n=10$), intermediate ($n=6$), and remission group ($n=3$). The TRD group had a significantly higher ratio of patients with bipolarity than the remission group, as confirmed by a Chi-square test ($p=0.028$). In more detail, the number of patients meeting the criteria of bipolar spectrum disorder were counted for the TRD ($n=2$), intermediate ($n=3$), and remission group ($n=0$). The numbers of patients meeting the criteria of bipolarity specifier were assessed for the TRD ($n=9$), intermediate ($n=5$), and remission group ($n=3$). One patient in the TRD and two patients in the intermediate group met the criteria of both bipolar spectrum disorder and bipolarity specifier. Table 4 shows the relationship between patients with or without bipolarity and two clinical variables such as the IES-R for onset-related events and the HDRS for all participants. The IES-R and HDRS scores were significantly higher in patients with bipolarity than in those without bipolarity.

**Table 4**
IES-R and HDRS scores in MDD patients with or without bipolarity.

| All patients | With bipolarity | Without bipolarity | *T* ratio [d.f.][a] | 95% CI[a] | *p*-Value[a] |
|---|---|---|---|---|---|
| 78 | 19 | 59 | | | |
| IES-R | 41.9 (18.3) | 24.9 (19.1) | 3.41 [76] | 7.09–26.94 | 0.001 |
| HDRS | 17.4 (9.1) | 11.6 (9.0) | 2.44 [76] | 1.07–10.51 | 0.017 |

*Note:* Variables represent mean (standard deviation: SD)
Abbreviations: IES-R, Impact of Event Scale-Revised; HDRS, Hamilton Depression Rating Scale; MDD, major depressive disorder

[a] Statistical analysis was performed by Student's *t*-test.

## 4. Discussion

In this study, we made three important clinical observations. First, the main finding of our study was that patients with TRD perceive their onset-related events as more serious psychological distress symptoms than patients with remitted MDD or MDD patients with mild residual depressive symptoms. Second, our results demonstrate that the severity of depressive symptoms shows a significant positive correlation with the severity of onset-related psychological distress symptoms in patients with MDD. Third, our study further shows that the clinical feature of bipolarity might be associated with severe depressive symptoms and onset-related psychological distress symptoms in patients with MDD.

The first main finding of our study was that patients with TRD think of their onset-related events as more serious psychological distress symptoms than those with remitted MDD or MDD patients with mild residual depressive symptoms. To our knowledge, this is the first study to demonstrate that patients with TRD perceive their experiences of onset-related events as serious psychological distress symptoms such as intrusion, avoidance, and hyperarousal. It has been reported that clinical factors including comorbid psychiatric disorders such as panic disorder and anxiety disorders, current suicidal risk, lack of full remission after a previous episode, age at onset younger than eighteen years, and bipolarity are related to TRD (Dudek et al., 2010; Souery et al., 2007). Regarding additional clinical factors, it is suggested that neuroticism and excessive pessimistic and negative thinking are involved in treatment resistant depression (Thase et al., 2001). Our findings may present not only a new perspective on clinical symptoms of TRD, but also suggest additional clinical factors associated with TRD.

Our results demonstrate that the severity of depressive symptoms is positively correlated with the severity of onset-related psychological distress symptoms in patients with MDD. This result should be interpreted in two ways: (1) Onset-related psychological distress symptoms directly affect depressive symptoms and may contribute to a worse prognosis for patients with MDD, although prospective cohort studies are needed to establish this concept. (2) The severity of the depressive state may affect cognitive distortions in depressed patients, and consequently elicit onset-related psychological distress symptoms. The possibility of a cognitive negative bias induced by the depressive state should be considered further (Beck, 2008). Patients with MDD tend to have pessimistic thoughts and recognize their future as hopeless when suffering from severe depression (Abramson et al., 1978). Consequently, it has been implied that cognitive distortions occur in depressed patients (Beck, 2008). From a neuroscience perspective, it was suggested that the human condition of depression could damage glial and neuronal cells in the hippocampus and reduce hippocampal volume (Campbell et al., 2004; Videbech and Ravnkilde, 2004). It was further reported that the morphologic change of the hippocampus could induce memory impairments in patients with depression (Sheline et al., 1999). Moreover, it was reported that the clinical condition of depression in particular induces impairment in declarative memory (Burt et al., 1995). It may be possible that patients with depression recall their past life events such as onset-related events with a bias due to distorted cognition. As a consequence of distorted cognition and memory impairment, patients with TRD may develop a more negative impression of their life events compared to mild or remitted MDD patients.

The present results showed that onset-related psychological distress symptoms are more severe in MDD patients with bipolarity than in those without bipolarity. In addition, our results suggest that TRD patients with bipolarity tend to experience severer onset-related psychological distress symptoms than TRD patients without bipolarity. It has been suggested that patients with clinical features of bipolarity share clinical similarities to patients with bipolar disorders rather than with unipolar depression on the basis of the concept of bipolar spectrum (Akiskal, 1995; Akiskal et al., 2006). Several studies suggest that patients with bipolar disorders have a higher prevalence of PTSD than those with MDD (Otto et al., 2004; Pollack et al., 2006). A high prevalence of traumatic experiences in patients with severe mental disorder such as bipolar disorder has been reported (Mueser et al., 1998). Moreover, the relationship between bipolar disorder and PTSD has received further support by findings of molecular biology, which implicates that low secretion of brain-derived neurotrophic factor (BDNF) in the human brain may be a risk factor for the onset of PTSD (Rakofsky et al., 2012), and the production of BDNF may also be reduced in patients with bipolar disorder (Rakofsky et al., 2012). The relationship between the clinical factor of bipolarity and onset-related psychological distress symptoms in depressed patients observed in the current study might contribute to adding a new perspective to the concept of mood disorders.

Our main finding that patients with TRD perceive their onset-related events as serious psychological distress symptoms such as intrusion, avoidance, and hyperarousal could not only be useful to improve understanding of clinical features of TRD, but also presents the potential for new perspectives in the treatment of TRD. Regarding the role of psychotherapy in the management of TRD, cognitive-behavioral therapy and interpersonal psychotherapy have been recommended as evidence-based treatments in combination with antidepressant medication (Flynn and Himle, 2011; Thase, 2013). On the basis of our finding, it may be effective to incorporate psychological intervention targeting psychological distress symptoms associated with onset-related events for patients with TRD. For example, psychotherapy techniques of prolonged exposure and cognitive restructuring may be effective in the treatment of TRD as well as PTSD (Marks et al., 1998). It was reported that prolonged exposure therapy improved symptoms of depression in patients with PTSD (Eftekhari et al., 2013). Using imaginal exposure for traumatic life-event memories or reappraising TRD patients' beliefs about their life events, which they thought as triggering the onset of depression, may be useful for the treatment of TRD.

In terms of pharmacotherapy for psychological distress, several case series reported that treatment with ifenprodil was effective for patients with PTSD, especially for treating flashbacks (Kishimoto et al., 2012; Sasaki et al., 2013). Ifenprodil is a neuroprotective drug that acts as an antagonist of the N-methyl-D-aspartate (NMDA) receptor, by binding to the GluN2B subunit (Williams, 2001). Ifenprodil also binds to the endoplasmic reticulum protein sigma-1 receptors (Hashimoto and London, 1993, 1995). Sigma-1 receptors play a role in the pathophysiology of neuropsychiatric diseases (Hashimoto and Ishiwata, 2006). Future studies examining treatment of TRD patients by psychotherapy and pharmacotherapy, such as ifenprodil treatment, may be needed.

### 4.1. Limitations

Three issues need to be discussed for the limitations. First, this study was not able to exclude the possibility that the severity of

onset-related psychological distress symptoms could cause the difficult therapeutic course in patients with TRD, because a cross-sectional design was implemented as the method of this study. To resolve this problem, prospective cohort studies are needed in the future. For the same reason, this study was not able to eliminate potential confounding factors, even if certain patient characteristics did not differ significantly between the three groups. Second, the limitation of the assessment method for onset-related psychological distress symptoms in patients with MDD should be acknowledged. Although the contents of the IES-R cover all aspects of psychological traumatic symptoms, the IES-R has been originally developed and used for a rating scale of PTSD severity. To establish validity and reliability of the assessment of onset-related psychological distress symptoms, further studies are needed to include a multilateral approach to assess symptoms of psychological distress in patients with MDD. Third, the recall bias might present an additional problem with this study, because the participants were requested to recall past life events.

## 5. Conclusion

This study confirmed that patients with TRD perceive their experiences of onset-related events as serious psychological distress symptoms. Future studies should be conducted to confirm distress symptoms in MDD or depressed patients with a wide range of clinical features, and to investigate the pathophysiology of psychological distress in patients with TRD through neuropsychological examinations and brain imaging techniques.

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**Conflict of interest**
We declare no conflicts of interest.

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# Understanding the pharmacogenetics of selective serotonin reuptake inhibitors

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***Introduction:*** The genetic background of antidepressant response represents a unique opportunity to identify biological markers of treatment outcome. Encouraging results alternating with inconsistent findings made antidepressant pharmacogenetics a stimulating but often discouraging field that requires careful discussion about cumulative evidence and methodological issues.
***Areas covered:*** The present review discusses both known and less replicated genes that have been implicated in selective serotonin reuptake inhibitors (SSRIs) efficacy and side effects. Candidate genes studies and genome-wide association studies (GWAS) were collected through MEDLINE database search (articles published till January 2014). Further, GWAS signals localized in promising genetic regions according to candidate gene studies are reported in order to assess the general comparability of results obtained through these two types of pharmacogenetic studies. Finally, a pathway enrichment approach is applied to the top genes (those harboring SNPs with $p < 0.0001$) outlined by previous GWAS in order to identify possible molecular mechanisms involved in SSRI effect.
***Expert opinion:*** In order to improve the understanding of SSRI pharmacogenetics, the present review discusses the proposal of moving from the analysis of individual polymorphisms to genes and molecular pathways, and from the separation across different methodological approaches to their combination. Efforts in this direction are justified by the recent evidence of a favorable cost-utility of gene-guided antidepressant treatment.

**Keywords:** antidepressant, depression, gene, genome-wide, genome-wide association studies, pathway, pharmacogenetics, pharmacogenomics, polymorphism, serotonin reuptake inhibitor

*Expert Opin. Drug Metab. Toxicol. [Early Online]*

## 1. Introduction

Antidepressant drugs, especially selective serotonin (5-HT) reuptake inhibitors (SSRIs), are largely used to treat a range of psychiatric disorders, the most common of which are mood disorders (65.3%), followed by anxiety disorders (16.4%) [1]. In the US, the rate of antidepressant treatment increased from 5.84% in 1996 to 10.12% in 2005, and regarded almost all sociodemographic groups [2]. Despite this widespread use, symptom remission during antidepressant treatment is reached in only one-third of patients and poor tolerability is a common cause of early treatment discontinuation [3]. No reliable clinical, environmental, demographic or biological predictor(s) of SSRI response and side effects have been identified so far, thus treatment choice and dose are usually determined according to a trial and error principle. This results in high personal and socioeconomic burden, indeed depressive disorders are responsible for the most part of disability-adjusted life years caused by mental disorders (40.5%), followed by anxiety disorders (14.6%) [4]. The observation of clustering of antidepressant response in relatives of affected

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