Neuropsychopharmacology (2017) 42, 2482–2492

www.neuropsychopharmacology.org

Dose-Related Effects of Adjunctive Ketamine in Taiwanese Patients with Treatment-Resistant Depression

Tung-Ping Su[*,1,2,3,4], Mu-Hong Chen[2,4], Cheng-Ta Li[1,2,4], Wei-Chen Lin[2,4], Chen-Jee Hong[1,2,4], Ralitza Gueorguieva[5,6], Pei-Chi Tu[1,2,3], Ya-Mei Bai[1,2], Chih-Ming Cheng[2] and John H Krystal[*,6,7,8,9]

[1]Division of Psychiatry, Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan; [2]Department of Psychiatry, Taipei Veterans General Hospital, Taipei, Taiwan; [3]Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan; [4]Institute of Brain Science, National Yang-Ming University, Taipei, Taiwan; [5]Department of Biostatistics, School of Public Health, Yale University, New Haven, CT, USA; [6]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA; [7]Department of Neuroscience, Yale University School of Medicine, New Haven, CT, USA; [8]Yale-New Haven Hospital, New Haven, CT, USA; [9]VA National Center for PTSD, VA Connecticut Healthcare System, West Haven, CT, USA

The antidepressant effects of ketamine are thought to depend on brain-derived neurotrophic factor (BDNF) genotype and dose. The purpose of this study was to characterize the dose-related antidepressant effects of ketamine in patients with treatment-resistant depression drawn from a Chinese population predominately possessing lower activity *BDNF* genotypes (Val/Met, Met/Met). We conducted a double-blind, randomized, parallel-group, placebo-controlled trial of a single ketamine infusion (saline, 0.2 mg/kg, 0.5 mg/kg). Patients ($N=71$; BDNF genotype: Val/Val ($N=12$, 17%), Val/Met ($N=40$, 56.3%), and Met/Met ($N=19$, 26.8%)) received mood ratings before infusion, after infusion, and for the subsequent 14 days. Plasma ketamine levels and BDNF genotypes were assessed. This study found a significant dose-related ketamine effect on scores on the Hamilton Depression Rating Scale (HAMD). The responder analysis (>50% reduction from baseline HAMD on at least 2 days between days 2 and 5) also revealed a significant dose-related effect (saline: 12.5%, 0.2 mg/kg: 39.1%; 0.5 mg/kg: 45.8%). This is the first report to our knowledge to demonstrate the dose-related efficacy of R/S-ketamine for treatment-resistant depression and the first to characterize ketamine effects in a genotyped Chinese population in which most (83%) patients possessed at least one copy of the lower functioning Met allele of the BDNF gene.
Neuropsychopharmacology (2017) **42,** 2482–2492; doi:10.1038/npp.2017.94; published online 14 June 2017

INTRODUCTION

The discovery and replication of the rapid and robust antidepressant effects of ketamine for treatment-resistant symptoms of depression may constitute an important treatment advance, addressing some of the limitations of current antidepressant treatment (Berman *et al*, 2000; Kishimoto *et al*, 2016; Krystal *et al*, 2013). In STAR*D, remission was achieved on average only after 2 months of citalopram treatment and all adjunctive treatments produced low remission rates in citalopram nonresponders, generally ˂30% (Gaynes *et al*, 2009). In contrast, ketamine produces clinical response in 50–75% of patients and remission in ~30% of patients 24 h following a single 0.5 mg/kg dose (Aan Het Rot *et al*, 2012).

*Correspondence: Dr T-P Su, Department of Psychiatry, Taipei Veterans General Hospital, No. 201, Section 2, Shih-Pai Road, Beitou District, Taipei 112, Taiwan. Tel: +886-2-28757027 (extn 302), Fax: +886-2-28733113, E-mail: tomsu0402@gmail.com or Dr JH Krystal, Department of Psychiatry, Yale University School of Medicine, 300 George Street 901, New Haven, CT 06511, USA, Tel: +1 203 785 6396, Fax: +1 203 785 6196, E-mail: john.krystal@yale.edu
Received 13 January 2017; revised 30 March 2017; accepted 30 April 2017; accepted article preview online 11 May 2017

The development of ketamine as a routine treatment for depression is limited by gaps in our knowledge about how to optimize its implementation. For example, the dose–response relationship for the antidepressant effects for R/S-ketamine has never been rigorously tested. One prior study of the more potent S-isomer of ketamine suggested that both 0.2 and 0.4 mg/kg were effective (Singh *et al*, 2015). R/S-ketamine, the form of ketamine currently available in the United States, is routinely dosed as a 40 min intravenous infusion of 0.5 mg/kg to treat depression based on prior work in healthy subjects (Berman *et al*, 2000; Krystal *et al*, 1994). However, it is possible that lower R/S-ketamine doses might retain efficacy while having lower propensity to produce perceptual, cognitive, euphorigenic, hypertensive, and nausea-stimulating effects.

In addition, the generalizability of ketamine efficacy to populations of diverse race and ethnicity has not been established. There has been particular interest in the efficacy of ketamine in the Han Chinese because of the high prevalence of a less functional Met allele of the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene (Choi *et al*, 2006; Itoh *et al*, 2004; Xu *et al*, 2012), known to influence the intracellular trafficking and secretion of BDNF (Chen *et al*, 2004). A preclinical study

(Liu *et al*, 2012) and small clinical pilot study (Laje *et al*, 2012) suggest that the presence of the Met allele attenuates the antidepressant response to ketamine. Thus, one might expect the antidepressant response to ketamine to be attenuated in this population. However, a small (15 patients per group) prior study of ketamine plus escitalopram in comparison with citalopram in Chinese patients did find evidence of efficacy in patients, although BDNF genopytpes were not reported (Hu *et al*, 2016).

The purpose of the current study was to conduct a double-blind, randomized, parallel-group, placebo-controlled study of the dose-related antidepressant effects of R/S-ketamine in genotyped Taiwanese patients. Patients were engaged in ongoing antidepressant treatments and still met criteria for treatment-resistant depression. This study found evidence of dose-related ketamine efficacy in this population, supporting the continued exploration of the 0.5 mg/kg dose as pharmacotherapy for treatment-resistant symptoms of depression.

MATERIALS AND METHODS

Screening Process and Patient Selection

Patients were recruited at the outpatient clinic of Taipei Veterans General Hospital (TVGH) from 2012 to 2015 (see Figure 1 for CONSORT diagram). This study was approved by the institutional review board of TVGH and the Department of Health of Taiwan. All patients provided written informed consent before study entry. Patients met DSM IV criteria for major depressive disorder (MDD), recurrent without psychotic features on the basis of the Mini International Neuropsychiatric Interview (MINI) (Sheehan *et al*, 1998), and who had failed to respond to more than two adequate antidepressant trials. The evaluation of the adequacy of prior antidepressant trials and the inadequacy of clinical response was determined on the basis of a semistructured patient interview and review of the medical record. Patients were excluded if they had a history of bipolar disorder, psychotic symptoms, substance dependence other than nicotine, and mild symptoms (17-item Hamilton Depression Rating Scale (HAMD) score <18 at screening or <13 before study entry; Hamilton, 1960). Patients with active medical disease were excluded on the basis of medical screening that included history, physical examination, laboratory tests (electrolytes, renal, liver, glucose, cholesterol), and electrocardiogram. Of the 74 patients enrolled, 3 subjects were excluded (hypertension ($N=1$), hyperglycemia ($N=1$), and remitted mood status ($N=1$)) from the study. No medication alterations were permitted in the 2 weeks before randomization and throughout the study. The study population is described in Table 1.

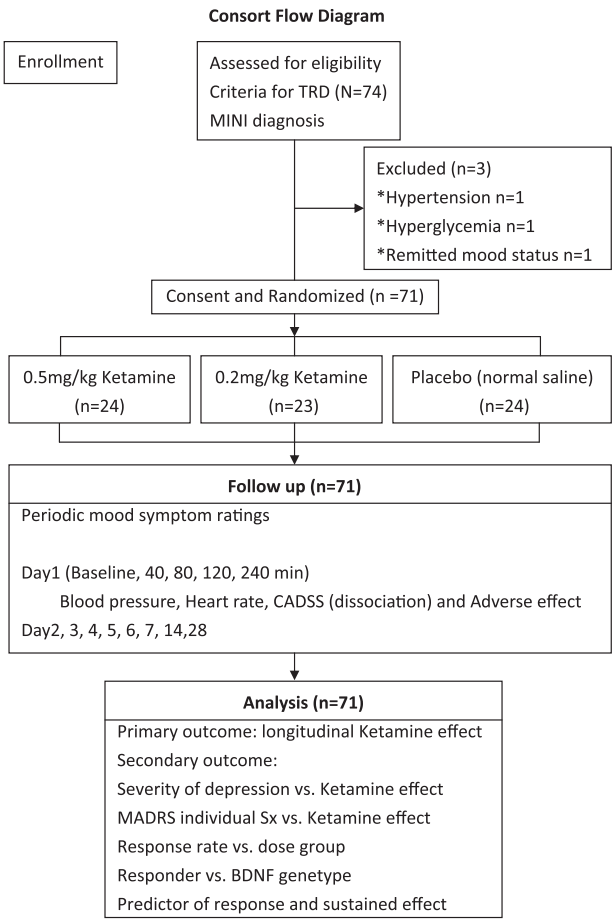


Figure 1 Consort flow diagram.

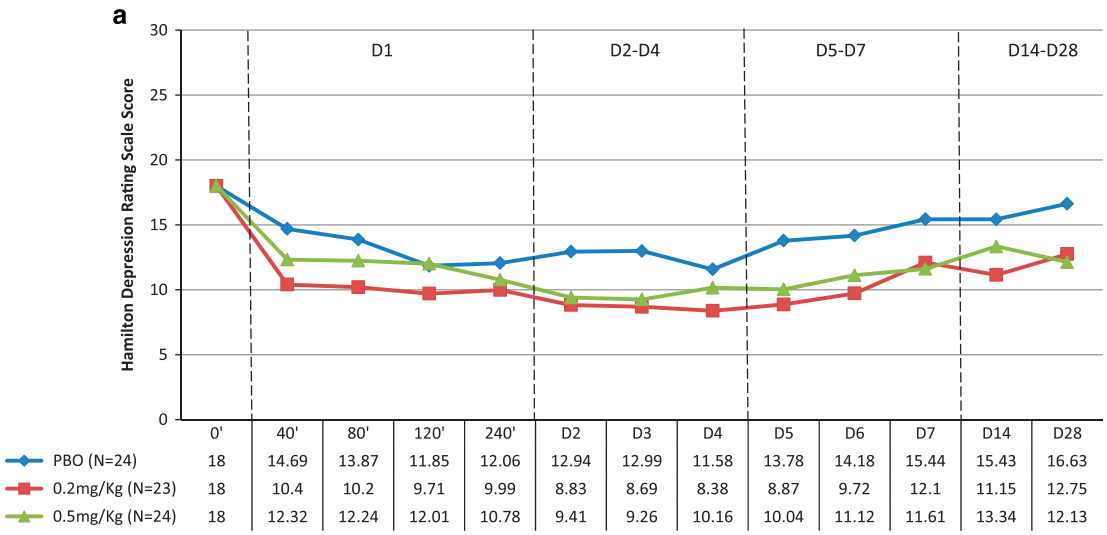
Study Design and Procedures

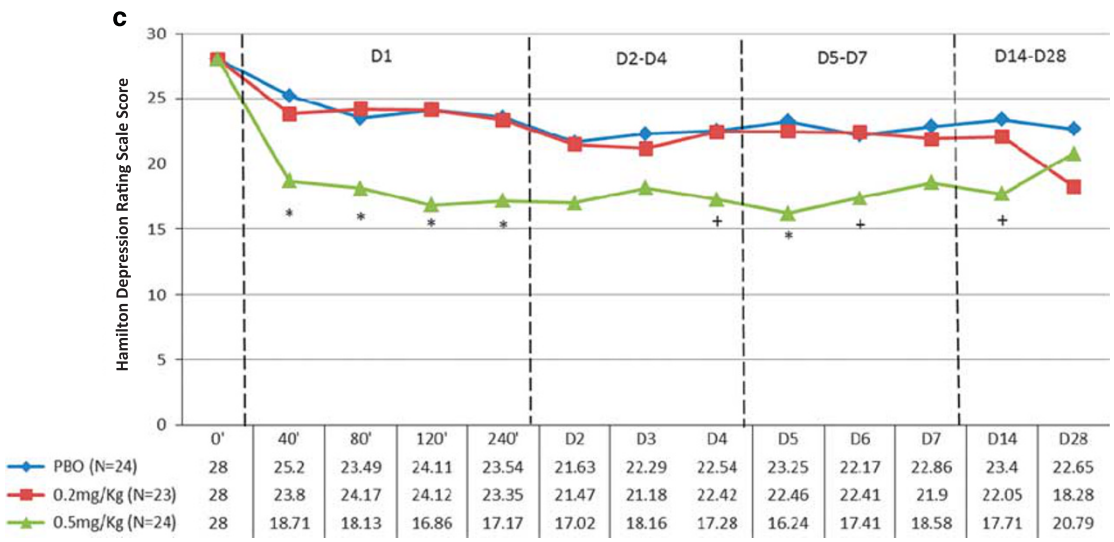
Patients completed a single 40 min intravenous infusion test day as part of a parallel-group design. Patients were studied under double-blind conditions and they were randomized to receive an infusion of saline or R/S-ketamine hydrochloride (Ketalar, Pfizer Pharmaceuticals) at doses of 0.2 or 0.5 mg/kg. Blood pressure, heart rate, and digit pulse oximetry were monitored repeatedly preinfusion and 10, 20, 30, 40, 80, 120, and 240 min following the initiation of ketamine infusion. HAMD was administered in person before the initiation of test infusions and 40, 80, 120, and 240 min later and telephone ratings were conducted 24, 48, 72, 96, 120, 144, and 288 h after infusion. We included telephone ratings in

Table 1 Characteristics of Patients with Treatment-Resistant Depression in Three Dosage Groups

Mean ± SD or *n* (%)	PBO *n* = 24 (%)	0.2 mg/kg *n* = 23 (%)	0.5 mg/kg *n* = 24 (%)
Age (y/o)	48.6 ± 8.2	45.0 ± 12.3	48.5 ± 11
Female	15 (62.5)	17 (73.9)	21 (87.5)
Height (cm)	162.2 ± 8.4	160 ± 9	158.6 ± 5.6
Weight (kg)	61.9 ± 11.5	55.1 ± 8.5	60.9 ± 10.2
BMI (kg/m^2)	23.6 ± 4.9	21.6 ± 3.5	24.2 ± 3.9
Education (years)	12.4 ± 3	13.1 ± 3.6	11.5 ± 3.7
Employed	6 (25.0)	7 (30.4)	6 (25.0)
Onset of first episode	38.1 ± 11.4	35.5 ± 12.4	35.5 ± 10.2
Duration of illness	10.9 ± 6.8	9.7 ± 8.7	13.2 ± 9
Suicidal history	13 (54.2)	7 (30.4)	14 (58.3)
Baseline HAMD-17	23.3 ± 4.1	23.1 ± 4.8	23 ± 4.9
Baseline MADRS	35 ± 4.9	35.1 ± 6.7	34 ± 7.3
Baseline BDI	30.9 ± 9.1	30.5 ± 8.9	28.3 ± 11.4

this study based on prior evidence that HAMD total scores from telephone interviews show a high level of correlation (interclass coefficient = 0.8) with face-to-face interviews (Simon *et al*, 1993). This high level of correlation holds even though assessments of some items that typically involve visual inspection during face-to-face interviews (eg, motor





retardation) depend entirely on self-report in the context of telephone interviews. Our impression is consistent with telephone administration of the HAMD within the NIMH STAR*D trial (Rush *et al*, 2004). For ketamine infusion test days, assessments were evaluated in relation to baseline. This plan has the consequence that some HAMD measures (sleep, weight) do not change during the test day. Subsequent assessments were based on the prior 24 h. To facilitate this, efforts were made to ensure that follow-up time points were consistent across test days. Consistent with our prior study (Berman *et al*, 2000), patients were discharged from testing 240 min after infusion, after all ketamine effects had abated.

Outcome Measures

The primary outcome was the HAMD. Psychotogenic effects of ketamine were assessed with the Brief Psychiatric Rating Scale (BPRS) positive symptom subscale that includes the following items: hallucinatory behavior, unusual thought content, suspiciousness, and conceptual disorganization (Hedlund and Vieweg, 1980; Overall and Gorham, 1962).

Laboratory Procedures

BDNF Val 66Met polymorphism/genotyping. Genomic DNA was extracted from EDTA-containing venous blood samples for BDNF Val66Met polymorphism genotyping. The DNA fragments of interest were amplified using PCR with the primers 5′-ACTCTGGAGAGCGTGAAT-3′ and 5′-AT ACTGTCACACACGCTC-3′. The PCR was performed in a total volume of 10 µl containing 50 ng of template DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 µM of dNTP, 10 pmol of each oligonucleotide, and 0.25 U of *Taq* DNA polymerase. Amplification conditions consisted of an initial 4 min denaturation step at 94 °C, 32 cycles of 30 s at 94 °C, 30 s at 58 °C, and 30 s at 72 °C, followed by a final extension of 10 min at 74 °C. The Val66Met polymorphism was differentiated with the *Nla*III restriction enzyme. Partial digestion was minimized by an internal restriction site and a control sample of digestible homozygous Val/Val. The genotyping was processed blinded to clinical data.

Plasma levels of ketamine. Plasma samples were obtained at baseline, and at 40, 80, 120, and 240 min after ketamine infusion via a separate intravenous line different from the one for ketamine administration. Samples were frozen at − 80 °C until analysis. Plasma levels of ketamine (K) and norketamine (NK) were analyzed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) with the extraction procedure consisted of protein precipitation of serum samples with 10% trichloroacetic acid (TCA) and subsequent centrifugation. The Agilent Triple Quad LC-MS/MS was used in the positive electrospray ionization multiple reaction monitoring (MRM) mode and quantification was accomplished using area ratios calculated using D_4-K and D_4-NK as the internal standard, the concentration of which was set at 1000 ng/ml. Data were acquired and analyzed using Mass Hunter software, version B01.03. Calibration curves were linear over a range of 10–500 ng/ml for K and NK ($r^2 \geqslant 0.995$), respectively. The limit of detection (LOD) and limit of quantification (LOQ) were 10 ng/ml for K and NK, respectively. The intraday precision and accuracy of three concentrations (low, middle, and high) of K and NK ranged from 2.1 to 8.9% and from − 0.5 to 8.3%, respectively.

Statistical Analyses

Descriptive statistics were calculated before statistical analysis and the normality of continuous variables was assessed using normal probability plots. Differences at baseline among groups were evaluated using Fisher's exact tests for nominal variables and one-way ANOVA for continuous variables.

A mixed effects regression model was used to assess ketamine effects on HAMD during the treatment period with group (0.5 and 0.2 mg/kg and placebo) as a between-subject factor, time (40, 80, 120, and 240 min on days 1, 2, 3, 4, 5, 6, 7, 14, and 28) as a within-subject factor, baseline HAMD score as a between-subject predictor, and all possible interactions. Unstructured variance–covariance matrix was used to account for correlations of repeated measures on the same individual over time. All available data on individuals were used. Adjusted *post hoc* comparisons of least square means were used to interpret significant effects in the model.

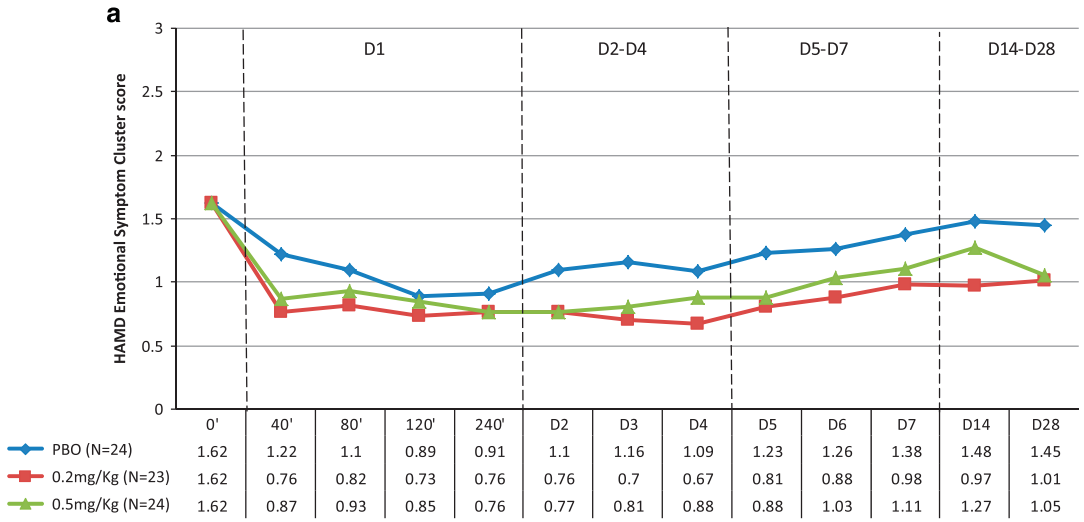
The effects of ketamine on features of depression rather than the overall severity of depression was explored by evaluating the effects on HAMD symptom factor scores separately using the same model as for the total score. A prior study identified three symptom clusters for the HAMD, based on data from the 4309 patients in the STAR*D trial (Chekroud *et al*, 2017): emotional symptoms (somatic anxiety, psychological anxiety, guilt and delusions, loss of interest, depressed mood), atypical symptoms (reduced libido, psychomotor slowing, suicidality, psychomotor agitation, hypochondriasis), and insomnia-related symptoms (energy/fatigability, delayed sleep onset, midnocturnal awakening, early morning awakening). Scores for each symptom cluster were calculated by averaging the individual items in the cluster.

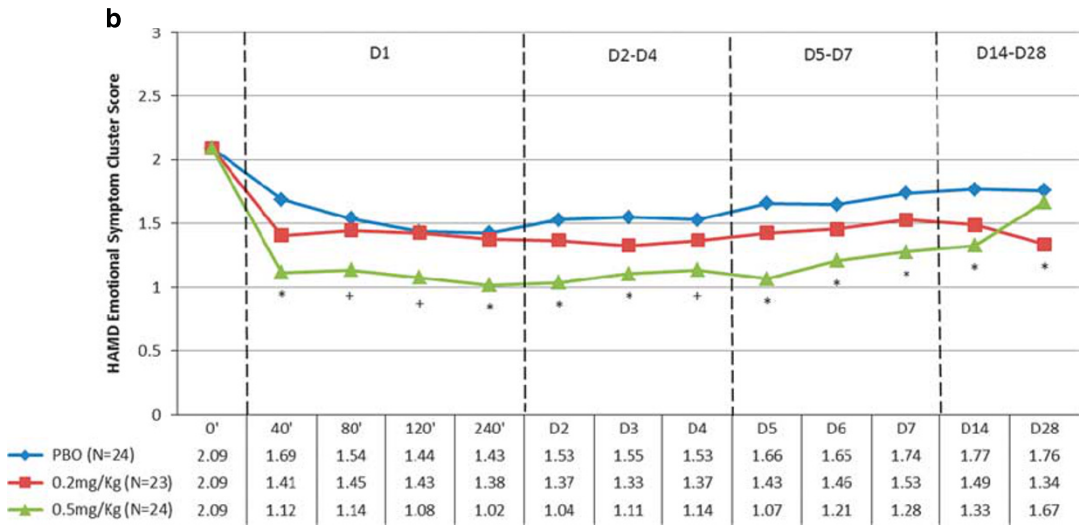
To evaluate the predictive effects of BDNF genotype on depression severity, genotype (Val/Val *vs* Met carrier) and its interactions with baseline severity and time were added to the mixed model described above. Because of the small number of subjects with Val/Val genotype in each treatment

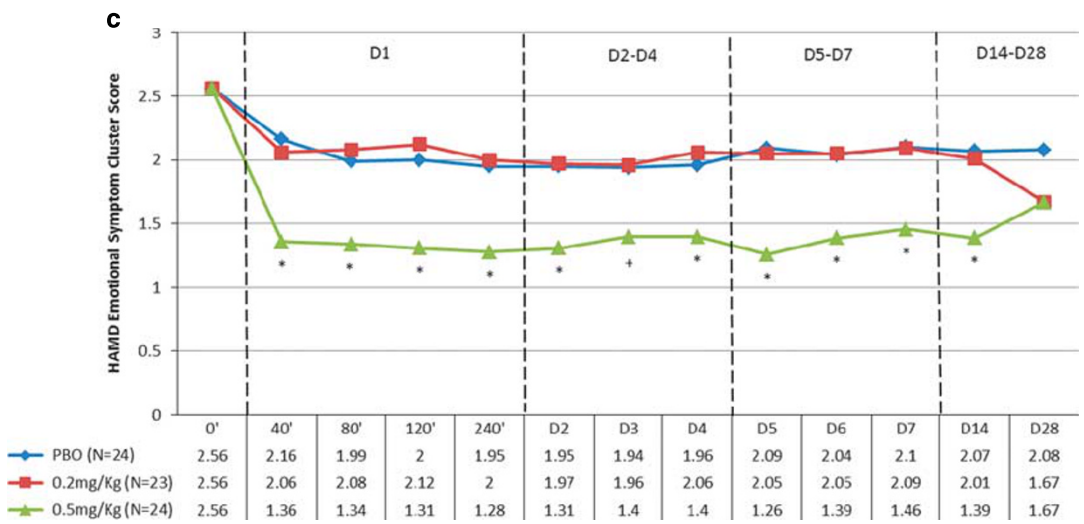
Figure 2 Least square means for Hamilton Depression Rating Scale-17 (HAMD) total scores by baseline HAMD severity level: low (a, top figure), average (b, middle figure), and high (c, bottom figure). The presented least square means are based on the model fit to the entire sample and hence the sample in each figure is the same. (a) Estimated least square means for HAMD scores by group at 'low' baseline depression severity, ie, HAMD scores = 1 SD below the mean (baseline HAMD score = 18). There were no significant differences between ketamine groups. (b) Estimated least square means for HAMD scores by group at 'average' baseline depression severity, ie, baseline HAMD score = 23. (c) Estimated least square means for HAMD scores by group at 'high' baseline depression severity, ie, baseline HAMD scores = 1 SD above the mean (baseline HAMD score = 28). Significant differences between ketamine groups, $^{*}p < 0.05$, $^{+}p < 0.1$ (total $n = 71$). PBO, saline placebo.

group (as low as 3), interactions of group and genotype were not evaluated.

Responder status was identified by response (⩾50% reduction of mood ratings) at any two daily HAMD measures during the period of 24 to 96 h (days 2 to 5) after infusion. Responder rates were compared among groups using Fisher's exact test. Separate Fisher's exact tests by genotype were also performed. Nonparametric mixed effects model with the same predictors as the one described above was used to assess ketamine effects on BPRS positive symptoms, as in prior ketamine studies (Krystal *et al*, 2005).







Ketamine and norketamine levels were compared between groups, across time points, and by responder status by fitting mixed effects models on log-transformed data similar to the model for the primary analysis.

Statistical tests were performed using SAS (SAS software, version 9.4; SAS Institute, Cary, NC). All tests were considered significant at 0.05 family-wise significance level.

Clinical trials registration: UMIN Clinical Trials Registry (UMIN-CTR): Registration number: UMIN000016985.

RESULTS

Study Subjects

As presented in Table 1, patients were predominately female between 40 and 50 years of age, and not obese. There were no significant differences in demographic data, medications, or comorbid diagnoses across groups. Before study entry, patients were treated predominately with the combination of at least one antidepressant and a second-generation antipsychotic (51%, $n=36$), a single antidepressant (28%, $n=20$), or two or more antidepressants (21%, $n=15$). Psychiatric comorbidities included chronic dysthymia (49.3%, $n=35$), generalized anxiety disorder (GAD, 50.7%, $n=36$), panic disorder (32.4, $n=23$%), social phobia (12.7%, $n=9$), and posttraumatic stress disorder (11.3%, $n=8$). Rates of psychiatric comorbidities did not differ by group. Across groups, 40 subjects (56.3% of the patients) had two or more comorbid psychiatric diagnoses, 22 (31%) had one comorbidity, and 9 (12.7%) had no comorbidities. At baseline, the rate of moderate-to-severe suicidality (score >6), as assessed using the MINI, was greater in the patients receiving 0.5 mg/kg than those with 0.2 mg/kg and placebo ($n=12$: 50%, $n=3$: 13% and $n=3$: 13%, respectively, Fisher's exact test p-value $=0.001$). Forty-eight of the subjects in this study participated in neuroimaging during their ketamine infusion. The neuroimaging results from these subjects were reported elsewhere (Li *et al,* 2016).

HAMD Score

There was a significant interaction between group, time, and baseline HAMD score in the mixed model assessing ketamine effects on depression severity as measured by the HAMD ($F(22, 64.9)=1.90$, $p=0.02$), indicating that dose effects differed by time point and baseline severity. The group by time interaction was also statistically significant ($F(22, 64.9)=1.74$, $p=0.04$). Therefore, we assessed group differences overall and by time point, and we report the results at different levels of baseline depression severity: 'low' baseline severity (ie, baseline HAMD fixed at 1 SD below the mean, baseline HAMD = 18; Figure 2a), 'average' baseline severity (baseline HAMD level = 23; Figure 2b), and 'high' baseline severity (baseline HAMD level fixed at 1 SD above the mean, baseline HAMD = 28; Figure 2c).

Overall, the 0.5 mg/kg dose was statistically significantly better than placebo (Tukey's-adjusted $p=0.008$) but the 0.2 mg/kg dose was not (adj. $p=0.20$), and the two ketamine doses were not significantly different from one another (adj. $p=0.37$). The results also show that the distinctive effects of ketamine 0.5 mg/kg relative to placebo or ketamine 0.2 mg/kg are more pronounced with increasing depression severity. Across time points, after Tukey–Kramer adjustment for three-dose comparisons at each baseline severity level, the *post hoc* tests showed that there were no significant ketamine effects at the lower baseline HAMD severity (all p-values >0.15) and significant ketamine efficacy for the 0.5 mg/kg dose compared with placebo at the mean (adjusted p-value $=0.01$) and higher baseline HAMD severity (adjusted p-value $=0.02$). At high baseline depression severity, the 0.5 mg/kg dose was more effective than the 0.2 mg/kg dose (adjusted p-value $=0.05$). The 0.2 mg/kg dose did not differ significantly from placebo (adjusted p-values >0.15). The between-group differences were most pronounced on day 1 and day 5 following ketamine infusion.

HAMD Factor Scores

There was a significant interaction between ketamine dose and baseline severity for the emotional symptom cluster scores ($F(2, 65)=3.21$, $p=0.05$; see Figure 3). For the atypical symptom cluster scores, there was a statistically significant interaction between group, time, and baseline atypical severity ($F(22, 65)=2.10$, $p=0.01$; see Figure 4). There were no statistically significant between-group differences for the sleep cluster (all p-values >0.26; data not presented).

For both the emotional and atypical symptom cluster scores, the group differences were more pronounced at higher baseline levels of severity for their respective symptom clusters (see Figures 3 and 4). For the emotional symptom cluster, the 0.5 mg/kg group was significantly different from the 0.2 mg/kg and placebo groups at high baseline depressive severity. At mean baseline severity, the 0.5 mg/kg differed significantly from the placebo group but not from the 0.2 mg/kg group. The three groups were not different from each other at low baseline severity on average across time points. For the atypical symptom cluster, the 0.5 mg/kg ketamine group was different from the placebo group at high and mean baseline atypical symptom level on the day following ketamine infusion.

Thus, it appears that the significant differences observed for total HAMD are due to changes on emotional and atypical symptoms, with changes in emotional symptoms

Figure 3 Least square means for Hamilton Depression Rating Scale-17 (HAMD) emotional cluster (somatic anxiety, psychological anxiety, guilt and delusions, loss of interest, depressed mood) symptom scores by baseline emotional cluster severity level: low (a, top figure), average (b, middle figure), and high (c, bottom figure). The presented least square means are based on the model fit to the entire sample and hence the sample in each figure is the same. (a) Estimated least square means for HAMD emotional cluster scores by group at 'low' baseline emotional cluster severity, ie, baseline emotional cluster scores = 1 SD below the mean. There were no significant differences between ketamine groups. (b) Estimated least square means for HAMD emotional cluster scores by group at 'average' baseline emotional cluster severity. (c) Estimated least square means for HAMD emotional cluster scores by group at 'high' baseline emotional cluster severity, ie, baseline scores = 1 SD above the mean. Significant differences between ketamine groups, $^{*}p<0.05$, $^{+}p<0.1$ (total $n=71$). PBO, saline placebo.

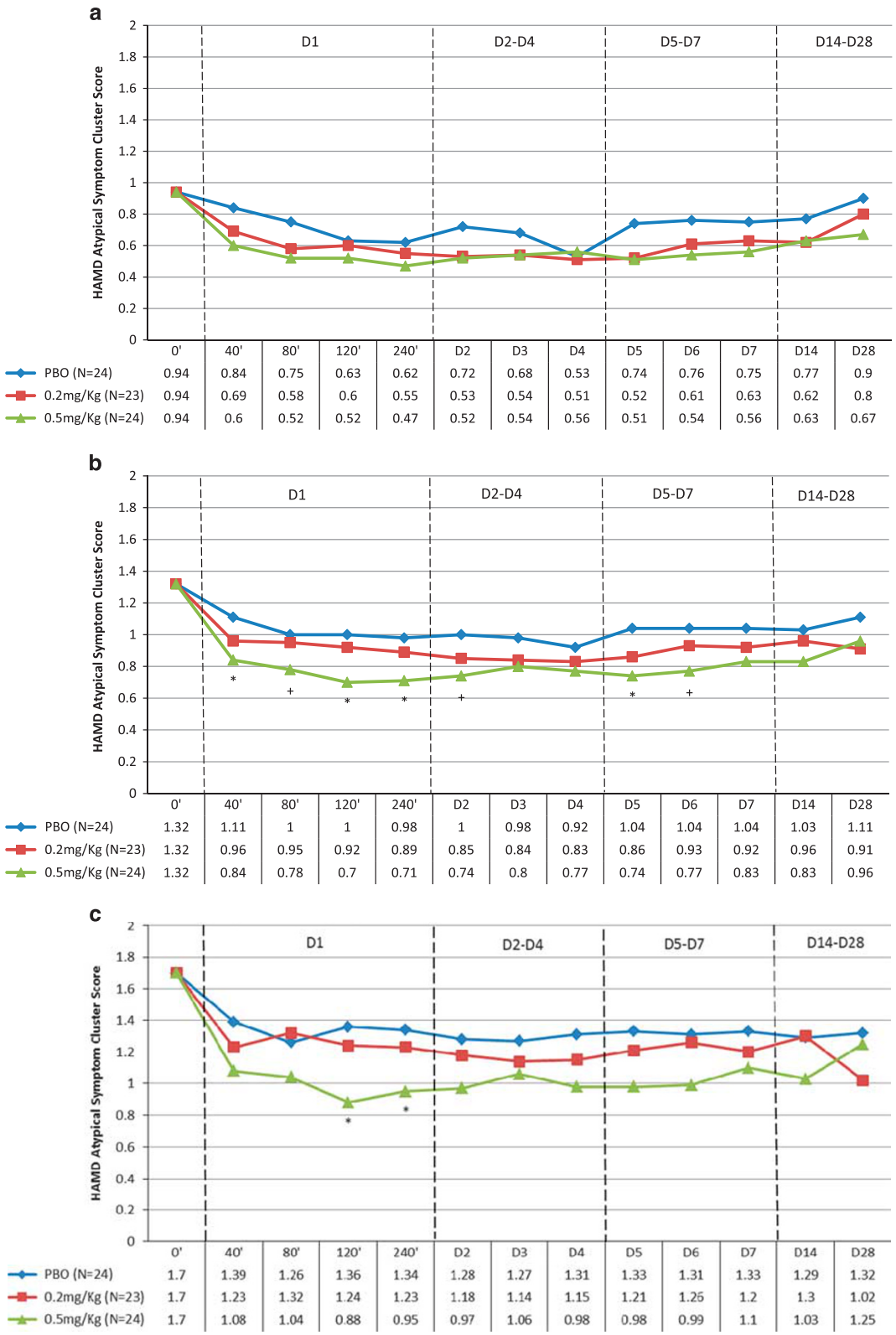


Figure 4 Least square means for Hamilton Depression Rating Scale-17 (HAMD) atypical symptom cluster (reduced libido, psychomotor slowing, suicidality, psychomotor agitation, hypochondriasis) scores by baseline atypical cluster severity level: low (a, top figure), average (b, middle figure), and high (c, bottom figure). The presented least square means are based on the model fit to the entire sample and hence the sample in each figure is the same. (a) Estimated least square means for HAMD atypical symptom cluster scores by group at 'low' baseline atypical cluster severity, ie, scores = 1 SD below the mean. There were no significant differences between groups (total $n = 71$). (b) Estimated least square means for HAMD atypical symptom cluster scores by group at 'average' baseline symptom cluster severity. (c) Estimated least square means for scores by group at 'high' baseline symptom cluster severity, ie, scores = 1 SD above the mean. Significant differences between ketamine groups, $^{*}p < 0.05$, $^{+}p < 0.1$ (total $n = 71$). PBO, saline placebo.

persisting throughout the study, whereas changes on atypical symptoms being rapid, but short lasting.

Response Rate

The 0.5 mg/kg group had 11 responders (45.8%), the 0.2 mg/kg group had 9 responders (39.1%), and the placebo group had 3 responders (12.5%) (Table 2). There was a significant difference in the response rate across the three groups (Fisher's exact test p-value = 0.03) with a significant linear trend test for the dose effect ($p = 0.01$). Responder rate was greater in the 0.5 mg/kg than PBO group (*post hoc* test, $p = 0.01$) and in the 0.2 mg/kg than PBO (*post hoc* test, $p = 0.05$) but not between the two ketamine groups ($p = 0.77$).

Response Rate by BDNF Val66Met Genotype

The distribution of genotypes of the Val66Met *BDNF* polymorphism in this Chinese study sample was Val/Val ($N = 12$, 17%), Val/Met ($N = 40$, 56.3%), and Met/Met ($N = 19$, 26.8%). Fisher's exact test showed no significant distribution of these genotypes ($p = 0.41$) in the three groups. We combined subjects with the Met/Met and Val/Met as a group to compare with the Val/Val subjects to see whether BDNF genotype predicted ketamine response. Although we had low statistical power to explore the question, we did not find significant differences between carriers of the Met allele and Val/Val patients (responder rate: 33.9% *vs* 25.0%, Fisher's exact test, $p = 0.55$). Adding BDNF genotype as a factor in the mixed model for HAMD scores did not change the significance of the main results and genotype was not a significant predictor of the outcome.

Ketamine and Norketamine Levels and Clinical Outcome

Plasma ketamine and norketamine levels are presented in Figure 5. Ketamine and norketamine levels were dose related, ie, significantly higher for the 0.5 mg/kg group than for the 0.2 mg/kg group ($F(1, 40.7) = 28.5$, $p < 0.0001$ and $F(1, 44.4) = 22.8$, $p < 0.0001$ respectively) (see Figures 3a and b). They also varied by time point ($F(3, 38.6) = 172.6$, $p < 0.0001$ and $F(1, 41.8) = 24.4$, $p < 0.0001$ respectively) but there were no significant differences by responder status (all p-values > 0.1).

Hemodynamic Effect of Ketamine

There were statistically significant dose-related ketamine effects on systolic blood pressure (SBP) ($F(8, 68) = 3.25$, $p = 0.004$) and diastolic blood pressure (DBP) ($F(8, 68) = 2.65$, $p = 0.01$) with largest increases occurring at the 40 min time point. In the 0.5 mg/kg group SBP increased from mean (SD) = 116.5 (3.2) to 129.3 (3.5) compared with from 117.3 (3.3) to 121.7 (3.6) in the 0.2 mg/kg group and from 115.7 (3.2) to 116.3 (3.5) in the placebo group. However, by the 2 h time point the levels were back to baseline (mean (SD) = 117.1 (3.0) in the 0.5 mg/kg group, 116.7 (3.1) in the 0.2 mg/kg group, and 117.3 (3.0) in the placebo group). Similarly, in the 0.5 mg/kg group DBP increased from mean (SD) = 74.4 (2.1) to 82.4 (2.2) compared with from 75.8 (2.1) to 78.3 (2.3) in the 0.2 mg/kg group and from 74.4 (2.1) to 75.9 (2.2) in the placebo group, and by the 2 h time point the levels were back to baseline (mean (SD) = 76.3 (2.0) in the 0.5 mg/kg group, 73.0 (2.0) in the 0.2 mg/kg group, and 73.9 (2.0) in the placebo group).

Heart rate also increased in a dose-related manner ($F(8, 59) = 3.12$, $p = 0.005$) with the largest increase occurring at the 2 h time point and then trending down. Heart rate increased from mean (SD) = 68.2 (1.8) to 77.2 (2.1) in the 0.5 mg/kg group, from 67.4 (1.9) to 77.3 (2.2) in the 0.2 mg/kg group, and from 64.6 (1.8) to 71.9 (2.1) in the placebo group.

Tolerability and Safety

There were no unexpected clinically significant adverse events in the study. There were also no significant dose-related psychosis as assessed by the BPRS 4 key positive symptom score (ANOVA-Type Statistic (ATS) for Dose effect (2) = 2.49, $p = 0.08$, for time effect ATS (4.14) = 1.13, $p = 0.34$, and for dose by time effect ATS (8.28) = 1.75, $p = 0.08$). Nausea occurred in 6 patients (8.5% of the sample). Ketamine infusion was discontinued in one patient because of transient behavioral effects that dissipated without intervention.

DISCUSSION

This study is the first to our knowledge to report the dose-related efficacy of R/S-ketamine and to describe its effects in a genotyped Chinese population comprising predominately patients bearing one or more Met allele of *BDNF*. The principal finding of this study was that ketamine had dose-related antidepressant effects, as measured reflected by HAMD score. The dose-related antidepressant effects of ketamine were moderated by baseline depression severity. Ketamine was not effective in patients with relatively mild depression. However, with greater baseline depression severity, the 0.5 mg/kg dose increasingly separates from placebo and 0.2 mg/kg. In this study, the 0.2 mg/kg dose was not significantly better than placebo in reducing HAMD scores, suggesting that any tolerability benefits provided by this lower ketamine dose are offset by reduced efficacy. Thus, in this study, 0.5 mg/kg was the optimal ketamine dose, supporting current clinical practice. The dose-related efficacy of ketamine in the current study is consistent with an earlier meta-analysis that suggested that, across studies, higher subanesthetic ketamine doses were more effective (Xu *et al*, 2016). Ketamine was very well tolerated in the current study, as in prior studies of this drug in depressed patients.

In the current study, a single dose of ketamine affected the symptoms of depression to varying degrees and for varying durations, suggesting differential modulation of brain circuits underlying these symptoms. The antidepressant effects of ketamine in the current study were rapid and most prominent for the emotional and atypical clusters of depression symptoms. The atypical symptom cluster improved rapidly, but this response was not sustained. Ketamine did not reduce the insomnia-related cluster of depression symptoms. This overall pattern of response is consistent with a prior study (Pennybaker *et al*, 2017).

However, daytime fatigue, which was included in the insomnia symptom cluster in the current study, has been reported to respond to ketamine in another ketamine trial (Saligan *et al*, 2016). Heterogeneity in the response of insomnia symptoms may have clinical relevance. For example, one *post hoc* analysis suggests that depressed patients who do not have a reduction in suicidal ideation after ketamine infusion have persistent insomnia, whereas patients who improve on this outcome showed improved sleep (Vande Voort *et al*, 2016). In animals, ketamine administered during wakefulness powerfully stimulates non-REM delta EEG activity during sleep (Feinberg and Campbell, 1993, 1995). It remains to be seen whether these EEG changes predict antidepressant responses to ketamine.

The results of the current study might suggest that the antidepressant potency of R/S-ketamine is less than that of S-ketamine, as reported previously in patient groups comprising predominately other racial and ethnic groups. This hypothesis is supported by the observation that 0.2 mg/kg of R/S-ketamine was not effective in reducing HAMD

Table 2 Comparison of Response Rates in Three Dose Groups

Dose group	Response/ responder rate		*P*-value[a]
	n	%	
Placebo	3	12.5	
0.2 mg/kg	9	39.1	0.03
0.5 mg/kg	11	45.8	

Response (⩾50% reduction of HAMD score from baseline at any two daily HAMD-17 measures during the period of 24 to 96 h after infusion).
[a]Fisher's exact test.

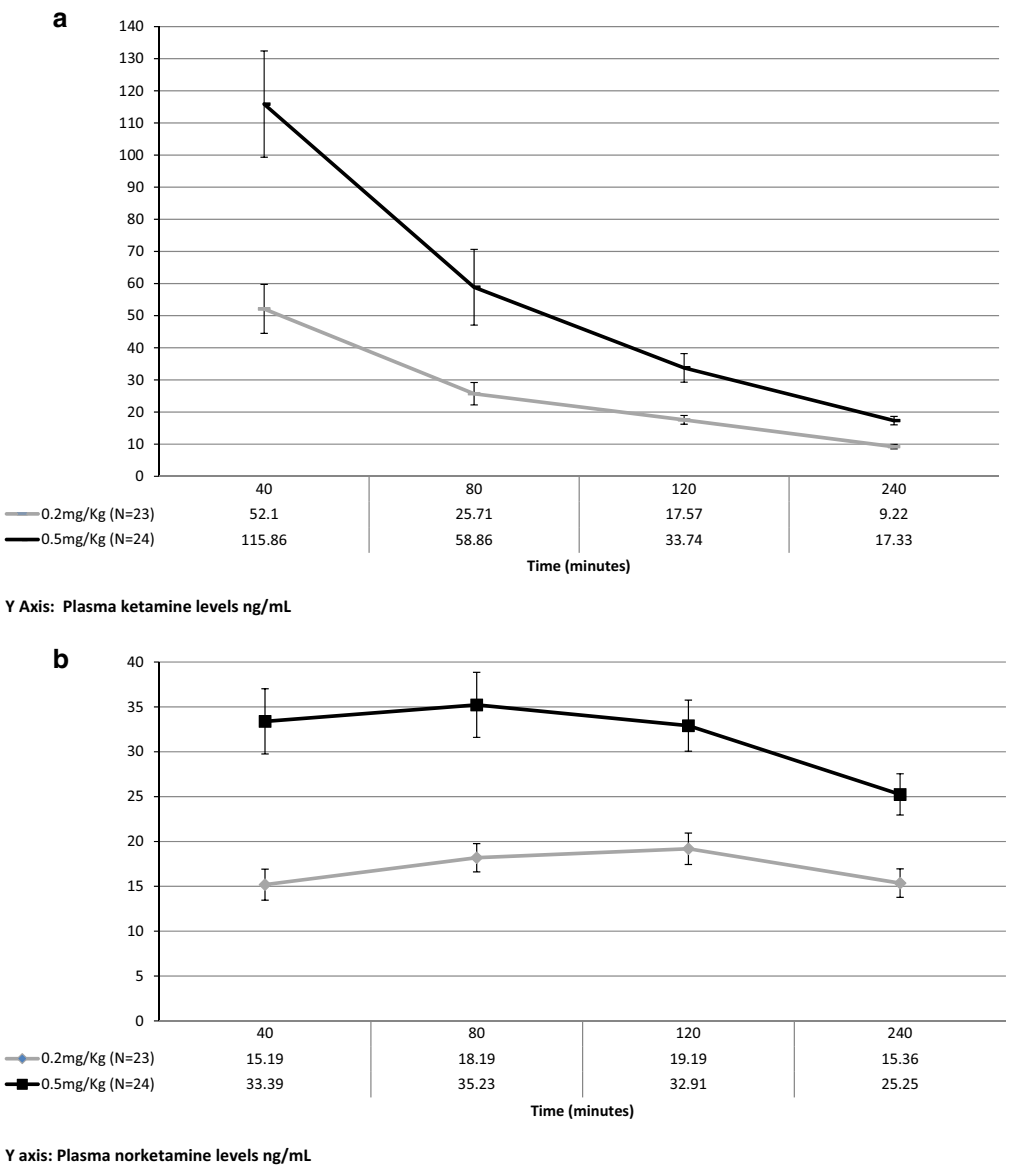


Figure 5 Plasma levels of ketamine (a, top figure) and norketamine (b, bottom figure) by dose for patients in this study. Plasma levels are presented as nM/ml and as mean ± SE.

scores in the current study, but 0.2 mg/kg of S-ketamine produced clinical improvement in a prior study (Singh *et al*, 2015). S-ketamine is at least twice as potent as R/S-ketamine at NMDA glutamate receptors (Hustveit *et al*, 1995). Therefore, the efficacy difference between 0.2 mg/kg R/S-ketamine in the current study and 0.2 mg/kg S-ketamine in the earlier study could be accounted for by the higher occupancy of NMDA-R by 0.2 mg/kg of S-ketamine that would be expected to be similar to higher R/S-ketamine doses. Thus, the difference between the current findings and the earlier findings are consistent with NMDA receptor antagonism as the mechanism of action of the antidepressant effects of ketamine, a hypothesis that was challenged recently (Zanos *et al*, 2016).

Ketamine showed significant efficacy in patients with more severe depression, but not mild depression. Depression severity also modifies the magnitude of the benefits associated with traditional antidepressant treatment (Kirsch *et al*, 2008). The inclusion of patients with relatively mild depression complicates the interpretation of the clinical significance of their data as, by definition, responders also meet remission criteria. The effect of baseline severity may have been missed in prior ketamine studies that tended to be smaller and that studied relatively homogenous groups with severe treatment-resistant symptoms of depression.

Overall, this study did not find clear evidence of reduced ketamine efficacy in patients distinguished by high rates of the less effective Met allele of the Val66Met polymorphism in the BDNF gene, consistent with an earlier positive report in Chinese patients (Hu *et al*, 2016). The rate of response to 0.5 mg/kg, ~ 50%, was low, but not remarkably so, relative to prior ketamine reports from treatment-resistant unipolar depression (Aan Het Rot *et al*, 2012). Similarly, this study reported lack of ketamine efficacy for insomnia-related symptoms of depression. It was not clear whether this response rate and lack of insomnia response was related to the inclusion of patients with low baseline severity, genotype, or other factors. For example, the peak plasma levels produced by ketamine 0.5 infusion in the current study (mean = 115 ng/ml) were noticeably lower than that reported in Caucasians in other studies (150–200 ng/ml) for reasons that are currently unclear (Zarate *et al*, 2012; Zhao *et al*, 2012). The levels of norketamine were not increased, suggesting that reduced ketamine levels did not simply reflect increased metabolism. In addition, we did not find evidence that the response to ketamine within dose group correlated with the plasma blood level. There is new interest in the ketamine metabolite 2R,6R hydroxynorketamine (HNK) in light of evidence that HNK levels may possess antidepressants in animals (Zanos *et al*, 2016).

Unfortunately, HNK levels were not measured in this study. Overall, this study provides compelling evidence that any reductions in ketamine efficacy associated with the *BDNF* Met allele are not sufficient to obscure its efficacy. The relatively low plasma ketamine levels in the current study raises the possibility that administration of higher ketamine doses might have increased the rates of clinical response in the current trial.

In conclusion, the current study provides the first evidence that ketamine has dose-related antidepressant effects that are moderated by baseline depression severity. It also supports the antidepressant efficacy of this drug in a Chinese population. Further research will be needed to determine whether the 0.2 mg/kg dose is effective in patients without the Met allele of the BDNF gene and whether increasing the ketamine dose to achieve the targeted plasma levels (150–200 ng/ml) would result in higher response rates.

FUNDING AND DISCLOSURE

The authors declare no conflict of interest. Dr Krystal acknowledges the following relevant financial interests. He is a co-sponsor of a patent for the intranasal administration of ketamine for the treatment of depression that was licensed by Janssen Pharmaceuticals, the maker of S-ketamine. He has a patent related to the use of riluzole to treat anxiety disorders that was licensed by Biohaven Medical Sciences. He has stock or stock options in Biohaven Medical Sciences, ARett Pharmaceuticals, Blackthorn Therapeutics, and Luc Therapeutics. He consults broadly to the pharmaceutical industry, but his annual income over the past year did not exceed $5000 for any organization. He receives over $5000 in income from the Society of Biological Psychiatry for editing the journal *Biological Psychiatry*. He has fiduciary responsibility for the International College of Neuropsychopharmacology as president of this organization.

ACKNOWLEDGMENTS

We thank Jennifer Vessicchio for her assistance with the preparation of this manuscript. We express our gratitude to all patients who kindly participated in this study. We thank all research assistants, physicians, pharmacists, and nursing staffs at D020 Unit of Taipei Veterans General Hospital for their assistance during the study process, without whom this work could not have been possible. We also very much appreciate Dr Guang Cheng for providing excellent opinions on our work. The study was sponsored by grants from the Ministry of Science and Technology, Taiwan (101-2314-B-010-060, 102-2314-B-010-060) and the Kun-Po Soo Medical Foundation. The participation of Dr Krystal was supported by the US Department of Veterans Affairs via its support for the VA National Center for PTSD, the US National Institute on Alcohol Abuse and Alcoholism (P50AA12870, M01RR00125), and the US National Center for Advancing Translational Science (UL1 RR024139). None of the aforementioned funding organizations had any role in the study design, data collection, analysis, interpretation of result, writing of the report, and the ultimate decision to submit the paper for publication.

REFERENCES

Aan Het Rot M, Zarate CA Jr, Charney DS, Mathew SJ (2012). Ketamine for depression: where do we go from here? *Biol Psychiatry* **72**: 537–547.

Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS *et al* (2000). Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* **47**: 351–354.

Chekroud AM, Gueorguieva R, Krumholz HM, Trivedi MH, Krystal JH, McCarthy G (2017). Reevaluating the efficacy and predictability of antidepressant treatments: a symptom clustering approach. *JAMA Psychiatry* **74**: 370–378.

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL *et al* (2004). Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci* **24**: 4401–4411.

Choi MJ, Kang RH, Lim SW, Oh KS, Lee MS (2006). Brain-derived neurotrophic factor gene polymorphism (Val66Met) and citalopram response in major depressive disorder. *Brain Res* **1118**: 176–182.

Feinberg I, Campbell IG (1993). Ketamine administration during waking increases delta EEG intensity in rat sleep. *Neuropsychopharmacology* **9**: 41–48.

Feinberg I, Campbell IG (1995). Stimulation of NREM delta EEG by ketamine administration during waking: demonstration of dose dependence. *Neuropsychopharmacology* **12**: 89–90.

Gaynes BN, Warden D, Trivedi MH, Wisniewski SR, Fava M, Rush AJ (2009). What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. *Psychiatr Serv* **60**: 1439–1445.

Hamilton M (1960). A rating scale for depression. *J Neurol Neurosurg Psychiatry* **23**: 56–62.

Hedlund JL, Vieweg BW (1980). The Brief Psychiatric Rating Scale (BPRS): a comprehensive review. *J Operational Psychiatry* **11**: 48–64.

Hu YD, Xiang YT, Fang JX, Zu S, Sha S, Shi H *et al* (2016). Single i.v. ketamine augmentation of newly initiated escitalopram for major depression: results from a randomized, placebo-controlled 4-week study. *Psychol Med* **46**: 623–635.

Hustveit O, Maurset A, Oye I (1995). Interaction of the chiral forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors. *Pharmacol Toxicol* **77**: 355–359.

Itoh K, Hashimoto K, Kumakiri C, Shimizu E, Iyo M (2004). Association between brain-derived neurotrophic factor 196 G/A polymorphism and personality traits in healthy subjects. *Am J Med Genet B Neuropsychiatr Genet* **124B**: 61–63.

Kirsch I, Deacon BJ, Huedo-Medina TB, Scoboria A, Moore TJ, Johnson BT (2008). Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. *PLoS Med* **5**: e45.

Kishimoto T, Chawla JM, Hagi K, Zarate CA, Kane JM, Bauer M *et al* (2016). Single-dose infusion ketamine and non-ketamine N-methyl-d-aspartate receptor antagonists for unipolar and bipolar depression: a meta-analysis of efficacy, safety and time trajectories. *Psychol Med* **46**: 1459–1472.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD *et al* (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* **51**: 199–214.

Krystal JH, Perry EB Jr, Gueorguieva R, Belger A, Madonick SH, Abi-Dargham A *et al* (2005). Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. *Arch Gen Psychiatry* **62**: 985–994.

Krystal JH, Sanacora G, Duman RS (2013). Rapid-acting glutamatergic antidepressants: the path to ketamine and beyond. *Biol Psychiatry* **73**: 1133–1141.

Laje G, Lally N, Mathews D, Brutsche N, Chemerinski A, Akula N *et al* (2012). Brain-derived neurotrophic factor Val66Met polymorphism and antidepressant efficacy of ketamine in depressed patients. *Biol Psychiatry* **72**: e27–e28.

Li CT, Chen MH, Lin WC, Hong CJ, Yang BH, Liu RS *et al* (2016). The effects of low-dose ketamine on the prefrontal cortex and amygdala in treatment-resistant depression: A randomized controlled study. *Hum Brain Mapp* **37**: 1080–1090.

Liu RJ, Lee FS, Li XY, Bambico F, Duman RS, Aghajanian GK (2012). Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. *Biol Psychiatry* **71**: 996–1005.

Overall JE, Gorham DR (1962). The brief psychiatric rating scale. *Psychol Rep* **10**: 799–812.

Pennybaker SJ, Niciu MJ, Luckenbaugh DA, Zarate CA (2017). Symptomatology and predictors of antidepressant efficacy in extended responders to a single ketamine infusion. *J Affect Disord* **208**: 560–566.

Rush AJ, Fava M, Wisniewski SR, Lavori PW, Trivedi MH, Sackeim HA *et al* (2004). Sequenced treatment alternatives to relieve depression (STAR*D): rationale and design. *Control Clin Trials* **25**: 119–142.

Saligan LN, Luckenbaugh DA, Slonena EE, Machado-Vieira R, Zarate CA Jr (2016). An assessment of the anti-fatigue effects of ketamine from a double-blind, placebo-controlled, crossover study in bipolar disorder. *J Affect Disord* **194**: 115–119.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E *et al* (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* **59**(Suppl 20): 22–33 quiz 34-57.

Simon GE, Revicki D, VonKorff M (1993). Telephone assessment of depression severity. *J Psychiatr Res* **27**: 247–252.

Singh JB, Fedgchin M, Daly E, Xi L, Melman C, De Bruecker G *et al* (2015). Intravenous esketamine in adult treatment-resistant depression: a double-blind, double-randomization, placebo-controlled study. *Biol Psychiatry* **80**: 424–431.

Vande Voort JL, Ballard ED, Luckenbaugh DA, Bernert RA, Richards EM, Niciu MJ *et al* (2016). Antisuicidal response following ketamine infusion is associated with decreased nighttime wakefulness in major depressive disorder and bipolar disorder. *J Clin Psychiatry* (doi:10.4088/JCP.15m10440; e-pub ahead of print).

Xu G, Lin K, Rao D, Dang Y, Ouyang H, Mai G *et al* (2012). Brain-derived neurotrophic factor gene polymorphism (Val66Met) and the early response to antidepressant in Chinese Han population. *Psychiatr Genet* **22**: 214–215.

Xu Y, Hackett M, Carter G, Loo C, Galvez V, Glozier N *et al* (2016). Effects of low-dose and very low-dose ketamine among patients with major depression: a systematic review and meta-analysis. *Int J Neuropsychopharmacol* (doi:10.1093/ijnp/pyv124; e-pub ahead of print).

Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI *et al* (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature* **533**: 481–486.

Zarate CA Jr, Brutsche N, Laje G, Luckenbaugh DA, Venkata SL, Ramamoorthy A *et al* (2012). Relationship of ketamine's plasma metabolites with response, diagnosis, and side effects in major depression. *Biol Psychiatry* **72**: 331–338.

Zhao X, Venkata SL, Moaddel R, Luckenbaugh DA, Brutsche NE, Ibrahim L *et al* (2012). Simultaneous population pharmacokinetic modelling of ketamine and three major metabolites in patients with treatment-resistant bipolar depression. *Br J Clin Pharmacol* **74**: 304–314.

Neuropsychopharmacology www.nature.com/npp

AUTHOR CORRECTION

Correction: Dose-Related Effects of Adjunctive Ketamine in Taiwanese Patients with Treatment-Resistant Depression

Tung-Ping Su[1,2,3,4], Mu-Hong Chen[2,4], Cheng-Ta Li[1,2,4], Wei-Chen Lin[2,4], Chen-Jee Hong[1,2,4], Ralitza Gueorguieva[5,6], Pei-Chi Tu[1,2,3], Ya-Mei Bai[1,2], Chih-Ming Cheng[2] and John H. Krystal[6,7,8,9]

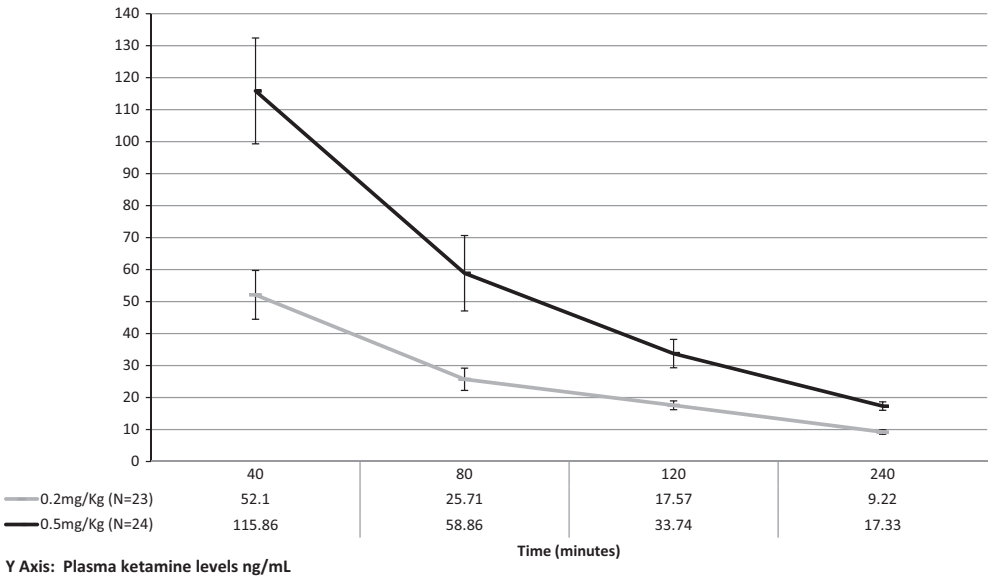
Neuropsychopharmacology (2018) 0:1–2; https://doi.org/10.1038/s41386-018-0253-z

Correction to: *Neuropsychopharmacology* (2017), https://doi.org/10.1038/npp.2017.94

Following the publication of this article the authors noted an error in Fig. 5. The units of blood concentration for ketamine and norketamine should be ng/mL, as per the assay details in Materials and methods section, and not nM/ml as presented in the figure. A revised Fig. 5 is in the correction article. The authors apologize for any inconvenience caused.

[1]Division of Psychiatry, Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan; [2]Department of Psychiatry, Taipei Veterans General Hospital, Taipei, Taiwan; [3]Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan; [4]Institute of Brain Science, National Yang-Ming University, Taipei, Taiwan; [5]Department of Biostatistics, School of Public Health, Yale University, New Haven, CT, USA; [6]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA; [7]Department of Neuroscience, Yale University School of Medicine, New Haven, CT, USA; [8]Yale-New Haven Hospital, New Haven, CT, USA and [9]VA National Center for PTSD, VA Connecticut Healthcare System, West Haven, CT, USA
Correspondence: T-P. Su (tomsu0402@gmail.com) or John H. Krystal (john.krystal@yale.edu)

Published online: 28 November 2018


140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
40
80
120
240
0.2mg/Kg (N=23)
52.1
25.71
17.57
9.22
0.5mg/Kg (N=24)
115.86
58.86
33.74
17.33
Time (minutes)
Y Axis: Plasma ketamine levels ng/mL

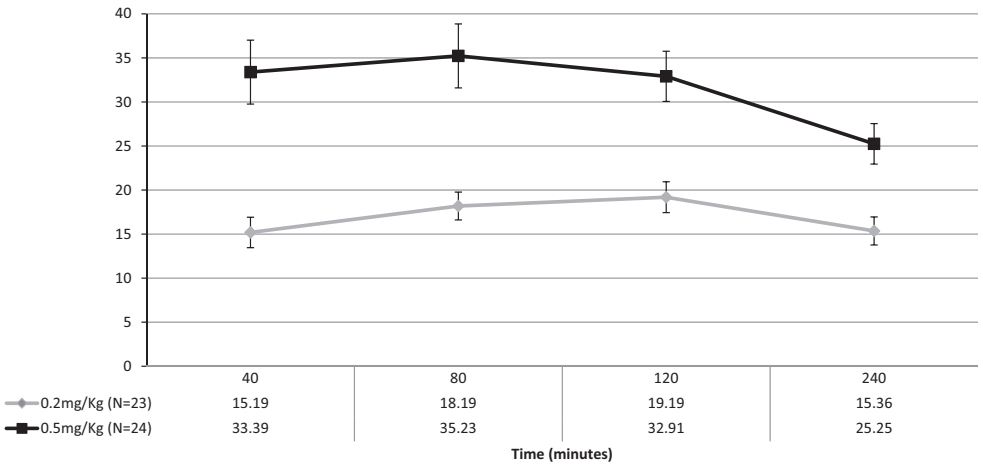

40
35
30
25
20
15
10
5
0
40
80
120
240
0.2mg/Kg (N=23)
15.19
18.19
19.19
15.36
0.5mg/Kg (N=24)
33.39
35.23
32.91
25.25
Time (minutes)
Y axis: Plasma norketamine levels ng/mL

Fig. 5