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REVIEW

Effects of glutamate positive modulators on cognitive deficits in schizophrenia: a systematic review and meta-analysis of double-blind randomized controlled trials

Y Iwata[1,2,3,8], S Nakajima[1,2,3,4,8], T Suzuki[3], RSE Keefe[5], E Plitman[1,6], JK Chung[1,6], F Caravaggio[1,6], M Mimura[3], A Graff-Guerrero[1,2,4,7] and H Uchida[3,4]

Hypofunction of *N*-methyl-D-aspartate (NMDA) receptors has been proposed to have an important role in the cognitive impairments observed in schizophrenia. Although glutamate modulators may be effective in reversing such difficult-to-treat conditions, the results of individual studies thus far have been inconsistent. We conducted a systematic review and meta-analysis to examine whether glutamate positive modulators have beneficial effects on cognitive functions in patients with schizophrenia. A literature search was conducted to identify double-blind randomized placebo-controlled trials in schizophrenia or related disorders, using Embase, Medline, and PsycINFO (last search: February 2015). The effects of glutamate positive modulators on cognitive deficits were evaluated for overall cognitive function and eight cognitive domains by calculating standardized mean differences (SMDs) between active drugs and placebo added to antipsychotics. Seventeen studies ($N = 1391$) were included. Glutamate positive modulators were not superior to placebo in terms of overall cognitive function (SMD = 0.08, 95% confidence interval = −0.06 to 0.23) (11 studies, $n = 858$) nor each of eight cognitive domains (SMDs = −0.03 to 0.11) ($n = 367$–940) in this population. Subgroup analyses by diagnosis (schizophrenia only studies), concomitant antipsychotics, or pathway of drugs to enhance the glutamatergic neurotransmission (glycine allosteric site of NMDA receptors or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors) suggested no procognitive effect of glutamate positive modulators. Further, no effect was found in individual compounds on cognition. In conclusion, glutamate positive modulators may not be effective in reversing overall cognitive impairments in patients with schizophrenia as adjunctive therapies.

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INTRODUCTION

Cognitive impairment represents a core feature of schizophrenia,[1] is evident before the first episode of psychosis (FEP),[2] and has been reported to be one of the strongest predictors of functional outcome in schizophrenia.[3,4] The primary treatment for schizophrenia is antagonism of dopamine receptors with antipsychotic medications. Unlike positive symptoms that are relatively well controlled by antipsychotics, cognitive symptoms are generally unresponsive to treatment.[5–7] Thus, there is an urgent need to develop novel compounds for the treatment of cognitive deficits in schizophrenia that act beyond the dopaminergic system.

The glutamate hypothesis of schizophrenia posits that dysfunction of neurotransmission mediated by the *N*-methyl-D-aspartate (NMDA) glutamate receptor might represent a primary deficit in the illness.[8,9] The most convincing link between NMDA receptor function and schizophrenia is the ability of NMDA receptor antagonists like ketamine to induce not only positive but also cognitive and negative symptoms in healthy volunteers[10–12] and to exacerbate psychosis in patients with schizophrenia.[13] Additionally, post-mortem studies have identified glutamate receptor irregularities in the brains of patients with schizophrenia and suggested a possible link between these abnormalities and cognitive deficits.[14,15] Taken together, these findings have led to the hypothesis that cognitive deficits in schizophrenia may arise from impaired NMDA neurotransmission.[4,16] As such, modulation of glutamate signaling could improve these difficult-to-treat symptoms.

During the last decade, drugs that enhance NMDA neurotransmission have been explored as a novel treatment approach for cognitive deficits in schizophrenia.[17–23] Two previous meta-analyses have reported the effects of glutamate modulators on cognitive deficits in schizophrenia. Tsai *et al.* noted beneficial effects of NMDA enhancing agents (that is, D-alanine, D-cycloserine (DCS), D-serine, glycine, and sarcosine) on cognitive deficits of schizophrenia (Cohen's $d = 0.28$, 13 studies, $n = 485$).[24] Choi *et al.* reported that glutamate receptor agonists (that is, CX516, DCS, and D-serine) had no effect on overall neurocognitive function and five cognitive domains (attention/vigilance, reasoning/problem solving, speed of processing, verbal learning, and visual learning) in schizophrenia (3–7 studies; sample sizes not reported).[25]

[1]Multimodal Imaging Group-Research Imaging Centre, Centre for Addiction and Mental Health, Toronto, ON, Canada; [2]Department of Psychiatry, University of Toronto, Toronto, ON, Canada; [3]Department of Neuropsychiatry, School of Medicine, Keio University, Tokyo, Japan; [4]Geriatric Mental Health Division, Centre for Addiction and Mental Health, Toronto, ON, Canada; [5]Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC, USA; [6]Institute of Medical Science, University of Toronto, Toronto, ON, Canada and [7]Campbell Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada. Correspondence: Dr S Nakajima, Multimodal Imaging Group & Geriatric Mental Health Division, Centre for Addiction and Mental Health, 250 College Street, Toronto, Ontario M5T 1R8, Canada.
E-mail: shinichiro_nakajima@hotmail.com
[8]These authors contributed equally to this work.
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Notably, in the past several years, a number of compounds have been identified to enhance glutamatergic signaling. Minocycline, a tetracycline with broad-spectrum antimicrobial activity, has been suggested to increase GluR1 subunit phosphorylation and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor potentiation.[26,27] L-carnosine, a co-localized dipeptide with glutamate,[28–31] and *N*-acetylcysteine, a precursor of glutathione, may enhance NMDA signaling via the redox site of the NMDA receptor.[32,33] Pregnenolone, a neurosteroid, elevates serum pregnenolone sulfate,[34] which in turn positively modulates NMDA receptors via a non-canonical G protein, phospholipase C, and a Ca^{2+} dependent mechanism.[35] These promising compounds were not included in the previous studies. Therefore, it is critically important to include those new drugs and conduct a more comprehensive meta-analysis in order to provide robust evidence on the effects of glutamate positive modulators on cognitive functions in patients with schizophrenia.

In this study, we conducted a meta-analysis on the effects of glutamate positive modulators on overall cognitive function and eight specific cognitive domains of clinical relevance in schizophrenia: (1) attention/vigilance, (2) cognitive control/executive function, (3) reasoning/problem solving, (4) social cognition, (5) speed of processing, (6) verbal learning, (7) visual learning, and (8) working memory in patients with schizophrenia.

MATERIALS AND METHODS

Literature search

The meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) group.[36] Two independent authors (YI and SN) independently performed the search (last search: 6 February 2015) and assessed eligibility. Three authors (YI, EP and SN) independently extracted data. Published articles from 1950 to February 2015 were searched for without language restrictions, using Embase, Medline, and PsycINFO. The Ovid search was conducted using the following search terms: (schizophreni* or psychosis) and (acetylcysteine/'α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid'/AMPA/benzoate/CX516/D-cycloserine/D-serine/glutamine/glutamate/'glutamate carboxypeptidase 2'/GCP2/glycine/'glycine transporter type 1'/GlyT1/'glutamate receptor, ionotropic, kainate'/GRIK/'kynurenine aminotransferase'/KAT/'metabotropic glutamate receptor'/mGluR/minocycline/'*N*-acetyl-aspartylglutamate'/NAAG/'*N*-methyl-D-aspartate'/NMDA/pregnenolone/sarcosine) and 'controlled trial'.

Inclusion criteria

Studies were included if: (1) they were double-blind randomized placebo-controlled trials, (2) they included patients with schizophrenia or related disorders, (3) study duration was 2 weeks or longer, (4) study drugs were used as adjunctive treatments to concomitant antipsychotic treatment, (5) study drugs were considered to act as glutamate positive modulators, (6) cognitive outcomes were measured using established cognitive tests, and (7) reported data were sufficient to calculate standardized mean differences (SMDs) of cognitive domains.

Exclusion criteria

Studies were excluded if they simply reported that their results were not significant without presenting raw data.

Outcome measures

In this study, we aimed to compare the effects of glutamate positive modulators on cognitive deficits in patients with schizophrenia or related disorders. We compared overall cognitive function (primary outcome) as well as eight specific cognitive domains (secondary outcomes), between active drugs and placebo that were added to antipsychotics. Modifying the Measurement and Treatment Research to Improve Cognition in Schizophrenia domains,[37] we classified cognitive function into eight cognitive domains: (1) attention/vigilance, (2) cognitive control/executive function, (3) reasoning/problem solving, (4) social cognition, (5) speed of processing, (6) verbal learning, (7) visual learning, and (8) working memory. Cognitive tests were classified into each cognitive domain (Supplementary Table S1). If cognitive tests could not be assigned to any domain, they were excluded.

Recorded variables

The variables for each study retrieved in the meta-analysis included characteristics of the subjects (that is, age, baseline symptom severity measured by the PANSS or the Clinical Global Impression score,[38] concomitant antipsychotics, diagnosis of subjects, duration of illness, and gender) and study design (that is, cognitive tests and outcomes, experimental drugs, duration of study, study locations, and sources of funding).

Data analysis

Meta-analysis. The primary meta-analysis as well as subgroup and sensitivity analyses were performed using Review Manager Version 5.2 (http://tech.cochrane.org/revman). The meta-regression was performed using Comprehensive Meta Analysis (www.meta-analysis.com). SMDs between active drugs and placebo were standardized by calculating the difference between the mean changes (that is, differences between post- and pre-treatment scores) divided by the pooled s.d. of the difference scores. In cases that s.d. values were not reported, we supplemented the missing values using one of the following options: (1) authors were contacted for additional data; (2) s.d. values were calculated from available data according to the *Cochrane Handbook for Systematic Reviews of Interventions* (http://www.cochrane-handbook.org.); or (3) when neither of the previous options were possible, s.d. values from similar studies that used the same drug were imputed. Effects were conventionally categorized as small (SMD = 0.2), moderate (SMD = 0.5) or large (SMD = 0.8),[39] with positive values indicating improvements in cognitive function. The inverse variance statistical method and random effects model were used to adjust for study heterogeneity.[40] Two-sided 95% confidence intervals (CIs) were used to assess significance, depending on whether the CIs included the null value.

In the analysis, we only included subjects who underwent cognitive tests. If the number of subjects who underwent cognitive tests was not presented, we used the number of subjects who completed the study.

The outcomes of overall cognitive function were derived from the composite scores of cognitive batteries or the average SMDs of cognitive domains if studies measured six or more of the eight cognitive domains. The outcomes of cognitive domains were derived as follows: (1) when one cognitive domain had two or more cognitive tests, average SMDs were used and (2) when one cognitive test had two or more outcomes, we used average SMDs of the relevant selected outcomes (selected outcomes are displayed in Supplementary Table S2). When studies reported outcomes of both cognitive domains and cognitive tests, the former was adopted. When studies included multiple doses of adjunctive medications, we computed SMDs of the mean of the groups.

Study heterogeneity was quantified for the primary outcome analysis using the I^2 statistic with $I^2 \geqslant 50\%$ indicating a significant heterogeneity. When heterogeneity was present, sensitivity analyses were conducted to assess potential influences of any one single study on the pooled SMD and associated *P*-values. The possibility of publication bias was also assessed using funnel plots, Egger's regression test,[41] and trim-and-fill procedure.[42]

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Moderator analyses. Moderator analyses were conducted to explore influences of study characteristics on the effects of glutamate modulators on cognitive function. Subgroup analyses were performed on overall cognitive function and eight cognitive domains for the following categorical characteristics: (a) by the pathway of drugs to enhance the glutamatergic neurotransmission (that is, the glycine allosteric site of NMDA receptors or AMPA receptors); (b) by concomitant antipsychotics (that is, clozapine or non-clozapine antipsychotics); and (c) by diagnosis (that is, schizophrenia or other related disorders). Meta-regression analyses were conducted on overall cognitive function for the following continuous characteristics: (a) age, (b) gender proportion, (c) duration of illness, (d) concomitant antipsychotic dose, (e) baseline PANSS total score, and (f) baseline Clinical Global Impression score.[38] Meta-regression was performed if at least five data sets were available in order to minimize the effect by chance.

Assessment of risk of bias. Included trials were assessed with the Cochrane Risk of Bias Tool for methodological quality of sequence generation, allocation concealment, blinding, incomplete outcome data, and selective reporting.[43]

The significance level for all tests was set at a *P*-value of < 0.05 (two-tailed). Continuous variables were described as mean ± s.d.

RESULTS

Included individual studies

Seventeen double-blind randomized placebo-controlled trials were included (total number of subjects, $N = 1391$).[22,23,34,44–57] The PRISMA flow diagram is displayed in Supplementary Figure S1. Study characteristics are summarized in Table 1. The average duration of the studies was 12.6 ± 8.7 weeks (range: 4–36 weeks), and the number of subjects amounted to 79.4 ± 58.9 (range: 18–214). Age of the subjects was 40.1 ± 7.4 years old, proportion of male was 70.4 ± 14.0%, duration of illness was 14.7 ± 7.3 years, concomitant antipsychotic dose was 652.2 ± 261.8 mg (chlorpromazine equivalent dose).[58] The baseline PANSS total and Clinical Global Impression scores were 72.7 ± 10.4 and 4.5 ± 0.5, respectively, indicating moderate illness severity. The numbers of the studies and subjects for each compound included in more than one study were as follows: CX516, 2 studies, $n = 124$; D-serine, 4 studies, $n = 350$; DCS, 4 studies, $n = 216$; and minocycline, 2 studies, $n = 146$. The numbers of studies and subjects included in the analyses of each cognitive outcome were as follows: overall cognitive function, 11 studies, $n = 858$; attention/vigilance, 14 studies, $n = 841$; cognitive control/executive function, 13 studies, $n = 743$; processing speed, 15 studies, $n = 940$; reasoning/problem solving, 8 studies, $n = 575$; social cognition, 5 studies, $n = 367$; verbal learning, 13 studies, $n = 875$; visual learning, 10 studies, $n = 752$; and working memory, 16 studies, $n = 932$. Studies were conducted in the North America ($n = 5$),[23,34,44,48,53] East Asia ($n = 4$),[22,45,56,57] multi-continental locations ($n = 4$),[46,47,51,52] Middle-East Asia ($n = 2$),[49,50] and unreported ($n = 2$).[23,54] Regarding sources of funding, 14 studies (82%) were supported from governmental grants.[22,23,34,44,45,47–52,55–57]

Risks of bias

The risks of bias of included studies are summarized in Supplementary Figure S2. Although all studies were randomized trials, the methodology of random sequence generation and allocation concealment was often unreported, leading to 'unclear risk' for selection bias in nine studies (53%). Similarly, blinding of outcome assessors was often unspecified, resulting in 'unclear risk' for detection bias in 12 studies (71%). Two studies (12%) were judged to have 'high risk' of attrition bias because of unbalanced dropout rates between the groups. One study (6%) did not report the data of cognitive tests as secondary outcomes and were judged to have 'high risk' of selective reporting. For other bias, one study (6%) did not specify the diagnostic criteria used and two studies (12%) were supported from industrial companies, which were judged to have 'high risk'. Taken together, only four studies (24%) showed a 'low risk' for bias.

Meta-analyses

Effects of glutamate positive modulator on cognitive function. As a whole, glutamate positive modulators were not superior to placebo in terms of overall cognitive function (SMD = 0.08, CI = − 0.06 to 0.23, $P = 0.57$) (Figure 1) and each of eight cognitive domains (Supplementary Figure S3) in patients with schizophrenia. Regarding individual compounds studied in more than one study, minocycline was effective for attention/vigilance (SMD = 0.42, CIs = 0.02 to 0.82, $P = 0.04$) (Supplementary Figure S3). In contrast, DCS had negative effects on visual learning (SMD = − 0.48, CIs = − 0.86 to − 0.09, $P = 0.01$). These results, however, did not survive after adjusting for multiple comparison testing (the significance level was set at a Bonferroni corrected *P*-value of $< 0.05/(10 \times 8)$ (10 compounds and 8 cognitive domains). (Supplementary Figure S3).

Moderator analyses

1. Subgroup analyses. Results of overall cognitive function are displayed in Supplementary Figure S4 (see Supplementary Figure S5 for each cognitive domain).

A. By the pathway of drugs to enhance the glutamatergic neurotransmission:

Glycine allosteric site of NMDA receptors

There were no differences between the drugs (benzoate, DCS, D-serine, glycine, and Org25935) and placebo in terms of overall cognitive function (Supplementary Figure S4).

AMPA receptors

Beneficial effects of the drugs (CX516 and minocycline) on attention/vigilance were found compared to placebo (four studies, $n = 205$, SMD = 0.32, CIs = 0.01 to 0.64, $P = 0.05$); the statistical significance did not survive after adjusting for multiple comparison testing in two subgroups and eight cognitive domains (a significance level of $P < 0.05/2 \times 8$) (Supplementary Figure S5).

B. By concomitant antipsychotics: No difference was found in subjects on non-clozapine antipsychotics between the drugs and placebo with respect to overall cognitive function (Supplementary Figure S4; no data for overall cognition available for those on clozapine).

C. By diagnosis of schizophrenia: Among the studies that included subjects with schizophrenia only, we found beneficial effects of glutamate positive modulators on attention/vigilance (seven studies, $n = 460$, SMD = 0.20, CIs = 0.01 to 0.39, $P = 0.04$), which, however, was not confirmed after adjusting for multiple comparisons in 8 cognitive domains (significance level of $P < 0.05/8$) (Supplementary Figures S5).

2. Meta-regression analyses. It was found that the higher the proportion of males in studies, the lower the SMDs of effects of glutamate modulators on overall cognitive function (11 studies, $n = 858$, slope = − 0.01, 95% CI: − 0.03 to − 0.002, $P = 0.03$) (Figure 2). There were no associations between the SMDs and age, duration of illness, concomitant antipsychotic dose, baseline PANSS total score, and baseline Clinical Global Impression score (Supplementary Figure S6).

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Table 1. Characteristics of included studies

Year	Author	Intervention	Dose (mg day^{-1})	Study period (week)	Randomized subjects (n)	Completer rate (%)	Age (year) mean (s.d.)	Gender (% male)
2014	Cain[44]	DCS	50	8	40	80.0	47.5 (12.4)	86.1
2014	Liu[45]	Minocyclin	200	16	92	68.5	27.4 (6.5)	62.1
2014	Schoemaker[46]	Org25935	4–8 and 12–16	12	214	89.3	38.1 (10.4)	61.7
2013	D'souza[47]	D-serine	30 per kg	36	104	64.4	36.5 (8.5)	75.0
2013	Lane[22]	Benzoate	1000	6	52	90.4	37.3 (8.8)	49.8
2012	Chengappa[48]	L-carnosine	500	12	70	84.3	46.5 (8.8)	62.9
2012	Weiser[49]	D-serine	2000	16	195	76.4	39.6 (12.2)	72.0
2010	Levkovitz[50]	Minocycline	200	24	54	38.9	25.0 (4.4)	76.4
2009	Marx[34]	Pregnenolone	500	8	21	85.7	51.1 (9.3)	50.4
2008	Berk[51]	NAC	2000	24	140	60.0	36.6 (10.9)	70.0
2008	Goff[23]	CX516	900	8	105	87.6	43.3 (10.2)	83.0
2007	Buchanan[52]	DCS/glycine	50/6000	16	165	80.6	43.5 (10.9)	80.3
2004	Duncan[53]	DCS	50	4	22	100.0	51.8 (6.0)	100.0
2001	Goff[54]	CX516	900 and 1200	4	19	94.7	39.8 (10.5)	84.2
1999	Goff[55]	DCS	50	8	47	83.0	43.9 (10.2)	73.9
1999	Tsai[56]	D-serine	30 per kg	6	20	100.0	41.1 (4.6)	55.0
1998	Tsai[57]	D-serine	30 per kg	6	31	90.3	32.8 (7.1)	54.8

Year	Author	Duration of illness (year) mean (s.d.)	Concomitant AP	AP dose (CPZ equivalent dose (mg)) mean (s.d.)	PANSS total scores mean (s.d.)	CGI mean (s.d.)	Inpatients or outpatients
2014	Cain[44]	NR	FGA and SGA	485.6 (300.4)	NR	NR	Outpatients
2014	Liu[45]	2.0 (1.2)	SGA	381 (89.5)	82.3 (11.8)	5.1 (1.3)	NR
2014	Schoemaker[46]	NR	SGA	NR	79.3 (11.0)	NR	NR
2013	D'souza[47]	10.5 (7.7)	FGA and SGA	336.4 (92.5)	58.1 (13.1)	NR	Outpatients
2013	Lane[22]	14.5 (6.1)	FGA and SGA	574.7 (378.7)	88.7 (12.5)	4.5 (0.7)	NR
2012	Chengappa[48]	NR	CLZ and FGA and SGA	612.7 (470.2)	56.4 (12.2)	NR	NR
2012	Weiser[49]	16.2 (11.0)	FGA and SGA	NR	75.5 (14.0)	NR	Inpatients and outpatients
2010	Levkovitz[50]	3.8 (3.9)	CLZ and SGA	NR	76.0 (13.3)	4.0 (0.8)	NR
2009	Marx[34]	NR	SGA	470.9 (147.9)	72.7 (10.6)	4.0 (0.3)	Outpatients
2008	Berk[51]	12.2 (8.9)	CLZ and FGA and SGA	656.5 (473.1)	64.2 (15.9)	4.0 (0.9)	Inpatients and outpatients
2008	Goff[23]	NR	CLZ and SGA	589.3 (246.4)	68.0 (16.4)	NR	Outpatients
2007	Buchanan[52]	20.7 (10.7)	FGA and SGA	1359.7 (1174.0)	NR	4.7 (0.7)	Inpatients and outpatients
2004	Duncan[53]	27.3 (6.0)	FGA	803.2 (703.5)	NR	NR	Inpatients and outpatients
2001	Goff[54]	19.8 (5.6)	CLZ	609.9 (189.9)	78.4 (17.2)	NR	Inpatients
1999	Goff[55]	18.5 (11.0)	FGA	914.3 (715.0)	NR	NR	Inpatients and outpatients
1999	Tsai[56]	20.3 (5.9)	CLZ	508.5 (219.0)	NR	5.3 (0.7)	Inpatients
1998	Tsai[57]	10.6 (6.4)	FGA and SGA	827.6 (635.5)	NR	4.6 (0.6)	Inpatients and outpatients

Year	Author	Study population	Site	Funding
2014	Cain[44]	18–65 Years old	USA, single site	Government
2014	Liu[45]	18–40 Years old, duration of illness <5 years	China, two sites	Government
2014	Schoemaker[46]	18–55 Years old, subject with predominant persist negative symptom, non-first episode psychosis	Europe, Latin America and Russia, 25 sites	Industry
2013	D'souza[47]	18–65 Years old, stable subjects	USA and India, two sites	Government
2013	Lane[22]	18–65 Years old, symptomatic subjects, PANSS $\geqslant 60$	China and Taiwan, two sites	Government
2012	Chengappa[48]	18–65 Years old, stable subjects	USA, multi-sites (number is not reported)	Government
2012	Weiser[49]	18–64 Years old, PANSS-N $\geqslant 18$	Israel, ten sites	Government
2010	Levkovitz[50]	18–35 Years old, PANSS $\geqslant 60$, early stage of illness	Israel, two sites	Government
2009	Marx[34]	18–65 Years old, duration of illness >1	USA, single site	Government
2008	Berk[51]	18–65 Years old, PANSS $\geqslant 55$ or at least two PANSS positive or negative items >3 or CGI $\geqslant 3$	Australia and Switzerland, five sites	Government
2008	Goff[23]	18–65 Years old	USA, five sites	Government
2007	Buchanan[52]	18–64 Years old, SANS $\geqslant 20$ or affective flattening score or alogia score of SANS $\geqslant 3$	Israel and USA, five sites	Government
2004	Duncan[53]	SANS $\geqslant 30$, deficits subjects	USA, single site	NR
2001	Goff[54]	Subjects taking CLZ	NR	Industry
1999	Goff[55]	SANS $\geqslant 30$	NR	Government
1999	Tsai[56]	SANS $\geqslant 45$, TRS, deficit subjects	China, single site	Government
1998	Tsai[57]	SANS $\geqslant 40$, TRS, deficit subjects	China, single site	Government

Year	Author	Primary outcomes	Cognitive battery	Cognitive measures
2014	Cain[44]	ADT, MATRICS, SANS	MATRICS	Attention/vigilance, reasoning/problem solving, social cognition, speed of processing, verbal learning, visual learning, working memory
2014	Liu[45]	SANS	MATRICS	Brief visuospatial memory test, CPT-IP, grooved pegboard, HVLT-revised, mazes (NAB), spatial span (WAIS III), stroop color word test, symbol coding (BACS), TMT A, verbal fluency, WCST

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Table 1. (Continued)

Year Author	*Primary outcomes*	*Cognitive battery*	*Cognitive measures*
2014 Schoemaker[46]	SANS, PANSS-N	CNS-VS	Executive functioning, memory score, perception emotions, reasoning, speed of processing, sustained attention, verbal memory, visual memory, working memory
2013 D'souza[47]	AIMS, BARS, CDS, cognitive function, PANSS, MMAA, QOL, SSPA, UPSA	N/A	CPT, digit span (WAIS III), digit symbol-coding (WAIS III), HVLT-revised, letter/number span or sequencing (WAIS), logical memory (WAIS III), spatial span (WAIS III), TMT A, Tower of London, WCST
2013 Lane[22]	PANSS	MATRICS	Category fluency, CPT, digit span (WAIS III), digit symbol-coding (WAIS III), maze (WAIS for Children III), MSCEIT, spatial span (WAIS III), TMT A, visual reproduction, word listing (WAIS III)
2012 Chengappa[48]	Cognitive function	N/A	Auditory digit span, finger tapping tests, set shifting test, strategic target detection test, word-list memory, working memory
2012 Weiser[49]	SANS, MATRICS	MATRICS	Brief visuospatial memory test, category fluency, CPT-IP, Letter/number span (WAIS III), mazes (NAB), RAVLT, spatial span (WAIS III), symbol coding (BACS), TMT A
2010 Levkovitz[50]	SANS	CANTAB	Intra dimensional/extra dimensional set-shifting (CANTAB), motor task (CANTAB), pattern recognition memory, rapid visual processing task, spatial recognition memory, spatial working memory (CANTAB), Tower of London
2009 Marx[34]	BACS, MATRICS, SANS	MATRICS and BACS	Brief visuospatial memory test, category fluency test, CPT - IP, digit sequencing (BACS), HVLT, letter/number span (WAIS), mazes (NAB), MSCEIT, spatial span (WAIS III), symbol coding (BACS), token motor (BACS), Tower of London, TMT A, verbal fluency, verbal memory (BACS)
2008 Berk[51]	PANSS	N/A	Digit Span (WAIS III), TMT A & B, verbal fluency, word learning
2008 Goff[23]	Cognitive function	N/A	Faces I and II (delayed) subtests, letter/number sequencing (WAIS), grooved peg board, HVLT, TMT B minus A, verbal fluency, WCST
2007 Buchanan[52]	Cognitive function, SANS	N/A	Brief visual spatial memory test, CPT, digit symbol-coding (WAIS III), grooved peg board, letter/number sequencing (WAIS), RAVLT, spatial working memory, verbal fluency, WCST
2004 Duncan[53]	ATRS, BPRS, cognitive function, SANS, SAS	N/A	CPT, Sternberg short term memory scanning paradigm
2001 Goff[54]	AIMS, Cognitive function, GAS, HAM-D, PANSS, SANS	N/A	Attention (SANS), finger tapping task, gordon diagnostic system, Randt Memory Test, Rey-Osterrieth and Taylor complex figure tests, TMT B, verbal fluency, WCST
1999 Goff[55]	AIMS, cognitive function, SANS, PANSS, HAMD, GAS, serum level of DCS and amino acids	N/A	Digit Span (WAIS III), finger tapping tests, Miller–Selfridge test, Sternberg Item Recognition Paradigm, Stroop color word test, verbal fluency
1999 Tsai[56]	AIMS, BACS, CGI, HDRS, PANSS, SANS, UKU, serum level of D-serine, WCST	N/A	WCST
1998 Tsai[57]	AIMS, BAS, CGI, HAMD, PANSS, SANS, UKU, serum level of D-serine, WCST	N/A	WCST

Abbreviations: ADT, Auditory Discrimination Test; AIMS, Abnormal Involuntary Movement Scale; AP, antipsychotics; ATRS, Abrams and Taylor Scale for Emotional Blunting; AVLT, Auditory Verbal Learning Task; BACS, Brief Assessment of Cognition in Schizophrenia; BARS, Barnes Akathisia Rating Scale; BPRS, Brief Psychiatric Rating Scale; CANTAB, Cambridge Neuropsychological Test Automated Battery; CDS, Calgary Depression Scale, CGI; Clinical Global Impression; CLZ, clozapine; CNS-VS, CNS-Vital Signs Neurocognitive Test Battery; CPT, Continuous Performance Test; CPT-IP, Continuous Performance Test-Identical Pairs Version; CPZ, chlorpromazine; CVLT, California Verbal Learning Test; DCS, D-cycloserine; DSM, Diagnostic and Statistical Manual of Mental Disorders; Dx, diagnosis; FGA, first generation antipsychotics; HAMD, Hamilton Depression Rating Scale; HVLT, Hopkins Verbal Learning Test; MATRICS, Measurement and Treatment to Improve Cognition in Schizophrenia; MMAA, Medication Management Ability Assessment; MSCEIT, Managing Emotions Branch of the Mayer-Salovey-Caruso Emotional Intelligence Test; N/A, not applicable; NAB, Neuropsychological Assessment Battery; NAC, N-acetylcysteine; NR: not reporeted; PANSS, Positive and Negative Syndrome Scale; QOL, Heinrichs-Carpenter Quality-of-Life Scale; RAVLT, Rey Auditory Verbal Learning Test; SAD, schizoaffective disorder; SANS, Scale for the Assessment of Negative Symptom; SAS, Simpson-Angus Scale; SGA: second generation antipsychotics; SSPA, Social Skills Performance Assessment; Sz, schizophrenia; TMT, Trails Making Test; TRS, treatment resistant schizophrenia; UKU, Udvalg for Kliniske Undersogelser Side Effects Rating Scale; UPSA, University of California San Diego Performance-Based Skills Assessment; WAIS, Wechsler Adult Intelligence Scale; WCST, Wisconsin Card Sorting Test.

Sensitivity analysis

Since significant heterogeneity of the included studies was found for social cognition ($I^2 = 56.0\%$) and visual learning ($I^2 = 51.0\%$), sensitivity analyses were performed. For social cognition, when one industry-sponsored study was excluded,[46] the heterogeneity disappeared ($I^2 = 0.00\%$) and the SMD was slightly reduced (SMD = 0.00 to −0.17). For visual learning, no single study significantly contributed to heterogeneity.

Publication bias

Results of Egger's test suggested the presence of publication biases in the analysis on attention/vigilance and working memory ($P = 0.05$ and 0.01, respectively). The SMDs were slightly reduced when the trim-and-fill method was used (SMD = 0.10 to 0.07 and SMD = 0.04 to −0.02, respectively). Forest plots are displayed in Supplementary Figure S7.

DISCUSSION

Main findings

To our knowledge, this is the first comprehensive meta-analysis to examine the effects of glutamate positive modulators on cognitive deficits in patients with schizophrenia. As a whole, glutamate positive modulators were not found to be superior to placebo as an adjunctive therapy to antipsychotics although 5 out of 17

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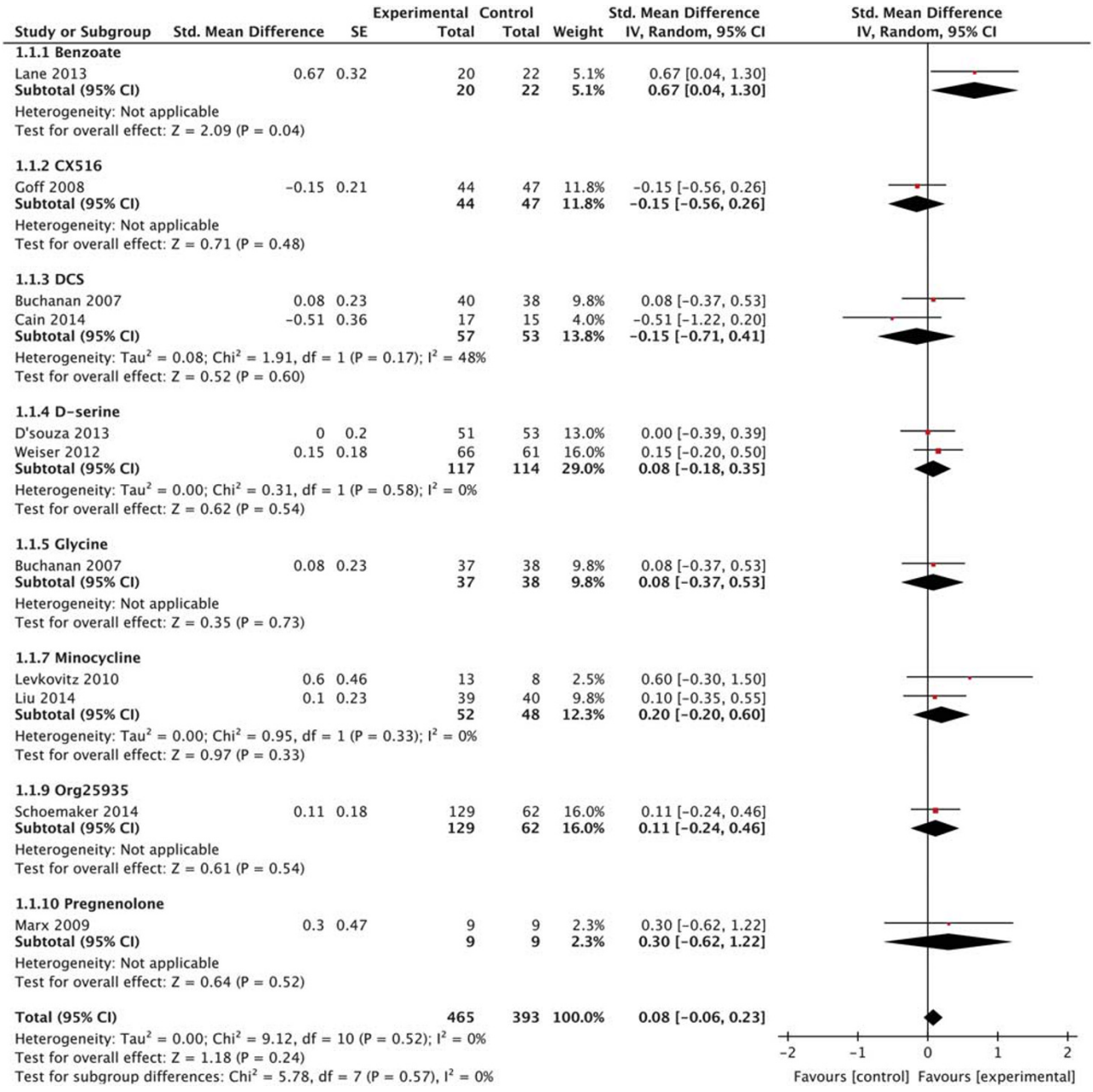

Figure 1. Effects of glutamate positive modulators on overall cognitive function. There were no significant differences in effects on overall cognitive function between glutamate positive modulators and placebo in patients with schizophrenia. CI, confidence interval; DCS, D-cycloserine; IV, inverse variance; SE, standard error; Std, standard.

individual studies have demonstrated their procognitive effects.[22,45,46,50,54]

This result is consistent with a recent meta-analysis by Choi *et al.*[25] Compared with the two previous meta-analyses, however, the present study has several strengths. First, the numbers of included individual studies and subjects are 17 and 1391, respectively, which is considerably larger than the earlier works (13 and 485, 7 and 342, respectively). Second, 10 compounds were included, which is also larger (3 and 5, respectively). This meta-analysis included five compounds (benzoate, L-carnosine, minocycline, Org25935, and pregnenolone) for the first time. Third, we covered extensive domains of cognitive function. The study by Tsai *et al.* employed PANSS cognitive subscale,[59] which cannot assess each cognitive domain, while the study by Choi *et al.* did not examine cognitive control/executive function, social cognition, and working memory. Finally, our calculation methods for the cognitive outcomes were more conservative. In the meta-analysis by Choi *et al.*, when the tests had multiple outcomes, only the one outcome with the largest effect size was chosen for the corresponding analysis. In contrast, our meta-analysis extracted outcomes from each test and averaged their SMDs for each outcome.

Despite the reported potential link between cognitive deficits and NMDA hypofunction in schizophrenia,[10–12,14,15] it still remains unclear whether cognitive deficits are related to glutamatergic signaling. For example, Ohnuma *et al.* did not find any relationship between cognitive functions and plasma levels of glutamatergic amino acid in this population.[60] In addition, to date, seven proton

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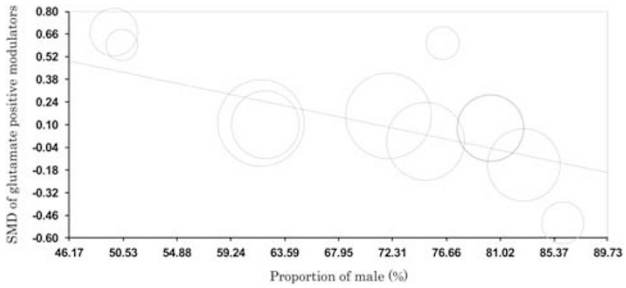

Figure 2. Meta-regression of effects of glutamate positive modulators on overall cognitive function in relation to proportion of male. Proportion of males had a negative correlation with SMDs of effects of glutamate positive modulators on overall cognitive function (11 studies, $n = 858$, slope = − 0.01, 95% CI: − 0.03 to − 0.002, $P = 0.03$). CI, confidence interval; SMD, standard mean difference.

magnetic resonance spectroscopy studies have examined the relationship between cognitive functions and glutamate levels in this patient population.[61–67] However, the results are inconsistent;[68] three studies did not find any relationships while the other four noted that executive functioning is negatively related to glutamate levels in the hippocampus/medial temporal lobe.[61,64,66,67] Thus, our null finding of the effects of glutamate positive modulators on cognitive deficits in patients with schizophrenia is consistent with previous work.

Findings by analyses of individual drugs and subgroup analyses

No significant effects were found in the analyses of individual drugs or subgroup analyses, while there was some suggestion that glutamate modulators may have beneficial effects on attention/vigilance. Glutamate positive modulators—in particular, AMPA receptor positive modulators—had a tendency to improve attention/vigilance in patients with schizophrenia; this finding did not survive after statistical corrections.

Cognitive functions have been reported to be one of the strongest predictors for functional outcome in patients with schizophrenia.[3,4] For example, one 7-year longitudinal study reported that three cognitive functions (attention, verbal memory, and processing speed) predicted functional outcomes of FEP.[69] Another 6-month longitudinal study examined neurocognitive predictors of remission in patients with FEP, reporting that only attention/vigilance at baseline was a predictor of remission of FEP amongst the seven Measurement and Treatment Research to Improve Cognition in Schizophrenia cognitive domains.[70] Given that attention/vigilance has a crucial role in predicting favorable outcomes in patents with schizophrenia, AMPA positive modulators in particular, which may have beneficial effects on attention/vigilance, might have a role in improving functional outcome.

AMPA receptors have been considered a promising target for the treatment of cognitive impairment in patients with schizophrenia because they have a critical role in synaptic plasticity, which is thought to be responsible for learning and memory.[71] Recently, one post-mortem study noted that AMPA receptor proteins, GRIA3 and GRIA4, were dysregulated in the auditory cortex of this population.[72] In addition, one genetic study reported that *GRIA3* gene mutations were related to moderate cognitive impairment in humans.[73] Furthermore, another line of evidence from animal studies has suggested a potentially compensatory role of AMPA receptors following NMDA receptor dysfunction.[74–76] For example, Jackson *et al.*[77] demonstrated that an increase in glutamate efflux by NMDA antagonists stimulated cortical AMPA receptors. Given that NMDA receptor dysfunction has been implicated in cognitive deficits in schizophrenia, enhancing AMPA signaling to further compensate this dysfunction may be promising for improving cognitive deficits. These findings corroborate our finding that AMPA positive modulators might improve attention/vigilance in schizophrenia. However, future studies are necessitated to investigate this relationship, given that there was a tendency that AMPA positive modulators might improve attention/vigilance.

Among several routes in the glutamate synapse that can potentially enhance glutamatergic neurotransmission,[20] the glycine allosteric site of NMDA receptors has been most examined for the effects of glutamate positive modulators on cognitive deficits in patients with schizophrenia. Out of 10 studies, 8 have reported negative results, and there are presumably several reasons for their lack of procognitive effects. For example, orally administered D-serine is metabolized substantially by D-amino acid oxidase, diminishing its oral bioavailability.[78] On the other hand, higher doses of D-serine may cause nephrotoxicity.[79] DCS has been suggested to have a narrow therapeutic window due to its partial agonist properties,[78] which may explain our finding that DCS might worsen visual learning impairments in schizophrenia. Thus, further research is needed to elucidate optimal dose ranges and route of administration of the drugs acting on glycine allosteric site in an effort to derive procognitive effects in schizophrenia.

Findings by meta-regression

Higher proportion of female gender was linked with greater improvements of overall cognitive function in our study. Previous reports have shown that female gender was related to better cognitive functioning throughout the illness stages.[80,81] Although it still remains unclear whether the higher cognitive reserve is related to the greater magnitude of procognitive effects induced by cognitive enhancers in female patients, our results suggest that female patients may benefit more from procognitive effects of glutamate positive modulators.

Limitations

The present report must be considered in light of various limitations. First, the number of included subjects and individual studies was still small. Second, we did not examine the long-term effects of glutamate positive modulators since duration of individual studies did not exceed 36 weeks. Third, the total number of subjects and studies varied across cognitive domains, as not all studies examined all cognitive domains. The results for specific cognitive domains that are based on a small number of studies or subjects need to be considered as preliminary. Fourth, it is worth noting that many of the drugs included in this study have different mechanisms of action even though each involves the glutamatergic system. As such, combining compounds with different glutamate-influencing mechanisms represents a limitation of our study. To somewhat address this limitation, we conducted subgroup analyses by the pathway of drugs to enhance the glutamatergic neurotransmission in which drugs were divided into the glycine allosteric site of NMDA receptor and AMPA receptor groups. However, the aforementioned limitation still exists for this subgroup analysis. Further research is necessitated to examine the relationships between procognitive effects and specific glutamate-influencing mechanisms of action. Fifth, some of the included compounds have been reported to have other mechanisms of action such as glutamatergic signal enhancers, anti-inflammation,[45] or neuroprotection.[34] Sixth, 15 out of 17 studies enrolled subjects within the chronic stage of the illness. It remains unclear whether these compounds have effects on subjects in the early stage of the illness (for example, FEP). Seventh, influences of concomitant antipsychotics are not clear. For example, 5 out of 17 studies did not discriminate between those taking clozapine, which has been reported to modulate glutamatergic signaling,[82,83] and those taking non-clozapine

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antipsychotics. Eighth, although we included only double-blind randomized placebo-controlled trials, only 24% of the studies had a 'low risk' of bias, which should be carefully taken into account. Ninth, a possibility of publication bias should not be dismissed. Finally, we did not examine adverse events, which clearly hinders us from making a balanced risk-and-benefit decision.

CONCLUSION

The findings from this meta-analysis indicate that glutamate positive modulators were not effective for overall cognitive deficits in patients with schizophrenia. Further research is required to elucidate the role of the glutamatergic system on the cognitive dysfunction observed in schizophrenia. Going forward, it is necessary to characterize a subgroup of patients for which glutamate modulators are specifically procognitive within this heterogeneous population.

CONFLICT OF INTEREST

YI has received manuscript fees from Wiley Japan within the past 3 years. SN has received fellowship grants from the CIHR and Japan Society for the Promotion of Science, and manuscript fees from Dainippon Sumitomo Pharma and Kyowa Hakko Kirin. TS has received manuscript or speaker's fees from Astellas, Dainippon Sumitomo, Eli Lilly, Elsevier Japan, Janssen, Meiji Seika, Novartis, Otsuka and Wiley Japan within the past 3 years. RSEK currently or in the past 3 years has received investigator-initiated research funding support from the Department of Veteran's Affair, Feinstein Institute for Medical Research, GlaxoSmithKline, National Institute of Mental Health, Novartis, Psychogenics, Research Foundation for Mental Hygiene and the Singapore National Medical Research Council. He currently or in the past 3 years has received honoraria, served as a consultant, or advisory board member for Abbvie, Akebia, Amgen, Astellas, Asubio, AviNeuro/ChemRar, BiolineRx, Biogen Idec, Biomarin, Boehringer-Ingelheim, Eli Lilly, FORUM, GW Pharmaceuticals, Helicon, Lundbeck, Merck, Minerva Neurosciences, Mitsubishi, Novartis, Otsuka, Pfizer, Roche, Shire, Sunovion, Takeda, Targacept and WWCT. RSEK receives royalties from the BACS testing battery, the MATRICS Battery (BACS Symbol Coding) and the Virtual Reality Functional Capacity Assessment Tool (VRFCAT). He is also a shareholder in NeuroCog Trials and Sengenix. EP has received the Ontario Graduate Scholarship and the Canada Graduate Scholarship. FC has received the Ontario Graduate Scholarship and the Canada Graduate Scholarship. MM has received grants and/or speaker's honoraria from Asahi Kasei Pharma, Astellas Pharmaceutical, Daiichi Sankyo, Dainippon-Sumitomo Pharma, Eisai, Eli Lilly, GlaxoSmithKline, Janssen Pharmaceutical, Meiji-Seika Pharma, Mochida Pharmaceutical, MSD, Novartis Pharma, Otsuka Pharmaceutical, Pfizer, Shionogi, Takeda, Tanabe Mitsubishi Pharma and Yoshitomi Yakuhin within the past 3 years. AG has received research support from the following external funding agencies: the Canadian Institutes of Health Research (CIHR), US National Institute of Health, Ontario Mental Health Foundation, Brain and Behavior Research Foundation, Mexico ICyTDF, CONACyT, Ministry of Economic Development and Innovation of Ontario, Ontario AHSC AFP Innovation Fund and W Garfield Weston Foundation. HU has received grants from Astellas Pharmaceutical, Eisai, Otsuka Pharmaceutical, GlaxoSmithKline, Shionogi, Dainippon-Sumitomo Pharma, Eli Lilly, Mochida Pharmaceutical, Meiji-Seika Pharma and Yoshitomi Yakuhin and speaker's honoraria from Otsuka Pharmaceutical, Eli Lilly, Shionogi, GlaxoSmithKline, Yoshitomi Yakuhin, Dainippon-Sumitomo Pharma, Meiji-Seika Pharma, Abbvie, MSD and Janssen Pharmaceutical within the past 2 years. Other authors have no financial or other relationship relevant to the subject of this manuscript.

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AUTHOR CONTRIBUTIONS

YI, HU, TS, RSEK, EP and SN led study design, literature review and interpretation and manuscript preparation. All authors have contributed to and approved the current version of the manuscript.

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