The Pharmacogenomics Journal (2020) 20:260–270
https://doi.org/10.1038/s41397-019-0101-5

ARTICLE

A functional HTR1A polymorphism, rs6295, predicts short-term response to lurasidone: confirmation with meta-analysis of other antipsychotic drugs

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Received: 7 October 2018 / Revised: 10 September 2019 / Accepted: 2 October 2019 / Published online: 21 October 2019

Abstract

Stimulation of the serotonin (5-HT)1A receptor (HTR1A) has been shown to contribute to the mechanism of action of some atypical antipsychotic drugs (APDs), including clozapine and lurasidone. A meta-analysis of rs6295, a functional polymorphism located at the promoter region of HTR1A, showed association with clinical response in schizophrenic patients treated with atypical APD. We have now tested whether other SNPs related to rs6295 predict response to lurasidone. We first evaluated whether rs358532 and rs6449693, tag SNPs for rs6295, predicted response to lurasidone, using data from two clinical trials of acutely psychotic schizophrenia patients with European (EUR, $n = 171$) or African (AFR, $n = 131$) ancestry; we then determined if those findings could be replicated in a third trial of lurasidone of similar design. Weekly changes (up to 6 weeks) in the Positive and Negative Syndrome Scale (PANSS) Total score and its five subscales were used to assess response. In EUR, a significant association, or trends for association, were observed for PANSS Total ($p = 0.035$), positive ($p = 0.039$), negative ($p = 0.004$), and disorganization ($p = 0.0087$) subscales, at week 1–6. There was a trend for replication with PANNS Total ($p = 0.036$) in the third trial. No significant association was observed in AFR or the placebo group. Meta-analysis of five studies, including the three with lurasidone, showed that rs6295 was associated with improvement in positive ($p = 0.023$) and negative ($p \le 0.0001$) symptoms in EUR patients with schizophrenia. This is the first study to show a significant association between functional *HTR1A* polymorphisms and treatment response to lurasidone. The meta-analysis provides additional evidence that rs6295 could be a race-dependent biomarker for predicting treatment response to APDs in schizophrenic patients with European Ancestry.

Introduction

Schizophrenia (SCZ) is a common, phenotypically diverse, and genetically complex syndrome with devastating functional consequences. While antipsychotic drugs (APDs) treat the key aspects of the syndrome, psychosis, negative symptoms, and cognitive impairment, many patients are only partial responders. Pharmacogenomic studies attempt to identify genetic markers which can predict response to specific APDs and if successful, can lead to fewer failed treatment assignment [1–5].

Atypical APDs with clozapine as the prototype have important advantages for the treatment of SCZ over typical APDs in both efficacy and side effects [6]. More potent blockade of serotonin $(5\text{-HT})_{2A}$ receptors (HTR2A) than dopamine (DA) D2 receptors (DRD2) is a shared property of many APDs, which lead to their advantages for efficacy and fewer side effects, such as extrapyramidal symptoms [7–11]. However, the atypical APDs have a variety of direct and indirect effects on other receptors, including HTR1A, HTR7, DRD1, and muscarinic receptors. SNPs for the *HTR2A, HTR7* and *DRD2* genes have been most intensively studied as biomarkers for overall response and response for specific domains, e.g., positive and negative symptoms [12–18]. However, results have been inconsistent [19].

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Supplementary information The online version of this article (https://doi.org/10.1038/s41397-019-0101-5) contains supplementary material, which is available to authorized users.

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The HTR1A in regions of the brain relevant to cognitive impairment, negative symptoms, and positive symptoms in SCZ, e.g., the prefrontal cortex (PFC), hippocampus (HIP), dorsal and ventral striatum, is a postsynaptic 5-HT heteroreceptor, whereas it is the presynaptic auto-receptor on 5-HT neurons in the dorsal raphe [20]. Blockade of HTR2A and DRD2 receptors increases cortical and striatal DA release via activation of HTR1A due to the release of 5-HT [21]. This mechanism may contribute to their efficacy for negative symptoms and fewer extrapyramidal symptoms produced by the atypical APDs [21, 22].

Altered HTR1A density has been initially identified in PFC and amygdala in postmortem specimens from SCZ patients [23, 24]. It has also been reported that tandospirone, a partial agonist of HTR1A, could ameliorate psychopathology, including negative and cognitive symptoms in patients with SCZ [25–29].

HTR1A is encoded at 5q11.2-13 and is made of 422 amino acids. rs6295, also known as C(−1019)G, is the most widely investigated functional variant in *HTR1A* associated not only with SCZ [19], but also with mood disorders [30, 31], anxiety disorders, and suicide [32, 33]. This may be related to its location at the extended promoter region where it may impact *HTR1A* gene expression [34, 35]. rs6295, when cloned into HEK-293 cells, was shown to exhibit higher transcriptional activity [36]. Associations between positive symptoms and rs6295 were also reported by Drago et al. [37], Kishi et al. [19], and Takekita et al. [38]. Associations between cognitive function and rs6295 were reported by Wesnes et al. [39], and Takekita et al [38]. Recently, Takekita et al. conducted a meta-analysis of the association of improvement in positive and negative symptoms of rs6295 using 1281 patients with SCZ from ten studies [40]. This association between rs6295 and treatment response to APDs was found only for SCZ patients of EUR or east Asian ancestry [40].

Lurasidone is a major atypical APD with high affinity for HTR1A with PA action [41–44]. It has been shown to improve psychopathology in SCZ in three 6-week, double-blind, randomized, placebo-controlled, phase 3 clinical trials [45–47]. A nonhypothesis driven GWAS based on a meta-analysis of these three clinical trials identified SNPs from ion channels, one of which reached genome wide significance, and synaptic adhesion molecules as the top predictors of efficacy [48, 49]. Here, we conducted a candidate SNP approach focused on the probable importance of HTR1A in the action of lurasidone by selection of rs358532 (C/A) and/or rs6449693 (G/A), proxy SNPs of rs6295, which are in high linkage disequilibrium (LD) with rs6295 (C/G), according to the 1000Genome project. Unlike rs6295, a transverse SNP, these are transition SNPs which are tagged in widely used microarray platforms such as Affymetrix 6.0 SNP array or Illumina Beadchip. We first evaluated whether rs358532 and rs6449693, predicted treatment response to lurasidone, based on two clinical trials [49] of acutely psychotic patients with European (EUR, $n = 171$) or African (AFR, $n = 131$) ancestry. We then determined if that association could be replicated in a third trial of lurasidone with a similar design. Finally, we conducted a meta-analysis of independent cohorts from several studies, including ours to determine whether rs6295 predicted treatment response to APDs which produce HTR1A agonism at clinically effective doses.

Material and methods

Subjects

The genomic DNA was collected from SCZ patients who participated in three 6-week randomized double-blind, placebo-controlled, multicenter registration trials of lurasidone [45–47]. All patients were acutely psychotic and diagnosed as SCZ by Structured Clinical Interview for DSM-IV. In the first two trials, the ethnicity of 171 (EUR) and 131 (AFR) patients was verified by principal component analysis (PCA) using 1000Genome sample as the reference [48]. One hundred (EUR) and 34 (AFR) patients from the third trial with a similar design was used as a replication dataset following ethnicity verification by PCA as well [49]. The demographic features of GWAS samples from three clinical trials are described in Table 1 and Supplementary Table 1. Treatment-resistant SCZ patients were identified by evaluation of history of treatment response to two or more trials of APDs and were excluded. Both trials showed significantly greater improvement than placebo at the end of 6 weeks in the lurasidone treated patients [45, 47]. Trial 3, efficacy of treatment of 100 SCZ patients with lurasidone (80 or160 mg/day) for 12 months was compared with that of quetiapine XR (QXR) (200–800 mg/day) in outpatients with acute exacerbation. The proportions of responders with improvement greater than 20% in PANSS Total were similar in all the clinical trials. All the participants were provided written informed consent. The trials were performed in accordance with the Helsinki Declaration of 1975 (as revised in 1983).

Evaluation of treatment response

The change (Δ) and % in PANSS Total were calculated each of the first 6 weeks with last observation carried forward (LOCF) in SCZ patients with European or African ancestry from Pearl 1, 2 and 3 trials of lurasidone and placebo. We also conducted the association test on Δ and % in PANSS five subscales; positive, negative, disorganization, excitement, anxiety and depression, based on the

Table 1 Demographic and clinical charateristics of GWAS sample ($n = 436$) from three clinical trials of lurasidone in schizophrenic patients with European or African ancestry

Clinical trials		Pearl 1 & 2 (Discovery)		Pearl 3 (Validation)	
Ethnicity		EUR	AFR	EUR	AFR
Number of patients (male/female)		171 (115/56)	131 (99/32)	100 (62/38)	34(26/8)
Number of subjects in dosage (40/80/120/160 mg/day)		59/41/71/0	57/25/49/0	0/52/0/47	0/17/0/17
PANSS Total[a]	Baseline	95.47 ± 0.67	94.63 ± 0.83	98.6 ± 9.90	97.21 ± 1.96
	Δ Change	−15.87 ± 1.30	−18.01 ± 1.42	−21.94 ± 1.80	−16.24 ± 3.00
	%Change	−16.55 ± 1.37	−18.89 ± 1.46	−22.5 ± 19.1	16.80 ± 3.11
Positive	Baseline	19.95 ± 0.24	20.54 ± 0.25	19.2 ± 3.00	21.65 ± 0.48
	Δ Change	−5.11 ± 0.37	−5.28 ± 0.39	−5.83 ± 4.33	−5.41 ± 0.89
	%Change	−25.67 ± 1.89	−25.55 ± 1.82	−30.0 ± 22.5	−25.22 ± 4.03
Negative	Baseline	22.54 ± 0.35	22.29 ± 0.40	23.3 ± 4.60	23.21 ± 0.90
	Δ Change	−3.26 ± 0.37	−4.21 ± 0.40	−3.81 ± 4.87	−3.71 ± 1.06
	%Change	−13.58 ± 1.73	−17.95 ± 1.76	−15.7 ± 22.30	−13.19 ± 4.31
Disorganization	Baseline	25.57 ± 0.32	24.08 ± 0.39	26.71 ± 4.15	23.32 ± 0.80
	Δ Change	−3.99 ± 0.32	−4.04 ± 0.39	−4.73 ± 5.07	−3.09± 0.62
	%Change	−15.41 ± 1.25	−16.27 ± 1.53	−17.80 ± 18.75	−14.67 ± 3.07
Excitement	Baseline	10.06 ± 0.23	10.47 ± 0.30	11.2 ± 3.07	10.44 ± 0.56
	Δ Change	−1.37 ± 0.29	−1.23 ± 0.35	−2.55 ± 3.70	−0.65 ± 0.40
	%Change	−9.64 ± 2.81	−7.96 ± 3.22	−20.96 ± 37.52	−5.75 ± 4.40
Anxiety and depression	Baseline	17.36 ± 0.26	17.24 ± 0.28	18.04 ± 3.39	18.59 ± 0.62
	Δ Change	−2.13 ± 0.32	−3.25 ± 0.36	−4.48 ± 4.39	−3.09 ± 0.62
	%Change	−11.4 ± 1.89	−17.67 ± 1.92	−23.61 ± 2.38	−16.52 ± 3.36

Clinical trials referred to three 6-week randomized double-blind, placebo-controlled, multicenter registration trials of lurasidone [45–47]. PANSS five subscales, positive, negative, disorganization, excitement, anxiety and depression were based on the five-factor model of SCZ psychopathology [50]

EUR European ancestry, *AFR* African ancestry

[a]Psychopathological data were presented as Mean ± SE

five-factor model of SCZ psychopathology [50], at week 1, 2, 4, and 6 from Pearl 1, 2 trials of lurasidone and placebo.

Genotyping

Details have been provided previously [47, 51]. DNA samples from the first two clinical trials were genotyped using Affymetrix SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA) [45, 47]. DNA samples from the third trial were genotyped with Illumina Omni5Exome-4v1beadchip [49]. The probes for rs6295 were unavailable in both array platforms. Due to the confusion brought by genotyping transverse SNP with minor allele frequency (MAF) close to 0.5 like rs6295 (C/G, 0.445) in EUR, we selected two proxy SNPs, rs358532 (C/A) and rs6449693 (G/A), which are highly in LD with rs6295 ($r^2 = 0.95/D' = 1$ in EUR and $r^2 = 0.80/D' = 0.90$ in AFR for rs358532; $r^2 = 1/D' = 1$ in EUR and $r^2 = 0.24/D' = 1$ in AFR for rs6449693), respectively, according to the report from 1000Genome (Supplementary Table 2). Genotyping data for rs6449693 or rs358532 were used for Pearl 1, 2 or 3 samples, respectively. The minor/major alleles and meta-analysis of the results from other studies used samples from EUR only. The phased reference genome data (1000Genome) in EUR were obtained to confirm that the minor A allele in rs358532 is linked to rs6295 G allele. The genotype call-rates for both proxy SNPs were 100% without significant deviation from Hardy–Weinberg Equilibrium ($p = 0.0001$ as a cutoff). The MAF in discovery samples were very close to the reported value from 1000Genome.

Association testing

A linear regression based on a recessive model after adjusting for age, gender, and dosage was conducted by PLINK 1.9 [52] for the association testing. Baseline psychopathology showed no significant contribution to the full model and was not included in the final model. A threshold of significance was set as 0.05 for the p-value calculated from the association between the genotype and change in PANSS Total and 0.01 for the association with PANSS five subscales, for Bonferroni correction for the multiple testing.

Association analyses by mixed model repeated measures

Multilevel multiple imputation is considered as a better approach for behavioral science data due to its flexibility to tailor the missing data handling procedure to match a specific set of analysis goals (https://stefvanbuuren.name/fimd/). Here we integrate R mice and lmer packages by conducting imputation, analysis by mixed-effects model, and pooling of the result by 'Rubin's rules', to determine the association of the candidate genetic variant, rs6449693, and dynamic change of PANSS total (TOT) or five domains for up to 6 weeks. The imputation method (meth) was a two-level normal model, '2l.norm', which could recover the intra-class correlation quite well, even for severe missing at random cases and high amounts of missing data in the outcome or the predictor [53]. We specified all predictors (rs6449693, PC1, PC2, PC3, gender, dose, time, baseline) as random effects. Patient ID (FID) was the class variable for '2l.norm'. The next step was to fit a linear model, 'PANSS ~ rs6449693(recessive) + PC1 + PC2 + PC3 + gender + dose + (1+1|time)' to each imputed dataset, and then to pool the results together. Time was a grouping factor and '1 + 1|time' was random intercept with fixed mean. The rest of other variables in this model were fixed factors. According to the margin plot of wk2 versus wk6 data (e.g., PANSS Total) as drawn by the margin plot function in R VIM package, There were different distributions of missing and observed data from Pearl 3 but not Pearl 1 & 2 (Supplementary Fig. 1), suggesting that the missing for repeated measures in Pearl 3 data was not completely random. We only apply this two-level model to imputation of missing data from Pearl 2 trial.

Meta-analysis

AY and JL conducted a literature search independently using PubMed, Web of Science, and Google scholar to identify articles published in English until July 10, 2018. Combinations of the following MeSH terms: ('Receptor, Serotonin, 5-HT1A'[MeSH] AND 'Antipsychotic Agents'[MeSH] AND 'Treatment Outcome'[MeSH] AND 'Genetic Association Studies'[MeSH]) were used as search queries. Finally, we manually checked related articles published in English. Any disagreement was resolved before the meta-analysis.

The following inclusion criteria were used to select studies: (1) investigation of the association between *HTR1A* functional polymorphisms, rs6295 or its proxy SNPs, or both and improvement after treatment with APDs; (2) the majority of patients had a diagnosis of SCZ based on DSM or ICD criteria (also including first-episode patients); (3) treatment response was evaluated using a standardized rating scale, such as PANSS at baseline and endpoint; (4) genotype distribution followed Hardy–Weinberg equilibrium; (5) the study was performed by the candidate SNPs approach; (6) the study was published in English in a peer-reviewed journal; (7) the study was based on an independent sample collection (nonoverlapping with each other); (8) all the patients were European origin. Based on these inclusion criteria, we selected three studies with five independent cohorts ($N = 523$) [54, 55]. Among them, two studies genotyped rs6295 directly [53, 54].

Meta-analysis was performed by R 'meta' package. Since clinical heterogeneity among the populations and treatments was expected, a random-effects model was used to analyze the data [56, 57]. The standardized mean difference between genotypes was collected from the original articles and its 95% confidence interval and standard deviation were either calculated or imputed using a correlation coefficient (0.80, this value was calculated by other studies) [58]. Study heterogeneity was measured using the chi-square and *I*-square statistics, with chi-square values of $p < 0.05$ and I^2 values of >50% indicating relevant heterogeneity. We only performed a meta-analysis separated by PANSS subscales, positive and negative symptoms since the original data from these two domains were the only ones available.

Results

HTR1A rs6295 or its related SNPs was associated with overall symptoms improvement only in SCZ patients with European ancestry

In EUR patients, the average of overall symptom improvement (Δ or % change in PANSS Total) or improvement in each subscale (Δ or % change in positive or negative symptoms) at LOCF or MMRM 6 weeks showed no significant difference between patients with AC and CC genotypes of rs358532 or between patients with GA and GG genotypes of rs6449693 ($p > 0.10$) (Table 2). Therefore, we conducted a recessive model of minor allelic association with phenotypes in subsequent analyses. This is also in accord with previous studies of association between rs6295 and treatment response to APDs [40].

In Fig. 1, overall symptoms improvement (ΔPANSS Total) after 6 weeks of treatment were significantly predicted by rs6449693 or rs358532, proxy SNPs of rs6295 in Pearl 1,2 studies ($\beta = 6.107$, $p = 0.046$) for both SNPs as they are in complete LD with each other. This significant association was validated for rs358532, a proxy SNP of rs6295 in Pearl 3 study ($\beta = 10.35$, $p = 0.036$). In contrast, rs6295 proxy SNPs did not predict overall improvement in AFR patients (all $p < 0.05$, Supplementary Table 1) or

Table 2 A rs6295 (tagged by rs6449693or rs358532) contributes to trajectory of early response to lurasidone in SCZ patients with European ancestry from Pearl 1 & 2 trial. B Association of rs6449693, a tag SNP of rs6295, with the dynamic change of PANSS Total and five domains by a linear mixed model of repeated measures for up to 6 weeks from Pearl 1 & 2 trial

Week	Week 1 ($N = 163$)					Week 2 ($N = 153$)					Week 4 ($N = 120$)					Week 6 ($N = 109$)				
Genotype of rs358532	A/A	A/C	C/C	A/C+C/C	*P/β (recessive)*	A/A	A/C	C/C	A/C+C/C	*P/β (recessive)*	A/A	A/C	C/C	A/C+C/C	*P/β (recessive)*	A/A	A/C	C/C	A/C+C/C	*P/β (recessive)*
Genotype of rs6449693	A/A	A/G	G/G	A/G+G/G		A/A	A/G	G/G	A/G+G/G		A/A	A/G	G/G	A/G+G/G		A/A	A/G	G/G	A/G+G/G	
Case number	41	77	45	122		39	74	40	114		27	61	32	93		24	54	31	85	
Gender (M/F)	28/13	51/26	31/14	82/40	0.89	26/13	49/25	26/13	75/38	0.998	18/9	41/20	21/10	62/30	0.996	15/9	37/17	20/10	57/27	0.873
Dose (40, 80, 120 mg)	8/8/25	32/20/25	19/10/16	51/30/41	0.033	8/8/23	32/20/22	18/9/13	50/29/35	0.028	6/8/13	26/18/17	13/8/11	39/26/28	0.329	6/8/10	22/16/16	12/8/11	34/24/27	0.704
Δ Change																				
PANSS Total	−4.54 ± 1.49	−9.23 ± 0.90	−7.27 ± 1.38	−8.50 ± 0.76	**0.035/ 3.510**	−7.79 ± 2.06	−12.57 ± 1.19	−14.90 ± 1.89	−13.39 ± 1.02	0.167/ 5.322	−15.07 ± 1.98	−18.75 ± 1.73	−22.47 ± 2.48	−20.03 ± 1.42	0.071/ 5.154	−15.04 ± 2.87	−23.50 ± 2.11	−23.35 ± 2.75	−23.45 ± 1.66	**0.028/ 7.705**
Positive	−1.90 ± 0.43	−2.69 ± 0.25	−2.04 ± 0.41	−2.45 ± 0.22	0.327/ 0.448	−2.62 ± 0.60	−3.92 ± 0.35	−3.80 ± 0.57	−3.88 ± 0.30	0.037/ 1.328	−5.00 ± 0.64	−5.66 ± 0.57	−6.09 ± 0.69	−5.81 ± 0.44	0.229/ 1.046	−5.50 ± 0.88	−7.52 ± 0.57	−6.68 ± 0.87	−7.21 ± 0.48	0.072/ 1.830
Negative	−0.05 ± 0.38	−1.38 ± 0.34	−1.51 ± 0.47	−1.43 ± 0.28	0.027/ 1.153	−0.77 ± 0.49	−2.35 ± 0.35	−3.08 ± 0.58	−2.61 ± 0.31	**0.004/ 1.726**	−1.56 ± 0.74	−3.31 ± 0.45	−4.81 ± 0.75	−3.83 ± 0.40	**0.003/ 2.303**	−1.79 ± 1.25	−4.22 ± 0.62	−5.32 ± 0.79	−4.62 ± 0.49	0.014/ 2.704
Disorganization	−1.05 ± 0.40	−2.52 ± 0.29	−1.84 ± 0.48	−2.27 ± 0.26	0.012/ 1.314	−2.15 ± 0.56	−3.22 ± 0.32	−3.70 ± 0.60	−3.39 ± 0.30	0.046/ 1.265	−3.30 ± 0.58	−4.48 ± 0.45	−5.22 ± 0.76	−3.30 ± 0.58	0.049/ 1.156	−3.38 ± 0.68	−5.67 ± 0.48	−5.06 ± 0.75	−5.45 ± 0.41	0.037/ 1.813
Excitement	−0.34 ± 0.45	−1.21 ± 0.25	−0.89 ± 0.44	−1.09 ± 0.22	0.125/ 0.701	−0.77 ± 0.47	−1.38 ± 0.35	−1.88 ± 0.53	−1.15 ± 0.29	0.143/ 0.800	−1.81 ± 0.70	−2.18 ± 0.44	−2.41 ± 0.54	−2.26 ± 0.34	0.432/ 0.481	−1.63 ± 0.72	−2.74 ± 0.54	−2.16 ± 0.57	−2.53 ± 0.40	0.245/ 0.800
Anxiety/ Depression	−1.20 ± 0.51	−1.44 ± 0.32	−0.98 ± 0.41	−1.27 ± 0.25	0.690/ −0.201	−1.49 ± 0.62	−1.70 ± 0.38	−2.45 ± 0.46	−1.96 ± 0.30	0.614/ 0.307	−3.41 ± 0.48	−3.13 ± 0.43	−3.94 ± 0.48	−3.41 ± 0.33	0.872/ −0.101	−2.75 ± 0.70	−3.35 ± 0.53	−4.13 ± 0.60	−3.64 ± 0.40	0.358/ 0.719
% Change																				
PANSS Total	−4.69 ± 1.60	−9.66 ± 0.96	−7.56 ± 1.44	−8.89 ± 0.81	**0.028/ 3.908**	−8.09 ± 2.18	−13.20 ± 1.26	−15.34 ± 1.88	−13.95 ± 1.05	0.139/ 5.770	−15.83 ± 1.99	−19.58 ± 1.78	−23.45 ± 2.49	−20.91 ± 1.45	0.059/ 5.583	−15.59 ± 3.06	−24.70 ± 2.20	−24.51 ± 2.85	−24.63 ± 1.73	**0.024/ 8.432**
Positive	−9.47 ± 2.09	−13.38 ± 1.26	−9.62 ± 1.99	−11.99 ± 1.09	0.351/ 2.170	−13.14 ± 2.85	−19.81 ± 1.75	−18.06 ± 2.64	−19.20 ± 1.46	0.039/ 6.561	−24.70 ± 2.60	−28.40 ± 3.04	−30.64 ± 3.31	−29.17 ± 2.29	0.201/ 5.646	−26.90 ± 4.07	−38.56 ± 2.95	−33.25 ± 4.10	−36.62 ± 2.40	0.053/ 9.820
Negative	0.16 ± 1.68	−5.20 ± 1.77	−5.90 ± 2.01	−5.46 ± 1.35	0.082/ 4.409	−2.58 ± 2.20	−9.89 ± 1.62	−12.74 ± 2.36	−10.89 ± 1.34	**0.005/ 7.499**	−5.13 ± 3.62	−14.12 ± 1.80	−20.47 ± 3.02	−16.30 ± 1.59	**0.001/ 11.410**	−5.21 ± 5.85	−18.52 ± 2.86	−23.21 ± 3.50	−20.23 ± 2.22	**0.007/ 14.470**
Disorganization	−3.98 ± 1.74	−9.79 ± 1.19	−7.23 ± 1.85	−8.85 ± 1.02	**0.099/ 5.550**	−8.02 ± 2.18	−12.60 ± 1.34	−14.11 ± 2.16	−13.13 ± 1.15	0.022/ 5.775	−12.86 ± 2.18	−17.10 ± 1.63	−20.27 ± 3.02	−18.19 ± 1.49	0.043/ 6.273	−13.00 ± 2.71	−22.19 ± 1.86	−19.55 ± 2.94	−21.23 ± 1.59	0.027/ 7.722
Excitement	−1.85 ± 5.03	−9.54 ± 2.51	−7.37 ± 4.28	−8.74 ± 2.23	0.198/ 6.299	−4.93 ± 4.89	−11.76 ± 3.18	−15.55 ± 5.25	−13.09 ± 2.76	0.165/ 7.556	−11.91 ± 7.89	−18.34 ± 3.95	−21.59 ± 4.51	19.46 ± 3.01	0.199/ 8.179	−10.50 ± 7.69	−22.12 ± 5.26	−18.42 ± 5.49	−20.77 ± 3.88	0.226/ 9.023
Anxiety/ Depression	−5.57 ± 3.12	−7.55 ± 1.73	−4.79 ± 2.31	−6.53 ± 1.38	0.888/ −0.418	−7.79 ± 3.67	−9.06 ± 2.11	−13.04 ±2.77	−10.46 ±1.68	0.584/ 1.982	−20.03 ±2.69	−16.92 ±2.41	−22.52 ±2.69	−18.85 ±1.84	0.695/ 01.468	−15.66 ±4.12	−17.99 ±3.30	−23.78 ±3.43	−20.10 ±2.45	0.474/ 3.577

Phenotype	Estimate (se)	Statistic	*p*-value
PANSS Total	−1.593 (0.892)	−1.786	0.074
Positive	−0.565 (0.32)	−1.764	0.082
Negative	−0.871 (0.309)	−2.822	0.005
Disorganization	−0.514 (0.299)	−1.719	0.086
Excitement	0.287 (0.22)	1.301	0.194
Anxiety/Depression	0.039 (0.254)	0.154	0.878

Genotype comparison of symptom improvement (Δ change or % change in psychopathology) after 1, 2, 4, and 6 weeks of treatment was conducted by a linear regresssion analysis based on a recessive model for minor allele after adjusting for covariates, such as age, gender, and dosage. A threshold of significance was set as $p < 0.05$ for PANSS Total. Bonferroni correction for multiple testing of five subscales was conducted. Only raw $p < 0.01$ was considered as significance with bold number fonts. Data were present as mean ± SE and *p*-value was present as raw without correction for multiple testing

The syntax for imputation was 'mice(long_format, meth = c("","","","","","","","","","","2l.norm"), pred = pred, maxit = 20, m = 10)'. The number (m) of imputed datasets was setup as 10. The number (maxit) of iterations for each imputation was setup as 20 so that the imputations for variable(s) reach convergence. The imputation method (meth) was a two-level normal model, '2l.norm'. We specified all predictors (rs6449693, PC1, PC2, PC3, gender, dose, time, baseline) as random effects. Patient ID (FID) was the class variable for '2l.norm'. The next step was to fit a linear model, 'PANSS ~ rs6449693 + PC1 + PC2 + PC3 + gender + dose + (1+1ltime)' to each imputed dataset, and then to pool the results together. Time was a grouping factor and '1 + 1ltime' was random intercept with fixed mean. The rest of other variables in this model were fixed factors.

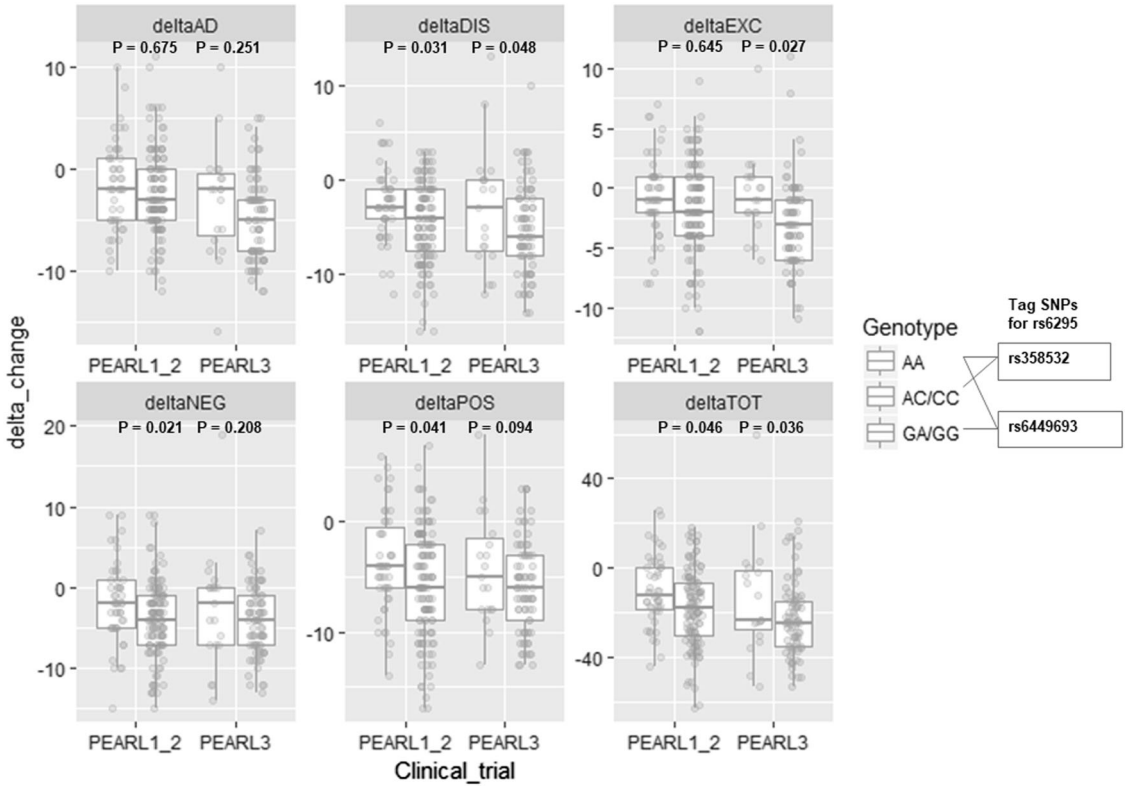

Fig. 1 Boxplots of association between rs6295, tagged by rs6449693 (Pearl 1, 2) or rs358532 (Pearl 3), and Δ change in PANSS Total and five subscales in SCZ patients with European ancestry after 6 weeks of treatment with lurasidone in Pearl 1, 2, and 3 trials. The Δ change in PANSS Total and five subscales was calculated with last observation carried forward (LOCF). Δ change = $PANSS_{LOCF6wk} - PANSS_{Baseline}$. Boxplots were created by R 'ggplot' package

patients treated with placebo (all $p < 0.05$, Supplementary Table 1), suggesting that this association was race-dependent and specific to the treated group. We then examined whether rs6295 proxy SNPs predicted improvement in specific domains of psychopathology, positive, negative, disorganization, excitement, and anxiety/depression, separately in EUR patients of Pearl 1,2 trials. There was a significant association between rs353832/rs6449693 and treatment response to lurasidone in positive symptoms (Δ positive, $\beta = 1.801$, $p = 0.041$ for Pearl 1,2), disorganization (Δ disorganization, $\beta = 1.628$; $p = 0.031$ for Pearl 1,2 and $\beta = 2.754$, $p = 0.048$ for Pearl 3) and a trend in that direction in positive symptoms (Δ positive, $\beta = 2.003$; $p = 0.094$ for Pearl 3) were identified in both datasets, but none of the p-value survived Bonferroni correction for multiple testing of individual domains. Similar results were found for improvement in negative symptom was observed in Pearl 1,2 ($\beta = 1.928$; $p = 0.02$) but not Pearl 3 ($\beta = 1.696$; $p = 0.208$).

Alternatively, we conducted imputation, analysis with a mixed-effects model, and pooling of the results to determine the association of the candidate genetic variant, rs6449693, and the changes in PANSS Total and five domains for up to 6 weeks in the Pearl 1 and 2 dataset. According to Table 2B, rs6449693, a tag SNP of rs6295, was significantly associated with the change in PANSS Negative (original $p = 0.005$), which survived Bonferroni correction. The negative value for the estimated fixed effect of rs6449693 indicates that the minor allele carriers (recessive mode of inheritance) had a worse response to treatment with lurasidone compared with those homozygous for major alleles. A trend of association with the same direction for the minor allele was also observed for PANSS Total and the Positive, and Disorganization domains in the Pearl 1 & 2 dataset.

rs6295 (tagged by rs6449693 or rs358532) contributes to trajectory of early response to lurasidone

It was noted that a significant association for response was identified as early as week one after initiating lurasidone ($\beta = 3.510$; $p = 0.035$ for PANSS Total). The association between rs6449696/rs358532 and improvement in negative symptom was significant at week one ($\beta = 1.153$; $p = 0.027$). This association was more robust at week 2 ($\beta = 1.726$; $p = 0.004$), and week 4 ($\beta = 2.303$; $p = 0.003$), suggesting HTR1A PA is crucial for negative symptom improvement. Major allele carriers had 2.61 in Δ change

(2.58% in % change), compared with minor allele carriers, with only 0.77 in Δ change (2.58% in % change) at week 2 in negative symptoms ($\beta = 1.726$; $p = 0.004$, Table 2). A trend of significant association between rs6449696/rs358532 and improvement in positive symptom was also observed at week two but not in week 1 and 4. All such associations were not identified in the corresponding placebo group ($p < 0.10$).

Meta-analyses confirmed the association in SCZ patients with European Ancestry

Meta-analysis was used to further test the association between functional *HTR1A* polymorphisms and treatment response to APDs in positive and negative symptoms in SCZ patients with European ancestry from independent cohorts (Supplementary Table 3).

SCZ patients ($n = 523$) with European ancestry from five independent cohorts were included in this meta-analysis [54, 55]. The results from using rs6295 or its proxy SNPs were included in this analysis. All these studies showed the distribution of genotypes followed Hardy–Weinberg equilibrium. A significant association was found between rs6295 and improvement in negative symptoms (five studies, SMD = 0.56, 95%CI = 0.33 to 0.80, $p =< 0.0001$, Fig. 2a) and positive symptoms (five studies, SMD = 0.33, 95% CI = 0.05–0.62, $p = 0.023$, Fig. 2b). Regarding heterogeneity, no significant heterogeneity was detected in any of the comparisons for rs6295 ($p = 0.42$ or 0.17, respectively, Fig. 2a, b)

Discussion

To our knowledge, this is the first study to evaluate the association between the tag SNPs for the functional *HTR1A* SNP rs6295 and treatment response to lurasidone, in acutely psychotic patients with SCZ. This is of interest because extensive evidence supports that HTR1A partial agonism is a major component of the pharmacology of lurasidone, as it is for some other atypical and APDs [42, 43, 59]. The significant association between rs358532/rs6449693, tag SNPs for rs6295, and Δ or % change in PANSS Total (LOCF) was only identified in EUR ($\beta = 6.107$, $p = 0.046$) but not in AFR ($\beta = -3.147$, $p = 0.399$) or the matched placebo groups ($\beta = 2.205$, $p = 0.739$), suggesting that this association is race-dependent and specific to the treated group. The direction of the association between minor allele of *HTR1A* rs6295 and treatment response is consistent with the result from the latest meta-analysis which reported G allele carriers of rs6295 had less improvement in response to APDs [40]. We replicated this association by using an independent dataset from the third trial of lurasidone which had a similar design to the discovery sample ($\beta = 10.35$; $p = 0.036$). Our subsequent analyses, separated by individual domains of psychopathology, provided further evidence of predicting symptom improvement in a domain-dependent manner (Fig. 1), suggesting that HTR1A-mediated signaling is most important in the neural circuitries related to 'negative', 'disorganization', and 'positive' symptoms, the three most important clinical features of SCZ and related psychotic disorders.

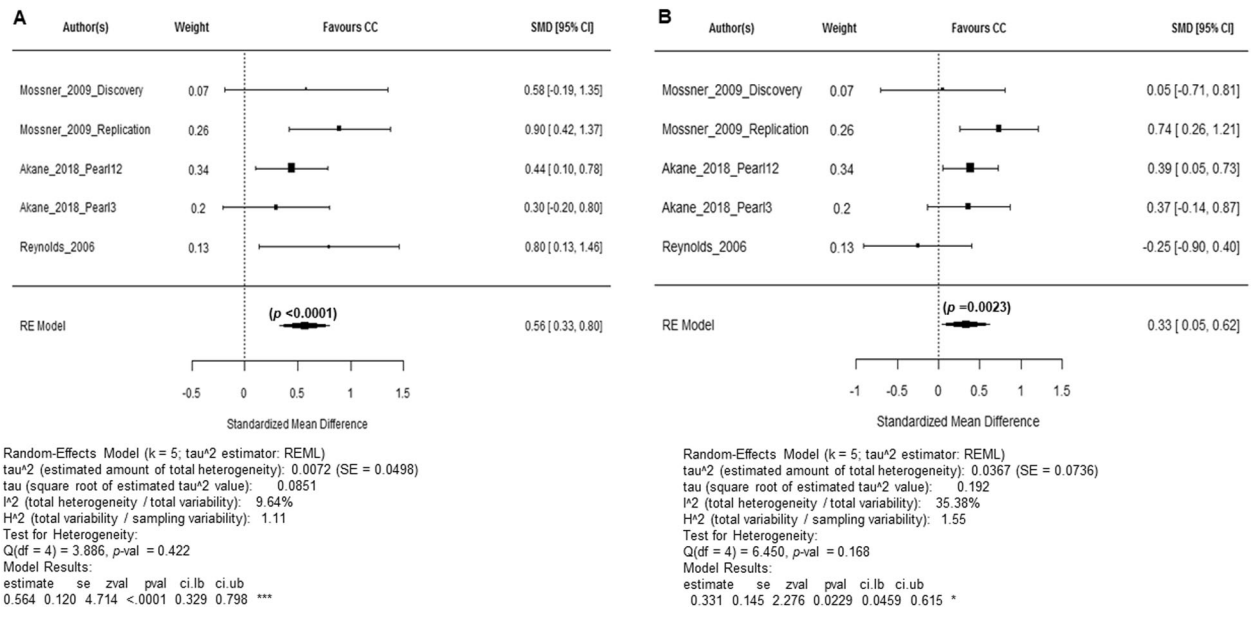

Fig. 2 Forest plots and summary statistics of the meta-analysis of five independent datasets to show the association between *HTR1A* functional polymorphism, rs6295, and improvement in PANSS negative symptom (**a**) and positive symptom (**b**) after treatment with antipsychotic drugs in SCZ patients with European ancestry. Forest plots were created by 'R meta' package

In addition, we presented the first evidence that the extent of this association is related to duration of the treatment, with the most significant association observed as early as one week for Disorganization, and as early as two weeks for Negative symptoms. Finally, we conducted a meta-analysis of five independent cohorts of EUR patients and showed that this functional HTR1A polymorphism or the tag SNPs were significantly associated with treatment response to APDs in positive ($p = 0.0023$) and negative symptom ($p < 0.0001$) improvement (Fig. 2a, b).

Importantly, the significant association between rs6295 and response to APDs was not restricted to negative symptom improvement. This is consistent with other research [54, 55] including a study reporting an association between rs6295 and General psychopathology subscale and total PANSS score in 63 drug-naive SCZ patients [54]. Drago et al. also found a significant association between rs6295 and improvement in positive symptoms ($p = 0.003$) in 96 acutely psychotic patients treated with haloperidol [60].

Hypodopaminergia has been suggested to be causally related to negative symptom [61, 62]. The association between a functional SNP of *HTR1A* and improvement in negative symptoms could be based on the increased release of DA in PFC induced by stimulation of HTR1A [21]. An association between HTR1A and negative symptom is consistent with an imaging study that the G allele (major allele) carriers, which is associated with increased amygdala reactivity to emotional stimuli [35], have greater improvement in negative symptoms with atypical APD treatment. Considering that disorganization is a manifestation of dysconnectivity in the brain of SCZ, and that G-carriers (major allele) of rs6295, who are predicted to have lower expression of HTR1A in PFC, leads to poor functional connectivity in DLPFC [63], and are more likely to respond to HTR1A agonism and show better response in negative symptoms and disorganization. Another possibility is that HTR1A activation promotes neurogenesis in HIP [64] ameliorate may be different cognition which is the basis of improvement in other dimensions.

The C(−1019) allele carriers have been suggested to have increased presynaptic HTR1A auto-receptors, enhancing inhibitory input to PFC 5-HT neurons [65]. This could lead to disinhibition of parvalbumin-positive or somatostatin-positive GABAergic interneuron in the PFC, increasing release of DA in PFC and greater improvement in negative symptoms in SCZ (Fig. 3). Enhanced inhibition due to increased HTR1A auto-receptors in the dorsal raphe nucleus may also increase inhibition of dopaminergic neurons in ventral tegmental area (VTA), which would be expected to diminish psychosis (Fig. 3). In addition, it might affect the balance between the pre/post HTR1A ratio, stimulating postsynaptic HTR1As to increase DA release in PFC [66].

We selected HTR7, one of a small number of neurotransmitter receptors for which lurasidone has high affinity, as a candidate gene to determine if its common variants are associated with clinical response to lurasidone. However, there was no significant association with annotated functional SNPs of the HTR7 and clinical response measures after correction for multiple testing (unpublished data). However, we previously demonstrated a significant inverse correlation between expression of HTR7 and the top genes identified by GWAS which predicted clinical response to lurasidone at 6 weeks; most of these encode synaptic adhesion and scaffolding proteins 4, suggesting that the HTR7-mediated signaling pathway may contribute to the mechanisms of action of lurasidone at the transcriptional level.

There are several limitations in our study. First, the small effect-size for rs6295 in the discovery sample, with nominally significant association to treatment response to lurasidone, in addition to the high MAF, increased the challenge to achieve replication in an independent dataset of smaller sample size. Nevertheless, we showed a trend for replication. Second, in our study, rs6295 was not actually genotyped but the tag SNPs were examined. Although rs6449693 might be better in terms of avoiding the confusion due to the C/G transverse SNP, the interpretation of the results needs caution in this perspective. Third, only five studies with the varied duration of treatment of APDs were included in the meta-analysis. It is debatable whether the conclusion made by the meta-analysis can be generalized to other APDs with HTR1A pharmacodynamic feature at a distinct duration of treatment. Fourth, caution is needed about generalizing the result of meta-analysis to other APDs because of the limited number of drugs in the first studies eligible for the meta-analysis. Also, the duration of treatment was different in the meta-analysis, ranging from 6 weeks to 3 months. Only five studies with nonuniform duration of treatment with APDs were included in the meta-analysis. It uncertain as to whether the conclusion from the meta-analysis can be generalized to other APDs with HTR1A pharmacodynamic feature with varying durations of treatment. Fifth, epigenetic factors may also affect treatment response. In Han Chinese inpatients presenting with their first psychotic episode, methylation of the specific CpG site adjacent to the functional polymorphism rs6295 was shown to be correlated with improvement in negative symptoms during treatment with APDs, including risperidone and clozapine [34]. Further study of genetic and epigenetic factors impacting the relation of rs6295 and clinical response is indicated.

In conclusion, we reported here that a common functional HTR1A polymorphism, rs6295, predicted response to lurasidone, which has a relatively high affinity for HTR1A. This study included the largest samples of Caucasian patients with SCZ to date and the first study for Africans.

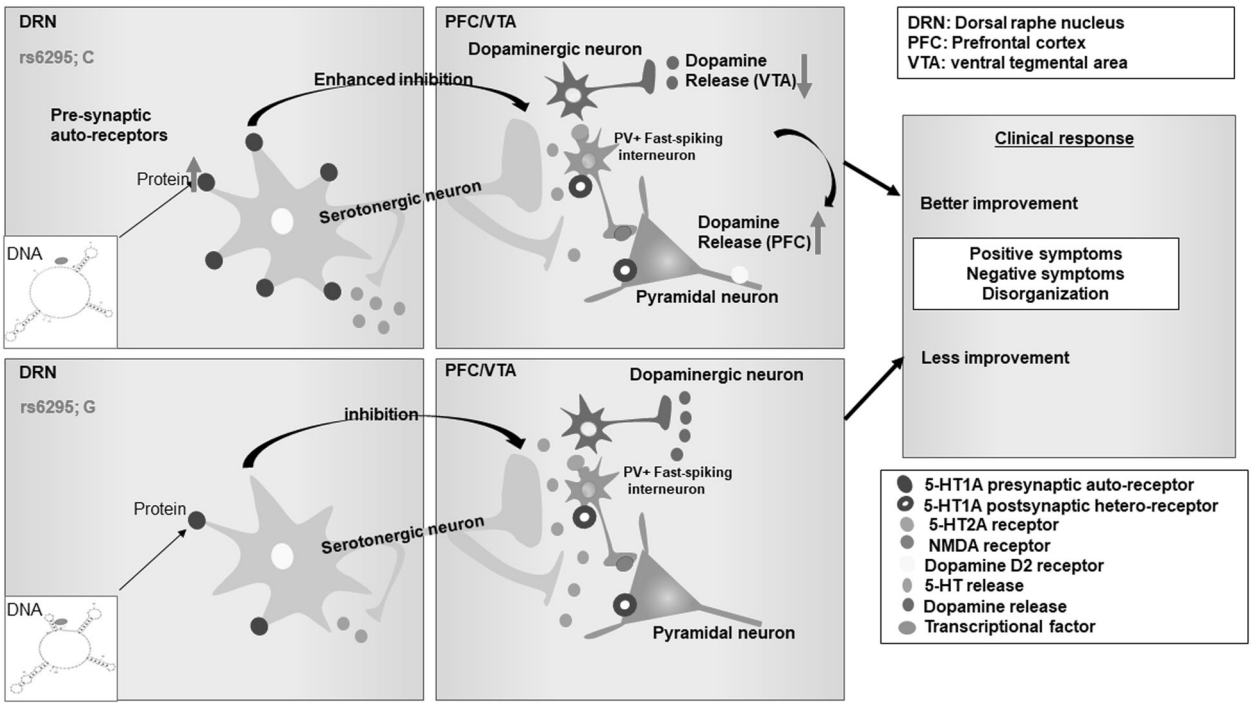

Fig. 3 Schematic diagram illustrated the functional impact of HTR1A polymorphism, rs6295 on the clinical response to lurasidone. SCZ patients from European ancestry harboring C allele of rs358532, corresponding to the C allele in rs6295, showed better improvement in PANSS positive, negative, and disorganization symptoms than homozygous G allele counterparts. C allele carriers of rs6295 have been shown to express more HTR1A auto-receptors in dorsal raphe nucleus (DRN), resulting in the enhanced inhibition of serotonergic firing activity at PFC (Albert, 2012). Disinhibition of parvalbumin-positive (PV+) GABAergic interneuron in PFC leads to increased release of dopamine in PFC. This may lead to a better improvement in negative symptoms in SCZ. Enhanced inhibition due to the increased HTR1A auto-receptors in DRN may also cause an enhanced inhibition of dopaminergic neuron in VTA, resulting in better improvement in positive symptoms in SCZ. Ameliorating positive and negative symptoms further supports improvement in disorganization. Transcriptional factor(s) binding is important to regulate the gene expression of HTR1A. Using mfold, we simulated the 2nd structure of transcriptional factors binding site of the palindromic sequence formed by rs6295 G allele but absence in rs6295 C allele. Having C allele in HTR1A promoter region, the transcriptional suppressor has no palindrome structure to bind to, resulting in enhanced inhibition by increased expression of presynaptic HTR1A auto-receptors

The results indicated that the tag SNPs for HTR1A functional polymorphism were significantly associated with improvement in PANSS positive, negative, and disorganization subscales in a race-specific and a time-dependent manner. We obtained a replication of the association in meta-analysis which included two treatment groups other than risperidone, but also involved patients of EUR ancestry. We further confirmed the association of rs6295 with improvement in positive and negative symptom in Caucasian patients with SCZ.

Acknowledgements The authors would like to thank all the patients, healthy volunteers, and psychiatrists who intensively cooperated with us to recruit patients in this study. This work was supported by a grant awarded to HYM by Sunovion.

Compliance with ethical standards

Conflict of interest HYM is a stockholder in ACADIA. HYM receives grant support from ACADIA, Allergan, Aptinyx, Eli Lilly, Janssen, Lundbeck, Neurocrine, Sumitomo Dainippon, and Sunovion. Other authors declare that they have no conflict of interest.

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