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REGULAR RESEARCH ARTICLE

TPH-2 Polymorphisms Interact with Early Life Stress to Influence Response to Treatment with Antidepressant Drugs

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Abstract

Background: Variation in genes implicated in monoamine neurotransmission may interact with environmental factors to influence antidepressant response. We aimed to determine how a range of single nucleotide polymorphisms in monoaminergic genes influence this response to treatment and how they interact with childhood trauma and recent life stress in a Chinese sample. An initial study of monoaminergic coding region single nucleotide polymorphisms identified significant associations of TPH2 and HTR1B single nucleotide polymorphisms with treatment response that showed interactions with childhood and recent life stress, respectively (Xu et al., 2012).
Methods: A total of 47 further single nucleotide polymorphisms in 17 candidate monoaminergic genes were genotyped in 281 Chinese Han patients with major depressive disorder. Response to 6 weeks' antidepressant treatment was determined by change in the 17-item Hamilton Depression Rating Scale score, and previous stressful events were evaluated by the Life Events Scale and Childhood Trauma Questionnaire-Short Form.
Results: Three TPH2 single nucleotide polymorphisms (rs11178998, rs7963717, and rs2171363) were significantly associated with antidepressant response in this Chinese sample, as was a haplotype in *TPH2* (rs2171363 and rs1487278). One of these, rs2171363, showed a significant interaction with childhood adversity in its association with antidepressant response.
Conclusions: These findings provide further evidence that variation in *TPH2* is associated with antidepressant response and may also interact with childhood trauma to influence outcome of antidepressant treatment.

Keywords: depression, antidepressive agents, stress, TPH2, polymorphism, single nucleotide

Introduction

Major depressive disorder (MDD) is a major economic and social burden owing to its high prevalence rate and disabling symptoms (Kessler et al., 2005). Although the newer antidepressant drugs are generally well tolerated and relatively effective, less than

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one-half of patients achieve remission with the first prescribed antidepressant (Rush et al., 2006). Partial remission results in continued suffering and higher costs (Tranter et al., 2002).

There have been many attempts to identify variables that could predict antidepressant response, with a variety of predictors proposed that include clinical, psychosocial, psychophysiological, neuropsychological, neuroimaging, and genetic factors (Bondy and Zill, 2004; Lohoff and Ferraro, 2010). While genetic polymorphisms may contribute 50% or more to antidepressant response, a more effective strategy should consider interactions between the various genetic factors (gene × gene) as well as their interaction with clinical and other environmental modulators (gene × environment) (Fabbri et al., 2014). In the main, genetic research focuses on genetic polymorphisms of target proteins that relate to mechanisms of antidepressant drug action (Serretti et al., 2005). As the monoaminergic theory remains the theoretical basis for the effect of most current antidepressant drugs, a number of genes coding for metabolic enzymes, transporters, and receptors in monoamine systems have been studied and reported to affect antidepressant drug response (reviewed by Schosser and Kasper, 2009; Fabbri et al., 2014). As the serotonin transporter and the 5-HT1A receptor are strongly implicated in antidepressant action, their genetic variability has been well studied in this respect (Illi et al., 2009). Investigations into an insertion–deletion (L/S)polymorphism(5-HTTLPR)associated with antidepressant response found that L-allele carriers have a faster and better response to antidepressants, if they are Caucasians, but allele frequencies and results of pharmacogenetic studies vary between different ethnic groups (Karlović et al., 2013). Other genes involved in serotonin and noradrenaline neurotransmission, including 5-HT6, 5-HT1A and 5-HT2A receptors, monoamine oxidase A, the noradrenaline transporter SLC6A2, and tryptophan hydroxylases (TPH1 and 2) among others also provide good candidates for pharmacogenetic study (Schosseret al., 2009; Kishi et al., 2010; Lin et al., 2014). The single nucleotide polymorphism (SNP) rs6295 of 5-HT1A receptor has been widely studied, because the G allele was associated with an upregulation of the receptor (Albert et al., 2004). TPH2 is the rate-limiting biosynthetic enzyme for serotonin that is preferentially expressed in the brain and has been implicated in the pathogenesis and treatment of major depressive disorder (Xu et al., 2012; Tsai et al., 2009). Several SNPs in this gene have been associated with affective disorders and their treatment response. Cichon et al. (2008) found that the minor alleles of SNPs rs11178997 (T-473A) and rs11178998 (A90G) in the 5' region were significantly overrepresented in bipolar affective disorder patients when compared with controls. In a recent meta-analyses, it was found that rs4760820 and rs11178998 demonstrated a strong genetic association with bipolar disorder (Gao et al., 2016). The promoter polymorphism rs4570625 (T-703G) of TPH2 has been found associated with paranoid schizophrenia in Han Chinese (Xu et al., 2014).Tsai et al (2009) reported that the proportion of intron 5 SNP rs2171363 heterozygotes had an increased chance of responding to an 8-week antidepressant treatment as compared with rs2171363 homozygotes (CC or TT).

Identifying environmental factors that may interact with different genetic pathways in determining antidepressant efficacy would be a valuable step towards personalized medicine in the treatment of depression. Environmental factors such as childhood maltreatment and stressful life events certainly show independent effects on antidepressant response (Nanni et al., 2012), and there are several reports of significant interactions with genetic polymorphisms in these effects (Uher, 2014). Thus, Mandelli et al. (2009) reported that stressors modulate the effects of a common functional polymorphism (5-HTTLPR) of the serotonin transporter gene (SLC6A4) on outcome of pharmacological treatment of depression. It was also found that variants in BDNF and ST8SIA may delay the early response to antidepressants in subjects not exposed to stressors at the illness onset, with a remarkable gene-environment interaction (Mandelli et al., 2014). Keers et al. (2011) also reported variation in the 5-HTTLPR and STin4 polymorphisms of SLC6A4 interacted with stressful life events to modify antidepressant effects. Our own studies identified that the *HTR1B* SNP rs6298 demonstrated interaction with recent stress in its association with antidepressant response, while rs7305115 of *TPH2* and rs5569 of the noradrenaline transporter gene *SLC6A2* interacted with childhood trauma to influence response to antidepressants (Xu et al., 2011, 2012).

Our previous studies focused on polymorphisms within exonic sequences of candidate genes involved primarily with serotonin and noradrenaline neurotransmission. In the current work, we extended this study, again using a candidate gene approach, but including intronic and promoter polymorphisms. At the same time, we aimed to replicate some significant results reported in Caucasians in our Chinese Han sample. Again, interactions between these polymorphisms and stressful life events were analyzed to obtain a better understanding of the role of both genetic and clinical factors in the response to antidepressant treatment.

METHODS

Subjects

The subjects were Chinese Han in- and out-patients referred to 5 hospitals in Beijing, Nanjing, Changsha, Yangzhou, and Huai'an. All recruited patients were 18 to 60 years old, had a baseline HDRS-17 score of >17, presented depressive symptoms for at least 2 weeks, and met DSM-IV for nonpsychotic MDD. All subjects were newly diagnosed or recently relapsed patients drug-free for over 2 weeks. The patients were diagnosed by 2 independent senior psychiatrists and confirmed by a third psychiatrist who was blind to the previous evaluations. Exclusion criteria included documented history of diagnoses on Axis 1 (including substance misuse, schizophrenia, schizoaffective disorder, bipolar disorder, generalized anxiety disorder, panic disorder, or obsessive compulsive disorder) of DSM-IV, personality disorder, mental retardation, pregnancy, lactation, primary organic disease and other medical illnesses impairing psychiatric evaluation, or a history of electroconvulsive therapy within the previous 6 months. Patients who suffered a manic episode during the 12 months after admission were excluded retrospectively. All patients were interviewed and diagnosed by 2 independent senior psychiatrists, and the diagnosis was confirmed by a third psychiatrist blinded to the previous evaluations. All subjects provided separate written informed consent for study participation, which was approved by each hospital ethical committee in accordance with the Declaration of Helsinki.

Antidepressant Treatment and Clinical Evaluation

MDD patients entering the study were given a single antidepressant drug (selective serotonin reuptake inhibitor [SSRI] or serotonin norepinephrine reuptake inhibitor [SNRI]) according to local clinical practice for at least 6 weeks. Subjects were divided into subgroups by drug type and sex for further analysis. A meeting was held for investigators from the different sites before the onset of the study for assessment, training,

and standardization of techniques. The assessing psychiatrists in different clinical centers achieved high inter-rater reliability, with an interclass correlation of at least 0.9. We interviewed each patient every 2 weeks using a standardized protocol across centers, recording treatment duration, dosage, outcome, compliance, and side effects. Severity of depressive symptoms was assessed using HDRS-17 by a trained senior psychiatrist who was blind to patients' genotypes. Adjustment of antidepressant dose and usage of concomitant anxiolytics have been described previously (Xu et al., 2011). The primary outcome was "response," defined by a reduction of ≥50% of the baseline HDRS-17 score after 6-week treatments (Xu et al., 2011). Patients who changed antidepressant drug or demonstrated nonadherence were retrospectively excluded from the study.

Two evaluation tools, the Childhood Trauma Questionnaire (28-item Short form, CTQ-SF) (Bemstein and Fink, 1998) and the Life Events Scale (LES) (Yang et al., 1999), were used to evaluate the occurrence of stressful life events that took place before the age of 16 years or during the previous year, respectively. Both of them are retrospective self-report questionnaires, and the details have been described previously (Xu et al., 2011). Control samples were not included in the present study; therefore, the control values of Fu et al. (2005) and Sun et al. (2008) were used to define normal CTQ-SF and LES values, respectively. The total CTQ-SF and NLES scores were dichotomized for use in the gene-environment interaction analyses. A total CTQ score ≥1 SD above the mean CTQ score (35.86 [8.25]) of control subjects (≥44.11) was designated "C high adversity"; <44.11 CTQ scores were designated "C low adversity" (Fu et al., 2005). An NLES score ≥1 SD greater than the mean NLES (13.09 [25.16]) of control subjects (≥38.25) was designated "N high adversity"; <38.25 NLES scores were designated "N low adversity" (Sun et al., 2008; Enoch et al., 2010).

Gene Selection and Genotyping Method

The 20 candidate genes tested were selected based on evidence for the involvement of the monoaminergic system in antidepressant mechanisms, and 64 SNPs in these genes were included on the basis of prior genetic or pharmacogenetic study. These were identified using dbSNP (Map to Genome Build: 36.3) and HapMap (Public Release #24), were screened using the tagging SNP method (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/), and then polymorphisms with reported minor allele frequency (MAF) values of ≥5% were prioritized. Most of the SNPs were located in gene exon, intron, or promoter regions. Genomic DNA samples were genotyped using Illumina Golden Gate assays (Illumina Inc). SNPs that were genotyped successfully in <90% of all samples or had an experimental MAF of <5% were excluded. Likewise, participants in whom <90% of the selected markers were successfully determined were also excluded from further analysis.

Statistical Analysis

Differences in clinical variables between responder and nonresponder groups were evaluated by Pearson's χ^2 test or ANOVA using SPSS version 13.0 (SPSS Inc). Haploview 4.0 was used to estimate Hardy-Weinberg equilibrium, MAF, linkage disequilibrium (both D' and r^2), and the percentage of nonmissing each marker (%Gene). Genetic polymorphisms were correlated with therapeutic effects by comparing allele, genotype, and haplotype distributions between responders and nonresponders using UNPHASED-3.0.13 (Dudbridge, 2003). One thousand random permutations were performed with UNPHASED 3.0.13 software to correct *P* values for multiple testing in the allelic, genotypic, and haplotype association analyses. Furthermore, G×E interactions in association with therapeutic outcome were assessed by logistic regression analysis, using age, HDRS-17 baseline score, sex, and drug type as covariates. Logistic regression analyses were used to assess the effects of CTQ-SF, NLES, genotype, and interactions between genotype and CTQ-SF or NLES on the categorical outcome of antidepressant response. For the G×E analysis, only those SNPs that provided significant findings in the initial association were included (as each SNP thus had a valid a priori hypothesis). Each SNP was tested against 2 environmental conditions. Benjamini and Hochberg Discovery Rate Multiple Testing Correction was applied to the statistical significance of $P < .05$ (Benjamini and Hochberg., 1995).

RESULTS

A total of 281 patients met the study entry criteria and completed 6 weeks of antidepressant treatment. Among those, 205 patients achieved response as defined by a 50% improvement in HAMD-17 score. Subgroups were SSRI (n = 164) vs SNRI (n = 117) and male (n = 116) vs female (n = 165). Of the 64 SNPs originally tested, 10 SNPs with MAF <5% or %Gene <95% were excluded, and 7 further SNPs were not in HW disequilibrium ($P < .001$), leaving 47 SNPs for inclusion in the analysis (Table 1).

The demographic and clinical characteristic of patients in the responder and nonresponder patient groups are shown in Table 2. There were no significant differences between the 2 subgroups in gender, age, years of education, or family history of mood disorder. However, the baseline HDRS-17 score was significantly different between the 2 groups ($F = 8.359$, $P = .004$), as shown in Table 2.

An analysis of single locus effects revealed that 2 SNPs, rs11178998 and rs7963717, in TPH2 had a statistically significant association with antidepressant response, as shown in Table 3. There was genotypic association for each of these with antidepressant response in the total group and in the SSRI subgroup, respectively, which withstood permutation testing. There was allelic association of one further TPH2 SNP (rs2171363) with antidepressant response in total group, withstanding permutation testing.

We examined the association of haplotypes derived from the SNPs in every gene with antidepressant response, limiting our analysis to haplotypes with a frequency ≥5%. Significant results were only found for TPH2. Table 4 shows the 2 haplotypes of *TPH2* in relation to antidepressant response. The A-A haplotype was significantly associated with antidepressant response in the total group and in the SSRI subgroup, which withstood permutation testing. Compared with the A-G haplotype, the A-A haplotype was associated with increased likelihood of poor response in the total group (OR = 0.6959, 95% CI = 0.4259–1.136) and in the SSRI subgroup (OR = 0.5526, 95% CI = 0.2898–1.054). With the 3 SNPs of TPH2, the G-G-A haplotype was associated with antidepressant response in the total group (OR = 1.986, 95% CI = 1.018–3.872), but not withstanding permutation testing.

Among 281 subjects, 208 completed the CTQ-SF, of whom 68 were categorized as high adversity based on their CTQ-SF score, and 219 completed the LES, of whom 78 were in the high adversity category based on their nLES score. In logistic regression analyses, there was no significant association between the categories derived from CTQ-SF or NLES and antidepressant outcome in the stepwise logistic regression model (results not shown). A further analysis searching for the interaction of these environmental measures with genotype in treatment response was then tested on the 3 SNPs with significant effects in the simple association analysis (rs11178998, rs7963717, rs2171363). A significant interaction between the TPH2 SNP rs2171363 and

CTQ-SF category was identified in the effect on antidepressant response, adjusting for age, gender, and HDRS-17 baseline scores as covariates. The results are shown in Table 5 (nonsignificant results not shown). In the regression model with CTQ-SF and rs2171363, the interaction term (CTQ-SF by rs2171363 GG genotype) was associated with reduced response to antidepressants, with the CTQ low adversity and rs2171363 AG/AA serving as reference (β = -2.432, SE = 1.060, OR = 0.088, 95%CI: 0.011–0.702). A further exploratory analysis searching for gene-environment interactions in the remaining SNPs identified no additional significant effects before any correction for multiple testing.

Discussion

In the initial investigation of associations of variants in monoamine neurotransmitter system gene with antidepressant response in patients with MDD, we found that SNPs in TPH2 were significantly associated with antidepressant response, and an interaction of one SNP in TPH2 and early life stress was also associated with antidepressant response.

There were genotypic associations for rs11178998, rs7963717, and rs2171363 in TPH2 with antidepressant response in the total group and, for the first two, in the SSRI subgroup. rs11178998 is located in the 5' UTR and has previously been reported

Table 1. Characteristics of Genotype Markers

Number	Gene	Name	%geno	HW P	MAF	Alleles
1	*ADRA2A*	rs12246561	100	1	0.06	A:C
2	*ADRA2A*	rs521674	100	.2271	0.297	T:A
3	*ADRB1*	rs17875446	99.6	.0792	0.146	G:T
4	*COMT*	rs2020917	99.6	.024	0.243	G:A
5	*COMT*	rs4680	98.2	1	0.277	G:A
6	*COMT*	rs165774	100	.1613	0.171	G:A
7	*COMT*	rs165599	99.6	.4011	0.479	A:G
8	*HTR1A*	rs6295	100	.6515	0.254	C:G
9	*HTR1B*	rs4140535	99.6	1	0.35	A:G
10	*HTR1D*	rs674386	100	.6239	0.292	G:A
11	*HTR2A*	rs7997012	99.6	1	0.234	G:A
12	*HTR2A*	rs1923884	100	.1605	0.498	G:A
13	*HTR2A*	rs2224721	99.6	.5167	0.323	C:A
14	*HTR2A*	rs9316233	100	.2275	0.283	G:C
15	*HTR2A*	rs2770296	98.6	.7639	0.253	A:G
16	*HTR2A*	rs1928040	99.6	.804	0.266	A:G
17	*HTR2A*	rs927544	100	.3008	0.286	A:G
18	*HTR2A*	rs6313	99.6	.402	0.411	A:G
19	*HTR2A*	rs6311	99.6	.6041	0.198	G:A
20	*HTR2C*	rs3795182	99.6		0.157	A:G
21	*HTR2C*	rs521018	100		0.173	A:C
22	*HTR3A*	rs1062613	99.3	.9186	0.1	G:A
23	*HTR3E*	rs10937162	98.9	.7334	0.317	G:C
24	*HTR3E*	rs6443950	100	.7589	0.173	A:T
25	*HTR4*	rs1432919	100	.164	0.288	G:A
26	*HTR4*	rs2278392	100	.2402	0.29	G:A
27	*HTR5A*	rs1881691	99.6	.3585	0.329	A:C
28	*HTR5A*	rs3734966	99.3	.5743	0.113	A:G
29	*HTR5A*	rs1800883	100	.0707	0.463	G:C
30	*HTR7*	rs1891311	100	.1058	0.183	A:G
31	*MAOA*	rs979606	99.6		0.407	G:A
32	*MAOA*	rs2064070	99.6		0.402	A:T
33	*TH*	rs10770140	98.2	.0153	0.085	A:G
34	*TH*	rs10840490	100	.0268	0.133	C:G
35	*TH*	rs7115640	100	.7755	0.069	G:A
36	*TPH1*	rs7933505	100	.8807	0.496	G:A
37	*TPH1*	rs1799913	100	.8787	0.493	C:A
38	*TPH1*	rs1800532	100	1	0.489	C:A
39	*TPH2*	rs7963717	100	.4763	0.16	A:C
40	*TPH2*	rs4570625	100	1	0.44	A:C
41	*TPH2*	rs11178998	100	.396	0.164	A:G
42	*TPH2*	rs7954758	100	.2892	0.157	A:G
43	*TPH2*	rs1386494	100	1	0.084	G:A
44	*TPH2*	rs2171363	100	.269	0.459	A:G
45	*TPH2*	rs1487278	99.6	.2022	0.305	A:G
46	*TPH2*	rs1386483	99.6	.579	0.443	A:G
47	*TPH2*	rs1487279	99.6	.3369	0.454	T:A

Abbreviations: HW P, Hardy-Weinberg equilibrium P-value; %geno, percentage nonmissing; MAF, minor allele frequency.
Alleles means the major and minor alleles.

Table 2. Demographic Characteristics of MDD Patients and Baseline HDRS-17 Scores between Responder and Nonresponder Groups

Demographic Characteristics	Responder (n = 205)	Nonresponder (n = 76)	F/ $\chi 2$	*P* Value
Age (y)	38.99 ± 12.93	36.18 ± 13.36	2.556	0.111
Gender, male (female)	39.5% (60.5%)	46.1% (53.9%)	0.978	0.323
Antidepressant, SSRI (SNRI)	55.1% (44.9%)	65.8% (34.2%)	2.590	0.108
Duration of illness (mo)	48.42 ± 75.63	49.78 ± 68.83	0.019	0.892
Family history of mood disorder, yes (no)	16.1% (83.9%)	23.7% (76.3%)	2.148	0.143
First episode, yes (no)	51.7% (48.3%)	46.1% (53.9%)	0.709	0.400
Education, (y)	11.28 ± 3.71	12.20 ± 3.71	3.400	0.066
Baseline HDRS-17 score	28.18 ± 5.68	26.01 ± 5.32	8.359	0.004

Table 3. Genetic Association Analysis (Genotypic/Allelic) of SNPs vs Response Status in Total Group and the SSRI Subgroups

Gene/rs#	Genotype/Allele	RES	NR	RES-freq(%)	NR-freq(%)	*P*	*P***	OR (95%CL)
Total group (n = 281)								
TPH2/	AA	149	45	72.68	59.21	.03002	.03996	1.833 (1.056–3.18)
rs11178998	AA/AG*	56	31	27.32	40.79	.03002		
TPH2/	AA	150	46	73.17	60.53	.04039	.03297	1.779 (1.022–3.096)
rs7963717	AC+CC*	55	30	26.83	39.47	.04039		
TPH2/	A*	210	94	51.22	61.84			
rs2171363	G	200	58	48.78	38.16	.02478	.01598	1.544 (1.055–2.258)
SSRI subgroup (n = 164)								
TPH2/	AA	81	26	71.05	52.00	.01834	.02098	2.266 (1.14–4.503)
rs11178998	AA/AG*	33	24	28.95	48.00			
TPH2/	AA	82	27	71.93	54.00	.02516	.02398	2.183 (1.095–4.352)
rs7963717	AC+CC*	32	23	28.07	46.00			

Table 4. Estimated Haplotype Frequency of the TPH2 SNPs and the Results of Haplotype Analysis in Responders and Nonresponders

Gene/rs#	Haplotype	RES	NR	RES-freq(%)	NR-freq(%)	*P*	*P***	OR (95%CL)
Total group (n = 281)								
TPH2/	A-A	87.9	48	21.54	31.58	.01153	.03996	0.6959 (0.4259–1.136)
rs2171363,	A-G*	121.1	46	29.68	30.26			
rs1487278	G-A	195.1	58	47.82	38.16			
TPH2/	A-A-A	28.85	17	70.70	11.18			
rs1386494,	G-A-A	58.71	31	14.39	20.39			
rs2171363,	G-A-G*	120.3	46	29.48	30.26			
rs1487278	G-G-A	195.4	58	47.90	38.16	.0364	.1239	1.986 (1.018–3.872)
SSRI subgroup (n = 164)								
TPH2/	A-A	44.53	32	19.53	32.00	.01203	.02697	0.5526 (0.2898–1.054)
rs2171363,	A-G*	65.47	26	28.72	26.00			
rs1487278	G-A	115.5	42	50.65	42.00			

Table 5. Results of the Interactions between rs2171363 and CTQ-SF on Antidepressant Response Adjusting for Age, Gender, and Baseline HDRS-17 Score

	β	SE	*P*	Odds Ratio	95% CI
Baseline HDRS-17 score	0.112	0.033	0.001	1.118	1.049–1.192
Gender	0.222	0.336	0.509	1.248	0.646–2.411
Age	-0.005	0.013	0.713	0.995	0.969–1.021
CTQ-SF	0.205	0.376	0.585	1.227	0.588–2.564
rs2171363	2.019	0.776	0.009	7.529	1.646–34.434
rs2171363 by CTQ-SF interaction	-2.432	1.060	0.022	0.088	0.011–0.702

Abbreviation: CTQ-SF, Childhood Trauma Questionnaire.

to be associated with bipolar disorder (Cichon et al., 2008). Furthermore, rs11178998 and rs7963717 were found to be in very high linkage disequilibrium in our study (Table 6), indicating that the finding may be due to the functional effect of a single SNP. There is also high linkage disequilibrium (D' = 1, $r^2 > 0.99$) between rs11178997 and rs11178998 reported in Chinese subjects, these SNPs also showing strong linkage with rs4570625 in the 5' region (Xu et al., 2013).

Results of a study investigating the effect of TPH2 promoter polymorphisms on transcriptional activity (Scheuch et al., 2007)

Table 6. Linkage Disequilibrium among SNPs in TPH2

TPH2	rs7305115[a]	rs4290270[a]	rs7963717	rs4570625	rs11178998	rs7954758	rs1386494	rs2171363	rs1487278	rs1386483	rs1487279 (D')
rs7305115[a]		0.69	0.41	0.70	0.41	0.39	1	0.98	0.94	0.71	0.66
rs4290270[a]	0.48		0.41	0.48	0.44	0.35	0.63	0.68	0.64	0.99	0.94
rs7963717	0.02	0.02		1	1	0.97	0.78	0.42	0.91	0.50	0.54
rs4570625	0.46	0.22	0.15		1	1	0.37	0.72	0.83	0.49	0.49
rs11178998	0.02	0.03	0.97	0.15		0.97	0.64	0.38	0.92	0.53	0.57
rs7954758	0.02	0.02	0.92	0.14	0.89		0.73	0.40	1	0.46	0.52
rs1386494	0.07	0.03	0.01	0.01	0	0		1	0.86	0.57	0.67
rs2171363	0.95	0.46	0.02	0.49	0.02	0.02	0.07		0.94	0.71	0.66
rs1487278	0.32	0.14	0.06	0.24	0.07	0.08	0.03	0.33		0.66	0.62
rs1386483	0.48	0.95	0.03	0.24	0.04	0.03	0.02	0.47	0.15		0.96
rs1487279 (r2)	0.44	0.87	0.04	0.23	0.05	0.04	0.03	0.42	0.14	0.88	

D' and r^2 value are shown above and below the diagonal, respectively.
[a] SNPs of TPH2 mentioned in the previous research (Xu et al., 2012) with the same sample.

indicated that the A-allele of rs11178997 significantly reduces TPH2 transcriptional activity via an effect on transcription factor binding, while rs4570625 was found to have no influence on transcription. However, a subsequent study (Chen et al., 2008) found both of these regulatory sequence SNPs influenced, in a synergistic manner, gene expression, while rs11178998 also had a strong effect on transcriptional activity independent of the other 2 SNPs. These data suggest that the functional effects of rs11178997 and/or rs11178998 may well contribute to the association of rs11178998 and rs7963717 with antidepressant response

Several other groups have shown association of rs4570625 with psychiatric disorders, including schizophrenia (Xu et al., 2014) and epileptic psychosis (Bragatti et al., 2014) and, interestingly in this context, an interaction of the SNP with maternal depression in infant response to fearful expression (Forssman et al., 2014). However, we find that this SNP is not significantly related to outcome measures in our study. This is likely due to the fact that while this SNP is in linkage disequilibrium with the functional rs11178997 and the closely linked rs11178998 that we measured, the MAFs are very different, resulting in $r^2 = 0.15$.

The third TPH2 SNP, rs2171363, significantly associated with antidepressant response in the total group, but not with the SSRI subgroup, was also associated with antidepressant response in another study of Chinese patients (Tsai et al., 2009). This effect may be additional to that of the 2 promoter SNPs; rs2171363 is found in a large intronic sequence approximately 30 kb removed from the 5' regulatory sequence and this SNP is not in strong linkage disequilibrium with the previous 2 (Table 6).

This rs2171363, but not the 2 other SNPs significantly associated with antidepressant response, shows a significant interaction with early life stress in this effect. This finding, in which the GG genotype and high childhood adversity were related to poor treatment response, is notable in light of our initial finding of such a gene-environment interaction with antidepressant response with this sample (Xu et al., 2012). The current findings appear to be a replication of the original effect; rs2171363 and the previously reported rs7305115 are in close linkage disequilibrium and are thus likely to show any association together. The mechanism underlying the association with antidepressant response is unclear; rs2171363 is intronic, while rs7305115 is a synonymous exonic SNP. However, there is a report that these are 2 of 5 SNPs within a closely linked haplotype block, each correlating with TPH2 mRNA expression (Lim et al., 2007).

Furthermore, the mechanism underlying the interactions between genetic variation in TPH2 and early stressors is also far from clear. One speculative explanation for the gene-environment interaction in our study might be that early life stress results in epigenetic changes such as DNA methylation and that this may interact with proximal polymorphisms, perhaps by influencing transcription factor binding, to modify gene expression. There is certainly evidence for this in terms of the relationship between early life stress and depression (Dalton et al., 2014) and has also been indicated as a factor influencing treatment response of some symptoms in schizophrenia (Tang et al., 2014).

The 2 TPH2 SNPs that showed no significant effects in our genotype and allele analysis, rs1487278 and rs1386494, have previously been reported to be associated with antidepressant response and treatment resistant depression, respectively (Tzvetkov et al., 2008; Anttila et al., 2009; Tsai et al., 2009). Haplotype analysis undertaken according to the haplotype blocks defined by the SNPs in this study has not shown any effect stronger than individual significant component SNPs.

The results of this study would benefit from being replicated in a further sample, and a second collection is currently

underway to address this. It would also be valuable to know whether the findings could be extrapolated to other ethnicities. As with all such studies, a larger sample would be more informative; this could enable the investigation of possible differential effects of sex, individual drugs, and further social or epidemiological variables as well as permitting better study of gene interactions with these factors and between genes. Furthermore, the differences in significant association between the whole sample and the SSRI subgroup may reflect the limited sample size rather than any subgroup specificity. We recognize that the retrospective and subjective determination of prior stressors is imprecise, and treating these as dichotomized categorical variables limits the analytical power. It is important to recognize that in this small study we have not investigated all the genetic factors that might contribute to functional effects on serotonin or noradrenaline neurotransmission, nor have we comprehensively assessed the genetic variability within the chosen candidate genes, having selected specific SNPs on the basis of prior study and likely or known functionality. Nevertheless, our findings provide an interesting and potentially valuable insight into possible genetic factors and gene-environment interactions that influence outcome in the treatment of depression.

In conclusion, we find 3 associations of genetic variability within TPH2 with response to treatment in our sample of Chinese subjects with depression. Only one of these demonstrates an interaction with early life stress, a finding which suggests a sequence-specific epigenetic consequence of this environmental factor that may be modified by the identified polymorphism, or one in close linkage. Further study needs to identify the effects of these genetic variants on gene expression and on the possible mechanisms relating early life stress to response to treatment with antidepressant drugs.

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Statement of Interest

None.

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