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Variants of Dopamine Beta Hydroxylase Gene Moderate Atomoxetine Response in Children with Attention-Deficit/Hyperactivity Disorder

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Abstract

Objective: Atomoxetine is the most widely used nonstimulant for the treatment of attention-deficit/hyperactivity disorder (ADHD). It selectively acts on the norepinephrine (NE) system. Dopamine beta hydroxylase (DBH) regulates the synthesis of NE. This study aimed to investigate whether variants in the DBH gene have an effect on the differential response to atomoxetine.

Methods: Children and adolescents with ADHD were enrolled in a prospective, open-label study of atomoxetine for 8–12 weeks. The dose was titrated to 1.2–1.4 mg/kg per day and maintained for at least 4 weeks. The primary efficacy measure was the investigator-rated ADHD Rating Scale-IV (ADHD-RS-IV). Three categorical evaluations of treatment effects (defined as response, robust response, and remission) were used. We used a candidate gene approach. Eight single nucleotide polymorphisms (SNPs) in DBH were selected and genotyped based on the functional annotation in literature. Their association with response or remission status was analyzed.

Results: Four SNPs were found nominally associated with response status (rs1076150, $p = 0.0484$; rs2873804, $p = 0.0348$; rs1548364, $p = 0.0383$; and rs2519154, $p = 0.0097$), two were associated with robust response (rs1076150, $p = 0.0349$; and rs2519154, $p = 0.0047$), and one was associated with remission (rs2519154, $p = 0.0479$). The association between rs2519154 and robust response was significant after correction of multiple comparison ($p = 0.0384$). Two haplotypes of linkage disequilibrium (LD) block1 (constituted by rs1108580, rs2873804, rs1548364, and rs2519154) were nominally associated with response and robust response status (CTAC: $p = 0.0301$ for response, $p = 0.0374$ for robust response; TCGT: $p = 0.0317$ for response, $p = 0.021$ for robust response), whereas one haplotype (GC) of LD block2 (constituted by rs2073837 and rs129882) was associated with robust response and remission status ($p = 0.0377$ for robust response; $p = 0.0321$ for remission), although none achieved significant threshold after multiple comparison.

Conclusions: Variants in DBH genes were associated with atomoxetine response in the treatment of ADHD. Further replication in larger samples would be warranted.

Introduction

Atomoxetine is the first approved nonstimulant for the treatment of attention-deficit/hyperactivity disorder (ADHD). It was marketed in 2002 in the United States and in 2007 in China, providing another potent medication for ADHD. In the clinical trials before and after marketing, atomoxetine showed superior effect on ADHD symptoms compared with placebo control (Kelsey et al. 2004; Michelson et al. 2002), with moderate effect size estimated to be 0.71.

Following studies showed that the effect of atomoxetine varied among patients. The response rate (defined as ≥40% reduction from baseline ADHD Rating Scale [ADHD-RS] scores) was reported to be 45% at the end of 6 weeks treatment (Newcorn et al. 2008). This figure was very close to that continuing treatment (48.4%) after clinical trials (Wilens et al. 2006), as one of the most common reason of discontinuation was lack of effectiveness.

Some other studies reported similar adherent rate of atomoxetine. In the COMPLY observational study performed in Germany, only 48.8% patients who took atomoxetine continued treatment

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after 12 months. Given the slow onset of the effect, and relatively low response rate, it was necessary to find predicting markers that could early identified patients who would benefit from atomoxetine treatment.

The first pharmacogenetic study of ADHD was published in 1999 (Winsberg et al. 1999). After this initiation, most of the following studies focused on methylphenidate (MPH). The candidate genes were selected from the pharmacodynamic, pharmacokinetic, and etiological pathways of ADHD. Possible associated genes involved were DAT1, DRD4, NET1, ADRA2A, CES1, HTT, and GRM7 (Seeger et al. 2001; Hamarman et al. 2004; Cheon et al. 2005; Faraone et al. 2005; Cheon et al. 2007; Polanczyk et al. 2007, da Silva, et al. 2008; Kooij et al. 2008; Mick et al. 2008; Purper-Ouakil et al. 2008; Stein and McGough 2008; McGough et al. 2009; Nemoda et al. 2009; Froehlich et al. 2010; Genro et al. 2010; Kieling et al. 2010; Kim et al. 2010; Polanczyk et al. 2010; Froehlich et al. 2011; Park et al. 2012a,b). Only two articles investigated atomoxetine response, which consistently reported association of genes in the NE system; that is, NET1 and ADRA2A (Ramoz et al. 2009; Yang et al. 2013).

Atomoxetine is a high selective inhibitor of the NE transporter, which may exert its therapeutic effect through change in the NE concentration in the synapses. The effectiveness of atomoxetine in ADHD patients led to the hypothesis that ADHD might be a noradrenergic disorder (Biederman and Spencer 1999). This hypothesis comes, not only from pharmacological evidence, but also from the fact that the noradrenergic system regulates many higher cognitive functions including attention (Solanto et al. 1998). Low levels of NE reduce motivation and performance in learning tasks (Viggiano et al. 2004). NE interacts with dopamine (DA) to regulate motor activity, as decreased DA reduces motor activity, whereas increased DA promotes activity.

Dopamine beta hydroxylase (DBH) is a synthetic enzyme for NE. Knocking out the DBH gene led to decreased NE levels in central neural system (Cryan et al. 2001), which suggested the important role of this enzyme in the maintenance of normal NE functions. Some antidepressants that act on NE system, such as reboxetine, have no effect in DBH knockout (KO) mice (Cryan et al. 2001), which made us speculate that any functional DNA variants in DBH genes, changing the activity of the enzyme, might modulate the response to atomoxetine in the treatment of ADHD. Discovery of such variants would make it possible to predict the treatment effect before it started. Up until now, there have been no studies investigating the association of the DBH gene with atomoxetine response in ADHD children.

We selected 8 single nucleotide polymorphisms (SNPs) in the DBH gene (rs1076150, rs1611115, rs1108580, rs2873804, rs1548364, rs2519154, rs2073837, and rs129882) to analyze their association with categorical assessments of atomoxetine response.

Methods

Participants

Children and adolescents who met the ADHD criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. (DSM-IV) were recruited from the Child and Adolescent Psychiatric Outpatient Department of Beijing University Sixth Hospital (American Psychiatric Association 1994). The diagnosis was first made by a child psychiatrist, and then validated by a semi-structured interview with the parents and the child, using Barkley's Clinical Diagnostic Interview Scale (Barkley 1998). This scale was based on DSM-IV criteria. It included questions about the 18 items regarding ADHD symptoms, onset of age, impairment of function, and exclusion criteria. We had used this scale in our previous pharmacogenetic study (Yang et al. 2004, 2013). All patients were required to meet the following symptom severity thresholds: A total score of investigator-rated ADHD-RS-IV no less than 25 for boys or 22 for girls, or a subtype corresponding subscale score ≥12 (DuPaul et al. 1998; Wang et al. 2007). The subjects were unmedicated, or had been medicated with MPH preparations or atomoxetine but had stopped for at least 1 or 4 weeks, respectively. All patients and their parents were Han Chinese. The exclusion criteria were: 1) Allergy to atomoxetine, 2) combined treatment with other psychotropic drugs or non-drug intervention for ADHD; 3) noncompliance with the blood draw. The study was approved by the Beijing University Sixth Hospital Institutional Review Board. Parents signed written informed consent. For younger adolescents, oral assent was acquired.

Clinical trial

The subjects received open-label treatment with atomoxetine for 8–12 weeks. The dose was titrated from 0.5 mg/kg/day in the 1st week, to 0.8 mg/kg per day in the 2nd week, and to 1.2 mg/kg per day in the 3rd–4th weeks. If necessary, the dose could be increased to 1.4mg/kg per day in the 5th week. Then the dose was maintained for at least 4 weeks. Those with side effects at any stage of titration could be maintained at the dose for 1–2 weeks. The total course of treatment was no more than 12 weeks. Treatment response was assessed at baseline, and at the end of the 1st, 2nd, and 4th weeks, and at the 8th week or at the end of the trial. Medication compliance was assessed by directly asking the parent at every visit. Patients who missed the whole or a partial dose for 3 consecutive days or for 10 total days were defined as noncompliant and were withdrawn from the trial.

Treatment response assessment

The primary efficacy measure was the investigator rated ADHD-RS-IV, (DuPaul 1998). It was rated based on both parent and teacher reports. The ADHD-RS-IV consists of 18 items corresponding to DSM-IV criteria for ADHD. The total symptom score as well as the inattention and hyperactivity-impulsivity subscales scores were used to evaluate the core symptoms of ADHD. This scale had been translated into Chinese. The validity and reliability of the Chinese version were demonstrated by Su et al. (2006).

In this study, we used categorical definitions of treatment response. A decrease of at least 25% on the ADHD-RS-IV total score from baseline to the end of the trial was defined as ''response'' (Swanson et al. 2001; Steele et al. 2006; Ramoz et al. 2009; Dickson et al. 2011). A decrease of 40% or more was defined as ''robust response'' (Newcorn et al. 2008). An average ADHDRS-IV item score ≤1 at the end of the treatment was defined as ''remission'' (Stein et al. 2003; Steele et al. 2006).

Genotyping

Eight SNPs in the DBH gene were selected via the ABI SNPbrowser™ (Table 1). These SNPs were either associated with ADHD in previous studies, or were tag SNPs selected by ABI SNPbrowser. We preferentially selected potential functional SNPs that located at coding regions, 5′ or 3′ untranslated regions, the boundary of exon and intron, and the 5′ regulatory region, including the promoter. Although a this was a bioinformatic analysis, all the eight SNPs were regulatory SNPs (http://rsnp.psych.ac.cn/).

Table 1. List of 8 SNPs Across the DBH Gene Investigated in this Study

NCBI SNP reference	*dbSNP allele*	*Public location*	*Location on gene region*	*SNP type*	*Residue change*	*MAF (HapMap-CHB)*
rs1076150	C/T	chr.9-136498761	Flanking_5′UTR	Regulatory	–	0.195
rs1611115	C/T	chr.9-136500515	Flanking_5′UTR	Regulatory	NA => NA	0.207
rs1108580	C/T	chr.9-136505114	Exon 2–intron2 splice junction	Silent mutation Regulatory	E [Glu] => E [Glu]	0.183
rs2873804	C/T	chr.9-136505644	Intron 2	Regulatory	–	0.244
rs1548364	A/G	chr.9-136507742	Intron 3	Regulatory	–	–
rs2519154	C/T	chr.9-136512275	Intron 5	Regulatory	–	0.125
rs2073837	A/G	chr.9-136522928	Intron 11	Regulatory	–	–
rs129882	C/T	chr.9-136523669	UTR 3′	Regulatory	NA => NA	0.366

SNP, single nucleotide polymorphism; DBH, dopamine beta hydroxylase; NCBI, National Center for Biotechnology Information; MAF, minor allele frequency; UTR, untranslated region.

The SNP was genotyped using TaqMan allelic discrimination assays (Livak 1999) on an ABI 7900HT instrument (Applied Biosystems, Foster City, CA), using predesigned and validated TaqMan assay reagent kits. The polymerase chain reaction (PCR) was performed following a standard protocol with 5 ng DNA in 5 mL reaction volumes for each sample. Thermal cycle included 95°C for 10 minutes, followed by 92°C for 15 seconds and 60°C for 1 minute for 40–45 cycles. SDS version 2.3 software (Applied Biosystems) was used for genotype identification. For quality control, 10% of the samples were genotyped as duplicates. Call rates for SNPs were 99.14%. Two to four negative test controls were set in every plate.

Statistical analysis

The SNPs were tested for Hardy–Weinberg equilibrium (HWE) by calculating the probability that the deviation from HWE could be explained by chance. None of the eight SNPs significantly deviated from HWE ($p > 0.05$). To evaluate the relationship of the SNPs, we used HaploView Program (http://www.broad.mit.edu/mpg/haploview) to calculate the pairwise value of linkage disequilibrium, D, D', and r^2. Blocks were defined using the algorithm of confidence interval (CI) by Gabriel et al. (2002). Two linkage disequilibrium (LD) blocks were detected (Fig. 1). Block 1 included four SNPs: rs1108580, rs2873804, rs1548364, and rs2519154, whereas Block 2 consisted of two SNPs: rs2073837 and rs129882. Baseline demographic and clinical features between differential response groups were compared using the SPSS 19.0 software, with categorical variables assessed by χ^2 test, and continuous variables by t test. The association among alleles, haplotypes, and treatment response to atomoxetine were evaluated using the χ^2 test by Haploview 4.0. The level of significance was 0.05 for all analyses. Five thousand permutation tests were used to control multiple comparisons. The odds ratio (OR) was calculated as the measure of effect size.

Results

Eighty seven subjects completed 8–12 weeks of treatment and provided both baseline and end-point assessments. The demographic and clinical features are presented in Table 2.

Single variant association

Using an ADHD-RS score decrease of ≥25% as the response criterion, 64 patients were responders and 23 were nonresponders. Among the eight SNPs used in the analysis, four showed nominal significant association with responder status, and one showed trend association (rs1076150, $p = 0.0484$; rs2873804, $p = 0.0348$; rs1548364, $p = 0.0383$; rs2519154, $p = 0.0097$; and rs1108580, $p = 0.0736$). rs2519154 kept a trend association after 5000 permutations performed for multiple test correction ($p = 0.0926$). Using the ''robust response'' criteria, 45 patients were robust responders and 42 were nonresponders. The abovementioned five SNPs also

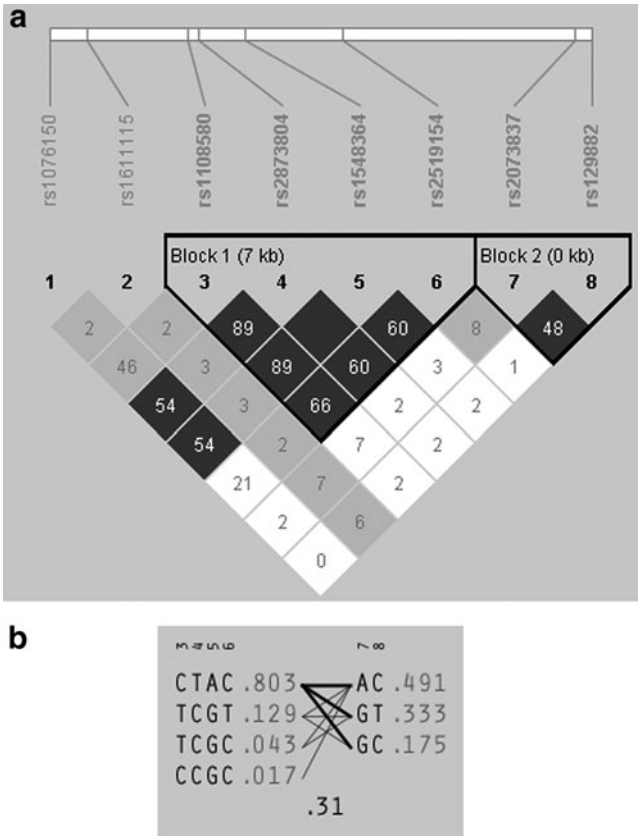

FIG. 1. **(a)** Linkage disequilibrium (LD) blocks of the dopamine beta hydroxylase (DBH) gene. The numbers marked in the cells were pairwise r^2. Dark gray represents ''stong evidence of LD,'' light gray represents ''uninformative,'' and white represents ''stong evidence of recombination.'' **(b)** Haplotypes and estimated frequency of the two LD blocks of the DBH gene.

Table 2. Demographic and Clinical Features of the Subjects According to Differential Response Status

	Response[a]				*Robust response*[b]				*Remission*[c]			
Features	*Yes* (n = *64*)	*No* (n = *23*)	χ^2/t	p *value*	*Yes* (n = *45*)	*No* (n = *42*)	χ^2/t	P *value*	*Yes* (n = *49*)	*No* (n = *38*)	χ^2/t	p *value*
Male, *n* (%)	53 (82.8)	19 (82.6)	0.000	1.000	38 (84.4)	34 (81.0)	0.186	0.667	41 (83.7)	31 (81.6)	0.066	0.798
Age, mean (SD)	9.1 ± 2.3	8.5 ± 2.0	0.785	0.438	9.3 ± 2.6	8.6 ± 1.8	1.046	0.302	9.4 ± 2.6	8.6 ± 1.8	1.121	0.269
IQ, mean (SD)	107.3 ± 16.1	97.7 ± 11.5	1.855	0.072	108.6 ± 16.2	100.3 ± 13.8	1.735	0.091	108.7 ± 16.0	100.2 ± 13.9	1.781	0.083
ADHD-Rating Scale, mean (SD)[d]												
Total	31.1 ± 8.2	33.1 ± 9.5	0.968	0.336	31.0 ± 8.4	32.4 ± 8.7	0.791	0.431	28.5 ± 7.9	35.7 ± 7.6	4.252	0.000
Inattention	17.9 ± 3.6	18.7 ± 4.2	0.790	0.432	18.1 ± 3.4	18.2 ± 4.1	0.097	0.923	17.5 ± 3.2	19.0 ± 4.2	1.778	0.080
Hyperactive-impulsive	13.2 ± 6.5	14.5 ± 6.8	0.806	0.422	12.9 ± 6.3	14.2 ± 6.9	0.972	0.334	11.0 ± 6.3	16.7 ± 5.5	4.421	0.000

[a]Response was defined as a decrease of at least 25% on the ADHD-RS-IV total score from baseline to the end of the trial.
[b]Robust response was defined as the decrease of ≥40% on the ADHD-RS-IV total score from baseline to the end of the trial.
[c]Remission was defined as the average ADHDRS-IV item score ≤1 at the end of the treatment.
[d]This indicated the baseline ratings.
IQ, intelligence quotient.

showed nominal significance or trend association (rs1076150, $p = 0.0349$; rs2873804, $p = 0.0665$; rs1548364, $p = 0.0564$; rs2519154, $p = 0.0047$; and rs1108580, $p = 0.0718$), with t rs2519154 still significant after 5000 permutations ($p = 0.0384$). The C allele was associated with being a nonresponder (93.6% vs. 77.6%, OR: 4.207, 95% CI: 1.465–12.076). Using remission criteria, 41 patients achieved remission, and 36 did not. rs2519154 and rs1076150 showed nominal significant or trend association with remission status (rs2519154, $p = 0.0479$; rs1076150, $p = 0.0969$), but none survived the 5000 permutation of multiple test correction ($p > 0.05$) (Table 3).

Haplotype association

Based on the current sample, we calculated blocks in which SNPs were in strong LD and might transfer to the next generation together. SNPs in a block could present the same trend of association with a phenotype as the causal variant. In this sample, we obtained two blocks. The LD plot and haplotype are illustrated in Figure 1a and b. Haplotypes of block 1 were nominally associated with responder status, with haplotype CTAC more prevalent in nonresponders (haplotype frequency: responders 76.4%, nonresponders 91.2%, $p = 0.0301$), whereas haplotype TCGT was more prevalent in responders (16.2% vs. 3.8%, $p = 0.0317$). These two haplotypes were also nominally associated with robust response (CTAC: 74.3% vs. 86.8%, $p = 0.0374$; TCGT: 18.6% vs. 6.8%, $p = 0.021$). One haplotype of block 2, GC, was nominally associated with robust response (23.3% vs. 11.3%, $p = 0.0377$). This haplotype was also nominally associated with remission (23.0% vs. 10.5%, $p = 0.0321$). But none achieved significance after a permutation test ($p > 0.05$) (Table 4).

Discussion

This study found SNPs and haplotypes of the DBH gene in association with atomoxetine response. Of the four associated SNPs (rs1076150, rs2873804, rs1548364, and rs2519154), rs2519154 survived the multiple test correction size. Variants in LD with a causal variant show elevated test statistics in association analysis. The trend association of the other SNPs and haplotypes in LD with rs2519154 suggested it to be a true association rather than inflation (Bulik-Sullivan et al. 2015).

ADHD was suggested to be an NE disorder (Biederman and Spencer 1999). NE neurons mainly originated from the locus coeruleus (LC) and projected to forebrain, cerebellum, and spinal cord (Cerbone and Sadile 1994). It has been widely acknowledged that ADHD children have functional alteration in the prefrontal cortex and cerebellum. LC cells have effects on the regulation of locomotor activity, attention and arousal, and information storage, as well as fear and anxiety (Mason 1981; Cerbone and Sadile 1994; Sadile 1996; Robbins et al. 1997). Low levels of NE reduce motivation and performance in learning tasks (Kobayashi et al. 2000).

DBH was the key enzyme in the biosynthesis process of NE; therefore, it was considered to be the candidate gene for ADHD susceptibility. Daly et al. (1999) first reported that the TaqI polymorphism in the fifth intron of DBH was associated with ADHD. Roman et al. (2002) and Smith et al. (2003) replicated this association. Hawi et al. (2003) performed haplotype analysis, and reported a haplotype containing that the A2 allele of the TaqI polymorphism was associated with ADHD. The meta-analysis of all the candidate genes for ADHD by Faraone et al. (2005) identified DBH to be one of the significant associated genes (OR = 1.33, 95% CI = 1.11–1.59).

One study investigated the DBH gene in association with MPH response in an adult sample (Contini et al. 2012), but no significant result was reported. They investigated seven genes; however, none got a significant result, even though some had been reported to be associated in previous studies. Because MPH had its effect mainly on the dopamine system, whereas atomoxetine played a major role on the adrenergic system, the response to atomoxetine might be more sensitive to the variability of DBH activity.

As a nonstimulant, the efficacy of atomoxetine has been well documented. It is a selective NE reuptake inhibitor. Although atomoxetine were considered to be effective and safe, there is considerable interindividual variability of the medication response among patients (Greenhill et al. 1996; Vaughan and Kratochvil 2006). Clinical treatment often used a trial and error approach, and gradual titration to the optimal dosage. We searched the literature and found only two studies investigating the genetic association of atomoxetine response. Ramoz et al. (2009) first investigated the SLC6A2 and CYP2D6 genes. The genomic regions across exon 2 and exon 4–9 of SLC6A2 were significantly associated with atomoxetine response in two independent samples. No association was found for the CYP2D6 gene. Another study was performed by our group. We found that rs3785143 in SLC6A2 had significant association with responder status, whereas rs2279805 was associated with remission status (Yang et al. 2012). The former SNP was

Table 3. Association of 8 SNPs in DBH Gene with Atomoxetine Response

		Response[a]					*Robust response*[b]					*Remission*[c]				
SNPs	*Alleles*	*Yes* (n=)	*No*	χ^2	p *value*	*Permutation* p *value*	*Yes*	*No*	χ^2	p *value*	*Permutation* p *value*	*Yes*	*No*	χ^2	p *value*	*Permutation* p *value*
rs1076150	T	108 (84.4%)	44 (95.7%)	3.896	0.0484	0.295	74 (82.2%)	78 (92.9%)	4.449	0.0349	0.2188	82 (83.7%)	70 (92.1%)	2.755	0.0969	0.5548
	C	20 (15.6%)	2 (4.3%)				16 (17.8%)	6 (7.1%)				16 (16.3%)	6 (7.9%)			
rs1611115	C	15 (11.7%)	6 (13.0%)	0.056	0.813	1	79 (87.8%)	74 (88.1%)	0.004	0.9488	1	84 (85.7%)	69 (90.8%)	1.039	0.3081	0.9536
	T	113 (88.3%)	40 (87.0%)				11 (12.2%)	10 (11.9%)				14 (14.3%)	7 (9.2%)			
rs1108580	C	102 (79.7%)	42 (91.3%)	3.2	0.0736	0.4256	70 (77.8%)	74 (88.1%)	3.241	0.0718	0.4522	78 (79.6%)	66 (86.8%)	1.577	0.2092	0.8442
	T	26 (20.3%)	4 (8.7%)				20 (22.2%)	10 (11.9%)				20 (20.4%)	10 (13.2%)			
rs2873804	T	97 (77.0%)	42 (91.3%)	4.457	0.0348	0.2324	68 (75.6%)	71 (86.6%)	3.367	0.0665	0.3796	76 (77.6%)	63 (85.1%)	1.564	0.2111	0.8448
	C	29 (23.0%)	4 (8.7%)				22 (22.4%)	11 (14.9%)				22 (24.4%)	11 (13.4%)			
rs1548364	A	99 (77.3%)	42 (91.3%)	4.291	0.0383	0.2348	68 (75.6%)	73 (86.9%)	3.641	0.0564	0.3608	76 (77.6%)	65 (85.5%)	1.772	0.1832	0.7982
	G	29 (22.7%)	4 (8.7%)				22 (24.4%)	11 (13.1%)				22 (22.4%)	11 (14.5%)			
rs2519154	C	91 (81.2%)	41 (97.6%)	6.684	0.0097	0.0926	59 (77.6%)	73 (93.6%)	8.006	0.0047	0.0384	66 (80.5%)	66 (91.7%)	3.913	0.0479	0.3578
	T	21 (18.8%)	1 (2.4%)				17 (22.4%)	5 (6.4%)				16 (19.5%)	6 (8.3%)			
rs2073837	A	63 (50.0%)	23 (52.3%)	0.067	0.7952	1	41 (46.6%)	43 (52.4%)	0.581	0.446	1	45 (47.9%)	39 (51.3%)	0.199	0.6553	1
	G	63 (50.0%)	21 (47.7%)				47 (53.4%)	39 (47.6%)				49 (52.1%)	37 (48.7%)			
rs129882	T	39 (30.5%)	19 (41.3%)	1.788	0.1812	0.837	28 (31.1%)	30 (35.7%)	0.414	0.5198	1	29 (29.6%)	29 (38.2%)	1.413	0.2345	0.8728
	C	89 (69.5%)	27 (58.7%)				62 (68.9%)	54 (64.3%)				69 (70.4%)	47 (61.8%)			

[a]Response was defined as a decrease of at least 25% on the Attention-Deficit/Hyperactivity Disorder Rating Scale-IV (ADHD-RS-IV) total score from baseline to the end of the trial.
[b]Robust response was defined as the decrease of ≥40% on the ADHD-RS-IV total score from baseline to the end of the trial.
[c]Remission was defined as the average ADHDRS-IV item score ≤1 at the end of the treatment.
SNP, single nucleotide polymorphism; DBH, dopamine beta hydroxylase.

TABLE 4. ASSOCIATION OF HAPLOTYPES IN DBH GENE WITH ATOMOXETINE RESPONSE

		Response[a]					*Robust response*[b]					*Remission*[c]				
Haplotypes	*Haplotype frequency*	*Yes*	*No*	χ^2	p *value*	*Permutation* p *value*	*Yes*	*No*	χ^2	p *value*	*Permutation* p *value*	*Yes*	*No*	χ^2	p *value*	*Permutation* p *value*
Block 1																
CTAC	0.803	97.8 (76.4%)	42.0 (91.2%)	4.702	0.0301	0.209	66.9 (74.3%)	72.9 (86.8%)	4.334	0.0374	0.2296	65.0 (85.5%)	74.8 (76.4%)	2.244	0.1341	0.7026
TCGT	0.129	20.7 (16.2%)	1.7 (3.8%)	4.613	0.0317	0.228	16.7 (18.6%)	5.7 (6.8%)	5.329	0.021	0.1406	6.7 (8.9%)	15.7 (16.0%)	1.962	0.1613	0.7702
TCGC	0.043	5.3 (4.1%)	2.3 (4.9%),	0.047	0.8287	1	3.3 (3.7%)	4.3 (5.1%)	0.211	0.6459	1	3.3 (4.3%)	4.3 (4.4%)	0.001	0.9807	1
CCGC	0.017	3.0 (2.3%)	0.0 (0.0%),	1.097	0.2949	0.9606	2.0 (2.2%)	1.0 (1.2%)	0.273	0.6014	1	1.0 (1.3%)	2.0 (2.0%)	0.133	0.7156	1
Block 2																
AC	0.491	63.0 (49.2%)	22.5 (48.9%)	0.002	0.9663	1	41.0 (45.6%)	44.5 (52.9%)	0.949	0.3299	0.9524	39.0 (51.3%),	46.5 (47.4%)	0.26	0.6104	1
GT	0.333	39.0 (30.5%)	19.0 (41.3%),	1.788	0.1812	0.837	28.0 (31.1%)	30.0 (35.7%),	0.414	0.5198	1	29.0 (38.2%)	29.0 (29.6%)	1.413	0.2345	0.8728
GC	0.175	4.5 (9.8%)	26.0 (20.3%)	2.566	0.1092	0.5726	21.0 (23.3%)	9.5 (11.3%)	4.32	0.0377	0.2356	8.0 (10.5%)	22.5 (23.0%)	4.594	0.0321	0.2124

[a]Response was defined as a decrease of at least 25% on the Attention-Deficit/Hyperactivity Disorder Rating Scale-IV (ADHD-RS-IV) total score from baseline to the end of the trial.
[b]Robust response was defined as the decrease of ≥40% on the ADHD-RS-IV total score from baseline to the end of the trial.
[c]Remission was defined as the average ADHDRS-IV item score ≤1 at the end of the treatment.
DBH, dopamine beta hydroxylase.

located in the sixth intron of SLC6A2. It was among the region of exons 4–9. Therefore, our result was consistent with Ramoz's. In the Yang et al. (2012) study, we also reported a haplotype with two SNPs at ADRA2A (rs1800544 and rs553668) in association with nonremission of ADHD symptoms after atomoxetine treatment.

There was no previous study investigating the association between DBH and atomoxetine response. Given the results in animals, namely that some effective antidepressants for ADHD via the NE system had no effect in DBH KO mice (Cryan et al. 2001), it is reasonable that functional variants in DBH gene might interfere with the response in human beings. The significant associated SNP rs2519154 of this study was located in the intron of the DBH gene (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2519154). Although it does not change the structure of the DBH enzyme, it is a regulatory SNP, which locates in the protein binding sites of encoded RNA, and has a distal regulation effect (http://rsnp.psych.ac.cn/quickSearch.do). The relationship of this SNP and the activity of the DBH enzyme need further research.

The limitations of this study included that the sample size was small; therefore many nominally associated SNPs and haplotypes of the DBH gene had not achieved significance after permutation correction. Validation of this result in large samples appears to be necessary.

Conclusions

Variants in the DBH gene, especially rs2519154, were associated with atomoxetine response in the treatment of ADHD. Given the small sample size, we still could not exclude a random association, and further replication in larger samples would be warranted.

Clinical Significance

This study suggested that DBH rs2519154 polymorphism was associated with the treatment response to atomoxetine in children and adolescents with ADHD. This SNP might be used as a predictor of atomoxetine response. Patients with the C allele were more likely to be nonresponders. The mechanism of how variants of the DBH gene moderate the atomoxetine response needs further research.

Disclosures

No competing financial interests exist.

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