Progress in Neuro-Psychopharmacology & Biological Psychiatry 43 (2013) 140–145

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

The DOPA decarboxylase (*DDC*) gene is associated with alerting attention

Bi Zhu [a], Chuansheng Chen [b,*], Robert K. Moyzis [c], Qi Dong [a,**], Chunhui Chen [a], Qinghua He [a], Jin Li [a], Jun Li [a], Xuemei Lei [a], Chongde Lin [a]

[a] State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, China
[b] Department of Psychology and Social Behavior, University of California, Irvine, CA, USA
[c] Department of Biological Chemistry and Institute of Genomics and Bioinformatics, University of California, Irvine, CA, USA

ARTICLE INFO

Article history:
Received 18 October 2012
Received in revised form 23 December 2012
Accepted 23 December 2012
Available online 29 December 2012

Keywords:
Alerting
Attention network test
Dopa decarboxylase gene
Haplotype

ABSTRACT

DOPA decarboxylase (*DDC*) is involved in the synthesis of dopamine, norepinephrine and serotonin. It has been suggested that genes involved in the dopamine, norepinephrine, and cholinergic systems play an essential role in the efficiency of human attention networks. Attention refers to the cognitive process of obtaining and maintaining the alert state, orienting to sensory events, and regulating the conflicts of thoughts and behavior. The present study tested seven single nucleotide polymorphisms (SNPs) within the *DDC* gene for association with attention, which was assessed by the Attention Network Test to detect three networks of attention, including alerting, orienting, and executive attention, in a healthy Han Chinese sample ($N = 451$). Association analysis for individual SNPs indicated that four of the seven SNPs (*rs3887825, rs7786398, rs10499695*, and *rs6969081)* were significantly associated with alerting attention. Haplotype-based association analysis revealed that alerting was associated with the haplotype G–A–T for SNPs *rs7786398–rs10499695–rs6969081*. These associations remained significant after correcting for multiple testing by max(T) permutation. No association was found for orienting and executive attention. This study provides the first evidence for the involvement of the *DDC* gene in alerting attention. A better understanding of the genetic basis of distinct attention networks would allow us to develop more effective diagnosis, treatment, and prevention of deficient or underdeveloped alerting attention as well as its related prevalent neuropsychiatric disorders.

1. Introduction

Attention is a central cognitive process involved in selectively concentrating on a certain aspect of the environment. Research on attention has potential applications in many areas, including education, human factors engineering, and treatment and rehabilitation of pathological conditions (Raz and Buhle, 2006). According to Posner and colleagues, there are three attention networks including alerting, orienting, and executive attention that can be measured by the attention network test (ANT) (Fan et al., 2002; Posner and Petersen, 1990). Alerting refers to the ability to achieve and maintain response readiness in preparation for incoming stimuli. Orienting is the ability to select specific information from sensory stimuli. Executive attention involves the mechanism for monitoring and resolving conflicts among thoughts, feelings and responses.

The ANT is a suitable task for detecting the neural and genetic correlates of three attention networks (Fossella et al., 2002). Previous twin studies suggested that the heritability of the three attention networks ranged from 0 to .72 (i.e., low for orienting, moderate for alerting, and high for executive function) (Fan et al., 2001; Wang et al., 2012). The three attention networks showed distinct neural networks, and are differentially related to specific neurotransmitters. Alerting is associated with activation of brain areas in the locus coeruleus, right frontal lobe and right parietal lobe; and it is influenced by the level of norepinephrine, also known as noradrenaline (Oberlin et al., 2005; Witte and Marrocco, 1997). Orienting is associated with activation of brain areas in the superior colliculus, superior parietal lobe, temporal parietal junction, and frontal eye fields; and it is influenced by acetylcholine (Thiel et al., 2005). Finally, executive attention is related to activation of brain areas in the prefrontal cortex, anterior cingulate, and basal ganglia; and it is modulated by dopamine (Posner, 2008; Raz and Buhle, 2006).

Abbreviations: DDC, DOPA decarboxylase; ANT, attention network test; *DRD4*, dopamine D4 receptor; *MAOA*, monoamine oxidase a; *TPH 2*, tryptophan hydroxylase 2; *COMT*, catechol-o-methyltransferase; *DAT 1*, dopamine transporter; ADHD, attention-deficit/hyperactivity disorder; AADC, aromatic L-amino acid decarboxylase; L-DOPA, L-dihydroxyphenylalanine; IMAGE, International Multicenter ADHD Genetics; LD, linkage disequilibrium; MAF, Minor allele frequency; HWE, Hardy–Weinberg equilibrium; LSD, least significant difference; CHB, Han Chinese in Beijing, China; CHD, Chinese in Metropolitan Denver, Colorado; JPT, Japanese in Tokyo, Japan; CEU, Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH) collection; ASW, African ancestry in Southwest USA.

* Correspondence to: C. Chen, Psychology and Social Behavior; 4201 Social & Behavioral Sciences Gateway, University of California, Irvine; Irvine, CA 92697-7085, USA. Tel.: +1 949 824 4184; fax: +1 949 824 3002.
** Correspondence to: Q. Dong, State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, 100875, Beijing, China.
E-mail addresses: cschen@uci.edu (C. Chen), psydongqi@126.com (Q. Dong).

0278-5846/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pnpbp.2012.12.020

Several previous studies detected associations between various genes with relevance for neurotransmission and specific attention networks using the ANT. For example, in a study of 200 normal adults, Fossella et al. (2002) found that polymorphisms in the *dopamine D4 receptor* (*DRD4*) gene and the *monoamine oxidase a* (*MAOA*) gene were related to efficiency of executive attention. Other researchers found associations between the executive attention network and the *tryptophan hydroxylase 2* (*TPH 2*) gene in normal subjects (Reuter et al., 2007), and between executive attention and the *catechol-o-methyltransferase* (*COMT*) Val108/158Met polymorphism in schizophrenic males (Opgen-Rhein et al., 2008). In addition, the *dopamine transporter* (*DAT 1*) gene showed a tendency for association with both alerting and executive attention in children with attention-deficit/hyperactivity disorder (ADHD), but these results did not survive the multiple testing corrections (Konrad et al., 2010).

The DOPA decarboxylase gene (*DDC*), which encodes the enzyme aromatic L-amino acid decarboxylase (AADC), is a very interesting candidate gene for mental activities and mental disorders, since it is involved in several neurotransmitter pathways (Borglum et al., 1999). The encoded protein catalyzes the conversion of L-dihydroxyphenylalanine (L-DOPA) to dopamine and of L-5 hydroxytryptophan to serotonin and L-tryptophan to tryptamine. Moreover, further downstream in the catecholamine pathway, dopamine transported into vesicles then converted to norepinephrine by dopamine β-hydroxylase. Although DDC is not a rate-limiting enzyme in the synthesis of dopamine, norepinephrine and serotonin, it is regulated at both pre- and post-translational levels (Ma et al., 2005). The *DDC* gene is located on the short arm of chromosome 7 and contains 15 exons and 14 introns.

Many previous studies showed that genetic variations in the *DDC* gene were significantly associated with ADHD, especially its inattentive symptoms. ADHD is a highly heritable and prevalent psychiatric disorder characterized by severe impairment in attention, hyperactivity and impulsivity (Banaschewski et al., 2010). Researchers reported that the *DDC* gene was strongly associated with ADHD in both childhood and adulthood (Ribases et al., 2009). A genome-wide association study also supported the association between the *DDC* gene and inattentive ADHD using over 900 ADHD Caucasian proband-parent trios (i.e., International Multicenter ADHD Genetics [IMAGE] project dataset) (Lasky-Su et al., 2008). Moreover, this association was also confirmed in a Chinese Han sample (Guan et al., 2009). There were also a few non-significant or only trend results reported such as an earlier analysis using a subsample of patients from the IMAGE sample (Brookes et al., 2006) or a study with a relatively small sample size (Hawi et al., 2001).

Thus, the *DDC* gene may act as a susceptibility gene for attention inefficiency. As addressed earlier, the *DDC* gene is involved with three important neurotransmitter systems (i.e., dopamine, norepinephrine, and serotonin), and these neurotransmitters are related to the attention networks. However, to date no study has examined possible associations between *DDC* variants and the attention networks. In this study, we analyzed seven SNPs selected to cover the whole *DDC* gene to explore whether there exists an association of the *DDC* gene with specific attention network inefficiency.

2. Method

2.1. Participants

451 healthy Han Chinese undergraduates were recruited (mean age = 19.95 years, *SD* = .89, range 18–22 years old; 57% female). The present sample included all subjects who completed the ANT from the Gene–Brain–Behavior Project at Beijing Normal University in China. All subjects were Han Chinese with normal or corrected-to-normal vision and had no neurological or psychiatric history based on their self-report[1]. They all signed written informed consent. This study was approved by the Institutional Review Board (IRB) of Beijing Normal University, China.

2.2. Genotyping

A 4 ml venous blood sample was collected from each subject. Genomic DNA was extracted according to standard methods within two weeks after the blood sample was collected. All samples were genotyped using the standard Illumina GoldenGate Genotyping protocol (see www.southgene.com.cn for details).

As depicted in Fig. 1 and Table 1, seven SNPs in the *DDC* gene on chromosome 7 were selected based on the HapMap data (www.hapmap.org), including *rs11238131, rs11238133, rs3887825, rs3807566, rs7786398, rs10499695*, and *rs6969081*. All seven SNPs met the criteria of a call rate of > 95%, Minor Allele Frequency (MAF) of > 0.05, and Hardy–Weinberg equilibrium (HWE) of $p > 0.05$. The allele frequencies in our sample were very similar to those of the Chinese in the HapMap dataset (see Table 1 and supplemental materials Figs. S2–S6). In order to sample the genetic diversity of the *DDC* gene, we selected the tag SNPs (tSNPs) defined by the HapMap project (www.hapmap.org [phase 3]), which are the minimum set of SNPs needed to sample most genetic diversity through linkage disequilibrium (LD). The tSNPs were defined by HapMap in 2007 using the four populations investigated at that time (European, African-Yoruban, Chinese, and Japanese ancestry), and a general r^2 value of 0.8 was used for identification. These seven SNPs were chosen to cover 84% of LD blocks in the *DDC* gene, as defined for the Chinese sample included in the HapMap Project (http://www.hapmap.org) and in the 1000 Genomes Project (http://browser.1000genomes.org). For SNPs *rs11238131* and *rs11238133*, there was one subject with missing genotype data in the current study.

2.3. Behavioral assessment

The attention network test (ANT) assesses the abilities of alerting, orienting, and executive function (see Fan et al., 2002, and Figure S1 for details). Subjects were seated 65 cm in front of the 17-in. computer screen, and responses were collected via two response buttons on the keyboard. They saw several arrows on the computer screen and had to decide the direction of the arrow in the middle. RT (ms) and performance accuracy (almost 100% accuracy in our study [mean = .98, *SD* = .02]) were recorded. It was a 4 × 2 × 3 × 2 design. The four factors were cue (no-cue, center-cue [i.e., a cue appeared in the middle], double-cue [i.e., two cues appeared up and down], spatial-cue [i.e., a cue appeared up or down]); the position of the target arrow (up or down); the direction of the arrows around the target arrow (congruent, incongruent, neutral); and the direction of the target arrow (right or left). The ANT consisted of a 24-trial practice block and two 96-trial experimental blocks. The alerting effect was calculated by subtracting the mean RT of the double-cue conditions from that of the no-cue conditions. The orienting effect was calculated by subtracting the mean RT of the spatial cue conditions (i.e., cues appeared up or down) from that of the center

[1] Although we did not administer a systematic battery of objective assessments for neurological diseases and mental disorders, the larger project included measures of two main mental health issues relevant to college students: depression and anxiety. They were measured with Beck's depression and anxiety inventories: BDI-II and BAI (Beck & Steer, 1990; Beck et al., 1996). According to the cutoff scores and interpretive labels provide by Beck and Steer (1990) and Beck et al. (1996), three subjects scored in the "severe" range (> 29) of BDI-II and 24 subjects scored in the "severe" range (> 26) of BAI. Because depression and anxiety were not significantly correlated with any indices of the attention network test in the current data, we did not exclude these subjects from our analyses. In addition, we administered Wechsler Adult Intelligence Scale-Revised Chinese version (WAIS-RC) (Gong, 1992). Mean IQ was 126 (S.D. = 8), with a range of 101 to 147. IQ was significantly associated with orienting attention ($r = .15$, $p = .001$), but not with either alerting ($r = .04$, $p = .41$) or executive attention ($r = .05$, $p = .28$). Even after controlling for IQ, however, the same four SNPs in the *DDC* gene (see Results) remained to be significant correlates of alerting attention.

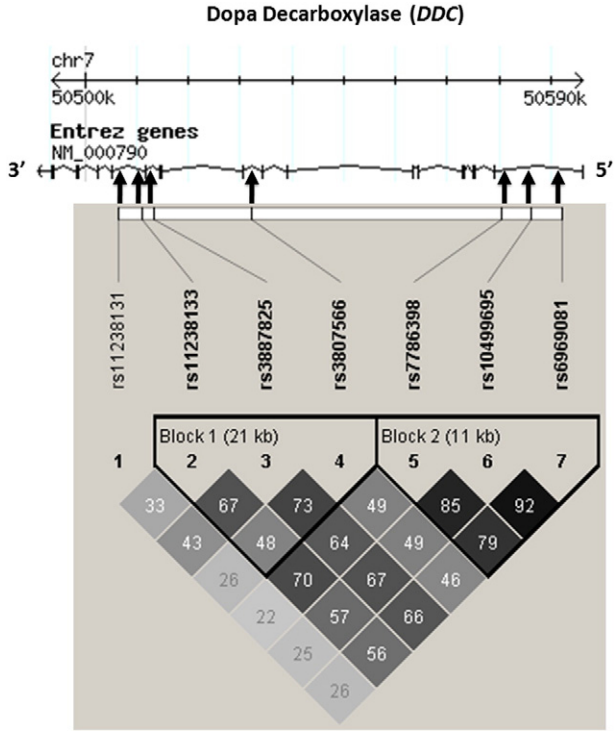

Fig. 1. Schematic representation of the *DDC* gene and linkage disequilibrium map of the seven SNPs used in the current sample. The *DDC* gene has 15 exons and 14 introns. Regions of high LD are shown in dark grey. Markers with lower LD are shown in light grey with the intensity decreasing with decreased r^2 value. Regions of low LD are shown in white. The numbers indicate the r^2 statistic value between the corresponding two SNPs.

cue (i.e., the cue appeared in the center). The executive effect was calculated by subtracting the mean RT of all congruent flanking conditions, summed across cue types, from the mean RT of the incongruent flanking conditions. It should be noted that alerting and orienting efficiency both represent benefits (i.e., decreases in RT), but executive represents the cost that is incurred (increased RT). Therefore, higher scores for indexes of alerting and orienting indicated higher abilities of alerting and orienting, but higher scores for the index of executive attention indicated poorer executive attention.

2.4. Data analyses

Quantitative trait genetic association analysis was carried out by using Plink v1.07 (Purcell et al., 2007), including allelic associations tests between individual SNPs and attention measures, and associations between haplotypes and attention measures. In order to test the group differences between different genotypes, ANOVA and the Fisher's least significant difference (LSD) post hoc tests (*t*-tests) were performed in SPSS 17.0. Pair-wise LD between all SNP markers was assessed using the Haploview 4.2 program (Barrett et al., 2005).

In a preliminary analysis, several separate two-way ANOVAs were conducted to test the effects of gender, each SNP, and their interaction on each attention measure. Results showed that the main effects of gender were non-significant: the *p* values were .33 for alerting, .50 for orienting, and .37 for executive scores. Perhaps due to the restricted age range, age was also not correlated with attention. Therefore, we did not include gender and age in subsequent analysis. The three attention measures (alerting, orienting, and executive scores) were analyzed individually. All significant associations were corrected for multiple testing by the max(T) permutations permutation approach in Plink (10000 permutation) for individual SNP analysis and haplotype-based association analysis considering all tests done for all three traits.

3. Results

In the current study, the mean scores for alerting, orienting, and executive attention were 6.67 ± 25.61, 33.52 ± 29.49, and 81.09 ± 36.02, respectively. Pair-wise correlations were not significant (i.e., $p > .05$), suggesting three independent attention networks as found in a previous study (Fossella et al., 2002). The mean reaction times for each condition in the ANT are shown in the supplementary materials Table S1.

Individual SNP analysis using Plink revealed significant associations for *rs3887825, rs3807566, rs7786398, rs10499695,* and *rs6969081* with alerting (see Table 2 for details). Except for *rs3807566*, the other four associations remained significant after correcting for multiple testing by max(*T*) permutation (corrected empirical *p*-value [max(*T*)/familywise]).

As shown in Fig. 1, the mean pair-wise r^2 values of seven SNPs within *DDC* were 0.57. According to the criteria of confidence intervals (Gabriel et al., 2002), two haplotype blocks across the *DDC* gene were revealed from the linkage disequilibrium (LD) data for these seven SNPs. The first block contained three SNPs *rs11238133, rs3887825*, and *rs3807566*, which covered 21 kb. The second block contained three other SNPs *rs7786398, rs10499695*, and *rs6969081*, which covered 11 kb.

Haplotype-based association analysis was performed for different combinations of SNPs within *DDC* in the current sample. As shown in Table 3, we found a major haplotype G-A-T (with a frequency of 52%) for *rs7786398–rs10499695–rs6969081* that showed a significant

Table 1
Allele frequencies of seven candidate SNPs in the *DDC* gene shown by ethnic groups. Data were from the present study and the HapMap data set (www.hapmap.org).

SNP	Base-pair position	Location	Reference/other allele[1]	Reference Allele frequencies					
				Present study Han Chinese	HapMap data				
					Chinese		Japanese	European	African
					CHB	CHD	JPT	CEU	ASW
rs11238131	50506083	Intron 11	G/A	0.47 ($N=450$)	0.56 ($N=168$)	0.46 ($N=168$)	0.51 ($N=170$)	0.69 ($N=226$)	0.58 ($N=106$)
rs11238133	50510408	Intron 11	C/A	0.49 ($N=450$)	0.54 ($N=168$)	0.49 ($N=170$)	0.62 ($N=172$)	0.67 ($N=226$)	0.68 ($N=106$)
rs3887825	50512587	Intron 10	G/A	0.40 ($N=451$)	0.45 ($N=168$)	NA	0.45 ($N=172$)	0.56 ($N=220$)	0.46 ($N=102$)
rs3807566	50531698	Intron 8	A/C	0.33 ($N=451$)	0.39 ($N=41$)	NA	0.34 ($N=43$)	0.35 ($N=57$)	NA
rs7786398	50580400	Intron 1	A/G	0.47 ($N=451$)	0.52 ($N=43$)	NA	0.60 ($N=44$)	0.51 ($N=56$)	NA
rs10499695	50586098	Intron 1	G/A	0.43 ($N=451$)	0.48 ($N=168$)	0.45 ($N=170$)	0.48 ($N=172$)	0.52 ($N=226$)	0.36 ($N=106$)
rs6969081	50591999	Intron 1	A/T	0.43 ($N=451$)	0.48 ($N=168$)	0.43 ($N=170$)	0.48 ($N=172$)	0.60 ($N=226$)	0.42 ($N=106$)

Note: On the HapMap Website (HapMap genome browser released 2 [phase 3]), these specific alleles of SNPs have different labels, due to different coding based on either the forward primer or the reverse primer. For example, the alleles of rs10499695 are A and G in the present study (the standard Illumina GoldenGate Genotyping protocol), but they are T and C on the HapMap Website; the alleles of rs11238131 are A and G in the present study (the standard Illumina GoldenGate Genotyping protocol), but they are T and C on the HapMap Website. In this table, we used the coding of all alleles based on the coding system of the Illumina system.
Population descriptors: CHB: Han Chinese in Beijing, China; CHD: Chinese in Metropolitan Denver, Colorado; JPT: Japanese in Tokyo, Japan; CEU: Utah residents with Northern and Western European ancestry from the CEPH collection; ASW: African ancestry in Southwest USA.

Table 2
Association between seven SNPs of the *DDC* gene and alerting attention.

SNP	Effective allele	Allelic test		maj	M	SD	*N*	het	M	SD	*N*	min	M	SD	*N*	LSD ($p<.05$)
		t	*p*													
rs11238131	G	1.39	.167	AA	4.20	(25.55)	121	AG	7.01	(25.27)	231	GG	8.96	(26.47)	98	
rs11238133	C	1.83	.068	AA	4.65	(26.45)	127	AC	5.65	(22.91)	209	CC	10.79	(28.90)	114	
rs3887825	G	2.80	.005	AA	4.19	(25.48)	164	AG	5.57	(24.80)	209	GG	15.10	(26.53)	78	AA<GG, AG<GG
rs3807566	A	2.39	.017	CC	4.16	(25.36)	205	AC	7.62	(25.37)	194	AA	13.42	(26.40)	52	CC<AA
rs7786398	A	2.61	.009	GG	3.86	(24.68)	135	AG	5.54	(24.44)	212	AA	12.83	(28.18)	104	GG<AA, AG<AA
rs10499695	G	2.73	.007	AA	3.82	(24.67)	149	AG	5.87	(24.85)	215	GG	13.78	(27.88)	87	AA<GG, AG<GG
rs6969081	A	2.60	.010	TT	3.47	(24.66)	147	AT	6.60	(25.57)	222	AA	12.85	(26.50)	82	TT<AA

Note: Significant *p*-values after correction for multiple comparisons by max(*T*) permutation are shown as underlined.
rs3887825, rs7786398, rs10499695, and rs6969081 remained significant after correcting for multiple testing by max(*T*) permutation (corrected empirical *p*-value [max(*T*)/familywise] = 0.020–0.031) maj = majority, het = heterozygote, min = minority. LSD = Fisher's least significant difference post hoc test.

inverse association with alerting ($t=-7.39$, $df=450$, $p=0.0068$). This association remained significant after correcting for multiple testing by max(*T*) permutation (corrected empirical *p*-value [max(*T*)/familywise] = 0.044). The haplotype A–G–A (with a frequency of 42%) for *rs7786398–rs10499695–rs6969081* showed a significant positive association with alerting ($t=6.06$, $df=450$, $p=0.0142$), but this association was no longer significant after multiple comparison correction. Additional haplotype analysis based on a different SNP combination (*rs11238133–rs3887825–rs3807566*) revealed a major C–G–A haplotype (with a frequency of 33%) in the current sample, which was positively associated with alerting ($t=5.08$, $df=450$, $p=0.0248$), but this association was no longer significant after correction.

No significant associations for seven SNPs in the *DDC* gene with orienting or executive attention measures were found ($p>.05$). In addition, the overall mean reaction time in ANT was 541 ± 67 ms. There were no significant differences among genotypes in the overall mean reaction time. The overall mean reaction time was significantly associated with orienting ($r=-0.11$, $df=449$, $p<0.05$), and executive attention ($r=0.27$, $df=449$, $p<0.05$), but not with alerting ($r=0.03$, $df=449$, $p=0.05$). Therefore, we also conducted individual SNPs analysis association with three attention networks, using the overall mean reaction time as a covariate. The same four SNPs in the *DDC* gene were still significantly associated with alerting attention after controlling for the covariate. There were no significant associations between the *DDC* gene and the orienting and executive attention measures with or without the covariate.

4. Discussion

In the present study, we chose seven SNPs in the *DDC* gene to investigate their genetic effects on three components of the attention network in normal Chinese adults. Results showed that four SNPs *(rs3887825, rs7786398, rs10499695* and *rs6969081)* were significantly associated with individual differences in the alerting efficiency of attention after max (*T*) permutation for multiple testing correction. Further, we identified a major haplotype G–A–T (with a frequency of 52%) for *rs7786398–rs10499695–rs6969081*, which showed a significant negative association with alerting, even after max (*T*) permutation for multiple testing correction. Moreover, two additional haplotypes were found to be positively related to alerting (i.e., A–G–A [with a frequency of 42%] for *rs7786398–rs10499695–rs6969081* and C–G–A [with a frequency of 33%] for *rs11238133–rs3887825–rs3807566*), which did not survive the correction. There was no association for the seven SNPs in the *DDC* gene with orienting and executive attention. These effects were independent of subjects' overall mean reaction time, age, and gender. These results further confirmed the critical role of the *DDC* gene in the alerting attention network.

Table 3
Association between major haplotype of the *DDC* gene and alerting attention.

rs11238133–rs3887825–rs3807566				rs7786398–rs10499695–rs6969081			
Haplotype	Frequency	*t*	*p*	Haplotype	Frequency	*t*	*p*
C–G–A	.33	5.08	.0248	G–A–T	.52	−7.39	*.0068*
A–A–C	.51	−3.74	.0537	A–G–A	.42	6.06	.0142
C–A–C	.09	−2.09	.1486	A–A–T	.04	−0.75	.7848
C–G–C	.07	.84	.3587				

Note: On the HapMap website, these specific SNPs alleles have different labels due to different coding based on either the forward primer or the reverse primer (see note to Table 1 for more details).
Significant *p*-value after max(*T*) permutation for multiple testing correction for the major haplotype is shown as underlined.

These findings can be integrated with multiple lines of human and animal research (including biochemical, pharmacological, genetic, and brain imaging studies) on the *DDC* gene, the related neurotransmitters, and specific attention networks. The enzyme of the *DDC* gene is required in the synthesis of dopamine, norepinephrine, and serotonin (Christenson et al., 1972). It is regulated at both pre- and post-translational levels (see Berry et al., 1996 for details). Three attention networks were related to three neurotransmitters respectively, alerting with norepinephrine, orienting with acetylcholine, and executive attention with dopamine. Pharmacological studies with human and monkeys showed that norepinephrine influences alerting but not orienting, while acetylcholine-related drugs influence orienting but not alerting (Posner, 2008). These findings may explain the association between the *DDC* gene and alerting but not orienting found in the current study.

Moreover, the *DDC* gene was found to be related to ADHD inattention symptoms (Guan et al., 2009; Lasky-Su et al., 2008; Ribases et al., 2009). There was direct evidence of altered DOPA decarboxylase activity in children and adults with ADHD using the positron emission tomography (Ernst et al., 1998, 1999). Using the ANT, several researchers reported alerting and executive attention deficits in children with ADHD (Johnson et al., 2008; Mullane et al., 2011). However, some other researchers suggested that the alerting network can best detect inattentive ADHD (Booth et al., 2007). For example, Booth et al. (2007) found that children with inattentive type ADHD showed a stronger alerting effect than those with the combined type ADHD, but no group differences were found in orienting and executive attention. A neuroimaging study also suggested that children with ADHD had a deficit in alerting, and this deficit was related to abnormal activities in the frontal and parietal brain regions (Cao et al., 2008). In line with the evidence presented above, the *DDC* gene was associated with alerting but not with orienting and executive attention in the current sample.

In the current study, we identified four SNPs (located in introns 1 and 10) and a three-marker haplotype (located in intron 1) in the *DDC* gene that were significantly related to alerting. These significant markers have not been used in previous studies. There were studies, however, linking markers in the same intron or adjacent location

with certain LD to ADHD and other disorders. For example, researchers reported that ADHD was associated with several genetic variations in the *DDC* gene such as polymorphisms on exon 1 (4 bp insertion), intron 3 (rs1466163), intron 8 (rs11575454) and intron 9 (rs6592952), and a haplotype block (rs11238131–rs6592961–rs1982406–rs2044859) on introns 1, 4, 6, 11 (Brookes et al., 2006; Guan et al., 2009; Hawi et al., 2001; Kirley et al., 2002; Ribases et al., 2009). Based on the LD data from the Asian samples in the HapMap Project and the 1000 Genomes Project, the four significant SNPs and the haplotype on introns 1 and 10 found in the current study are located in the LD blocks, which encompassed the associated SNPS reported in the previous studies about the *DDC* gene and attention-related phenotypes. Genetic variations located in intron 1 of the *DDC* gene were also found to be related to nicotine dependence (Ma et al., 2005; Yu et al., 2006), and interestingly nicotine could potentiate alerting attention in humans (Hahn et al., 2007; Thienel et al., 2009). In addition, the *DDC* gene has a high level of expression in the basal ganglia, which have been shown to modulate frontal-posterior connectivity involved in attention(van Schouwenburg et al., 2010).

Our analysis suggested that the particular haplotype (G–A–T) of rs7786398–rs10499695–rs6969081 located on intron 1 decreased alerting in the attention network test. Although these three SNPs are intronic and hence their biochemical functions are not straightforward, several previous studies have shed light on this genetic variant. One previous study found that a DNA sequence upstream of *DDC* possesses enhancer-link properties and is essential for normal neuron-specific expression in Drosophila (Johnson et al., 1989). Mutations that abolish the activity of the *DDC* gene have been described in a recessively inherited disease, called aromatic L-amino acid decarboxylase deficiency (Lee et al. 2009). As the second enzyme in the catecholamine biosynthetic pathway, the deficiency of the enzyme DOPA decarboxylase leads to a reduced level of downstream biogenic amines, including dopamine, norepinephrine, epinephrine and serotonin. Previous studies also described chromosomal aberrations in the region encompassing the *DDC* gene, e.g., a duplication (dup(7)p11.2–p12) associated with mild cognitive deficit (Leach et al. 2007). Taken together, these studies provided evidence that common variants in the *DDC* gene may be involved in the genetics of alerting.

Finally, given that *DDC* is involved in the synthesis of dopamine and subsequently norepinephrine and that dopamine is believed to modulate executive function while norepinephrine is believed to modulate alerting, it is somewhat counterintuitive that the *DDC* gene is linked only to alerting, not to executive function. There may be several explanations of this apparent paradox. First, the dopamine system is a complex system involving several subsystems and many genes. It is possible that different genes play different roles in modulating dopamine levels in different brain regions, which in turn affect different components of the attention network. For example, previous studies showed that *DRD4* VNTR was related to executive attention (e.g., Fossella et al., 2002). Second, we studied healthy young college students, who were likely to have normal executive function. Indeed, as Fig S7 shows, we had a restricted distribution of scores on executive function (but not for alerting and orienting), which might have contributed to our not finding an association between the *DDC* gene and executive function. It is plausible that when clinical samples such as children with ADHD who have a special deficit in executive attention are included (e.g., Johnson et al., 2008; Mullane et al., 2011), one may find an association between the *DDC* gene and executive function. Finally, because the relationship between the dopamine level and cognitive performance follows an "inverted-U-shaped" function (Cools and D'Esposito, 2011), complex patterns of associations between dopamine-related genes and cognitive performance occur depending on environmental factors as well as gene–gene and gene–environment interactions. Future research is needed to untangle these complex relationships.

Although the current findings add to the growing body of evidence for genetic bases of individual differences in attention (Fossella et al., 2002; Greenwood et al., 2005; Parasuraman et al., 2005), several limitations of this study need to be noted. First, the biochemical and physiological functions of the haplotype polymorphisms were not directly explored in the present study. Additional molecular functional studies are needed to investigate the details of mechanism of this association. Second, it should be noted that the current study is based on a healthy Han Chinese sample. Previous studies have inconsistent results about the racial specificity (e.g., African Americans versus European Americans) of the association between the *DDC* gene and certain behavioral phenotypes (Ma et al., 2005; Yu et al., 2006). The SNPs in the *DDC* gene have different minor allele frequency (MAF) and constructed different LD blocks in different ethnic populations based on the HapMap Data (www.hapmap.org, see Table 1 and Figs S2 to S6). In order to obtain confirmatory evidence, this association should be replicated in future studies using different ethnic samples. Third, although alerting is a particularly important component of attention, it is not well studied as compared to other components of attention, such as executive attention (Raz and Buhle, 2006). Finally, future studies should also map the *DDC* genetic variation of alerting attention onto brain activity.

5. Conclusions

In conclusion, this study provided the first evidence of the association between the *DDC* gene and alerting attention in healthy Chinese individuals and excluded several confounding factors including overall mean reaction time, age, and gender.

Acknowledgments

This study was supported by the 111 Project of the Ministry of Education of China (B07008) and the National Natural Science Foundation of China (grant 31200850).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.pnpbp.2012.12.020.

References

Banaschewski T, Becker K, Scherag S, Franke B, Coghill D. Molecular genetics of attention-deficit/hyperactivity disorder: an overview. Eur Child Adolesc Psychiatry 2010;19:237–57.
Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.
Beck AT, Steer RA. Manual for the Beck Anxiety Inventory. San Antonio, TX: Psychological Corporation; 1990.
Beck AT, Steer RA, Brown GK. Beck Depression Inventory-II. San Antonio, TX: The Psychological Corporation; 1996.
Berry MD, Juorio AV, Li XM, Boulton AA. Aromatic L-amino acid decarboxylase: a neglected and misunderstood enzyme. Neurochem Res 1996;21:1075–87.
Booth JE, Carlson CL, Tucker DM. Performance on a neurocognitive measure of alerting differentiates ADHD combined and inattentive subtypes: a preliminary report. Arch Clin Neuropsychol 2007;22:423–32.
Borglum AD, Bruun TG, Kjeldsen TE, Ewald H, Mors O, Kirov G, et al. Two novel variants in the DOPA decarboxylase gene: association with bipolar affective disorder. Mol Psychiatry 1999;4:545–51.
Brookes K, Xu X, Chen W, Zhou K, Neale B, Lowe N, et al. The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. Mol Psychiatry 2006;11:934–53.
Cao Q, Zang Y, Zhu C, Cao X, Sun L, Zhou X, et al. Alerting deficits in children with attention deficit/hyperactivity disorder: event-related fMRI evidence. Brain Res 2008;1219:159–68.
Christenson JG, Dairman W, Udenfriend S. On the identity of DOPA decarboxylase and 5-hydroxytryptophan decarboxylase (immunological titration-aromatic L-amino acid decarboxylase-serotonin-dopamine-norepinephrine). Proc Natl Acad Sci U S A 1972;69:343–7.
Cools R, D'Esposito M. Inverted-U-shaped dopamine actions on human working memory and cognitive control. Biol Psychiatry 2011;69:113–25.

Ernst M, Zametkin AJ, Matochik JA, Jons PH, Cohen RM. DOPA decarboxylase activity in attention deficit hyperactivity disorder adults. A [fluorine-18]fluorodopa positron emission tomographic study. J Neurosci 1998;18:5901–7.
Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Jons PH, Cohen RM. High midbrain [18F]DOPA accumulation in children with attention deficit hyperactivity disorder. Am J Psychiatry 1999;156:1209–15.
Fan J, McCandliss BD, Sommer T, Raz A, Posner MI. Testing the efficiency and independence of attentional networks. J Cogn Neurosci 2002;14:340–7.
Fan J, Wu Y, Fossella JA, Posner MI. Assessing the heritability of attentional networks. BMC Neurosci 2001:2-14.
Fossella J, Sommer T, Fan J, Wu Y, Swanson JM, Pfaff DW, et al. Assessing the molecular genetics of attention networks. BMC Neurosci 2002;3:14.
Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225–9.
Gong Y. The manual of Wechsler Adult Intelligence Scale Revised in China. China: Changsha Hunan Medical University Press; 1992.
Greenwood PM, Fossella JA, Parasuraman R. Specificity of the effect of a nicotinic receptor polymorphism on individual differences in visuospatial attention. J Cogn Neurosci 2005;17:1611–20.
Guan L, Wang B, Chen Y, Yang L, Li J, Qian Q, et al. A high-density single-nucleotide polymorphism screen of 23 candidate genes in attention deficit hyperactivity disorder: suggesting multiple susceptibility genes among Chinese Han population. Mol Psychiatry 2009;14:546–54.
Hahn B, Ross TJ, Yang Y, Kim I, Huestis MA, Stein EA. Nicotine enhances visuospatial attention by deactivating areas of the resting brain default network. J Neurosci 2007;27:3477–89.
Hawi Z, Foley D, Kirley A, McCarron M, Fitzgerald M, Gill M. Dopa decarboxylase gene polymorphisms and attention deficit hyperactivity disorder (ADHD): no evidence for association in the Irish population. Mol Psychiatry 2001;6:420–4.
Johnson KA, Robertson IH, Barry E, Mulligan A, Daibhis A, Daly M, et al. Impaired conflict resolution and alerting in children with ADHD: evidence from the Attention Network Task (ANT). J Child Psychol Psychiatry 2008;49:1339–47.
Johnson WA, McCormick CA, Bray SJ, Hirsh J. A neuron-specific enhancer of the Drosophila dopa decarboxylase gene. Genes Dev 1989;3:676–86.
Kirley A, Hawi Z, Daly G, McCarron M, Mullins C, Millar N, et al. Dopaminergic system genes in ADHD: toward a biological hypothesis. Neuropsychopharmacology 2002;27:607–19.
Konrad K, Dempfle A, Friedel S, Heiser P, Holtkamp K, Walitza S, et al. Familiality and molecular genetics of attention networks in ADHD. Am J Med Genet B Neuropsychiatr Genet 2010;153B:148–58.
Lasky-Su J, Neale BM, Franke B, Anney RJ, Zhou K, Maller JB, et al. Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. Am J Med Genet B Neuropsychiatr Genet 2008;147B:1345–54.
Leach NT, Chudoba I, Stewart TV, Holmes LB, Weremowicz S. Maternally inherited duplication of chromosome 7, dup(7)(p11.2p12), associated with mild cognitive deficit without features of Silver-Russell syndrome. Am J Med Genet A 2007;143A: 1489–93.
Lee HF, Tsai CR, Chi CS, Chang TM, Lee HJ. Aromatic L-amino acid decarboxylase deficiency in Taiwan. Eur J Paediatr Neurol 2009;13:135–40.
Ma JZ, Beuten J, Payne TJ, Dupont RT, Elston RC, Li MD. Haplotype analysis indicates an association between the DOPA decarboxylase (DDC) gene and nicotine dependence. Hum Mol Genet 2005;14:1691–8.
Mullane JC, Corkum PV, Klein RM, McLaughlin EN, Lawrence MA. Alerting, orienting, and executive attention in children with ADHD. J Atten Disord 2011;15:310–20.
Oberlin BG, Alford JL, Marrocco RT. Normal attention orienting but abnormal stimulus alerting and conflict effect in combined subtype of ADHD. Behav Brain Res 2005;165: 1-11.
Opgen-Rhein C, Neuhaus AH, Urbanek C, Hahn E, Sander T, Dettling M. Executive attention in schizophrenic males and the impact of COMT Val108/158Met genotype on performance on the attention network test. Schizophr Bull 2008;34:1231–9.
Parasuraman R, Greenwood PM, Kumar R, Fossella J. Beyond heritability: neurotransmitter genes differentially modulate visuospatial attention and working memory. Psychol Sci 2005;16:200–7.
Posner MI. Measuring alertness. Ann N Y Acad Sci 2008;1129:193–9.
Posner MI, Petersen SE. The attention system of the human brain. Annu Rev Neurosci 1990;13:25–42.
Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
Raz A, Buhle J. Typologies of attentional networks. Nat Rev Neurosci 2006;7:367–79.
Reuter M, Ott U, Vaitl D, Hennig J. Impaired executive control is associated with a variation in the promoter region of the tryptophan hydroxylase 2 gene. J Cogn Neurosci 2007;19:401–8.
Ribases M, Ramos-Quiroga JA, Hervas A, Bosch R, Bielsa A, Gastaminza X, et al. Exploration of 19 serotoninergic candidate genes in adults and children with attention-deficit/hyperactivity disorder identifies association for 5HT2A, DDC and MAOB. Mol Psychiatry 2009;14:71–85.
Thiel CM, Zilles K, Fink GR. Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. Neuropsychopharmacology 2005;30: 810–20.
Thienel R, Voss B, Kellermann T, Reske M, Halfter S, Sheldrick AJ, et al. Nicotinic antagonist effects on functional attention networks. Int J Neuropsychopharmacol 2009;12: 1295–305.
van Schouwenburg MR, den Ouden HE, Cools R. The human basal ganglia modulate frontal-posterior connectivity during attention shifting. J Neurosci 2010;30:9910–8.
Wang Z, Deater-Deckard K, Cutting L, Thompson LA, Petrill SA. Working memory and parent-rated components of attention in middle childhood: a behavioral genetic study. Behav Genet 2012;42:199–208.
Witte EA, Marrocco RT. Alteration of brain noradrenergic activity in rhesus monkeys affects the alerting component of covert orienting. Psychopharmacology (Berl) 1997;132:315–23.
Yu Y, Panhuysen C, Kranzler HR, Hesselbrock V, Rounsaville B, Weiss R, et al. Intronic variants in the dopa decarboxylase (DDC) gene are associated with smoking behavior in European-Americans and African-Americans. Hum Mol Genet 2006;15: 2192–9.