RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Interactions Between *MAOA* and *SYP* Polymorphisms were Associated with Symptoms of Attention—Deficit/Hyperactivity Disorder in Chinese Han Subjects

Qian Gao,[1,2] Lu Liu,[1,2] Hai-Mei Li,[1,2] Yi-Lang Tang,[3] Zhao-Min Wu,[1,2] Yun Chen,[1,2] Yu-Feng Wang,[1,2]* and Qiu-Jin Qian[1,2]*

[1]Peking University Sixth Hospital/Institute of Mental Health, Beijing, China

[2]Key Laboratory of Mental Health, Ministry of Health, Peking University, Beijing, China

[3]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia

Manuscript Received: 16 February 2014; Manuscript Accepted: 25 September 2014

As candidate genes of attention—deficit/hyperactivity disorder (ADHD), monoamine oxidase A (*MAOA*), and synaptophysin (*SYP*) are both on the X chromosome, and have been suggested to be associated with the predominantly inattentive subtype (ADHD-I). The present study is to investigate the potential gene–gene interaction (G × G) between rs5905859 of *MAOA* and rs5906754 of *SYP* for ADHD in Chinese Han subjects. For family-based association study, 177 female trios were included. For case-control study, 1,462 probands and 807 normal controls were recruited. The ADHD Rating Scale-IV (ADHD-RS-IV) was used to evaluate ADHD symptoms. Pedigree-based generalized multifactor dimensionality reduction (PGMDR) for female ADHD trios indicated significant gene interaction effect of rs5905859 and rs5906754. Generalized multifactor dimensionality reduction (GMDR) indicated potential gene–gene interplay on ADHD RS-IV scores in female ADHD-I. No associations were observed in male subjects in case-control analysis. In conclusion, our findings suggested that the interaction of *MAOA* and *SYP* may be involved in the genetic mechanism of ADHD-I subtype and predict ADHD symptoms.

© 2014 Wiley Periodicals, Inc.

Key words: ADHD; *MAOA*; *SYP*; gene; interaction

How to Cite this Article:
Gao Q, Liu L, Li H-M, Tang Y-L, Wu Z-M, Chen Y, Wang Y-F, Qian Q-J. 2015. Interactions Between *MAOA* and *SYP* Polymorphisms were Associated with Symptoms of Attention—Deficit/Hyperactivity Disorder in Chinese Han Subjects.
Am J Med Genet Part B 168B:45–53.

INTRODUCTION

Although there has been a rapid growth in exploration of the etiology of attention-deficit/hyperactivity disorder (ADHD) in the past decades, the complex mechanisms of ADHD remain poorly understood. Genetic influences play an important role in the neuropathology of ADHD, which is of a high heritability of approximately 0.76 [Faraone and Mick, 2010]. The existed findings from ADHD genetic studies are somewhat inconsistent and disappointing. Candidate gene studies have targeted various pathways of ADHD, including dopamine (DA), norepinephrine (NE), and serotonin (5-HT). However, the effect size of each risk variant is small [Faraone et al., 2005]. Up to date, no genome-wide association studies (GWAS) of ADHD have revealed any genetic variant passed the statistical threshold for genome-wide significance [Neale et al., 2008; Neale et al., 2010a, b; Yang et al., 2013]. However, some important findings in GWAS implicated genes participated in ADHD might be connected with process of neuronal plasticity

Grant sponsor: National Basic Research Development Program of China; Grant number: 973 program 2014CB846104; Grant sponsor: National Natural Sciences Foundation of China; Grant numbers: 81071109, 81301171; Grant sponsor: Program for New Century Excellent Talents in University; Grant number: NCET-11-0013.

*Correspondence to: Yu-Feng Wang, M.D., Ph.D., Peking University Sixth Hospital, 51, Huayuan Bei Road, Haidian District, Beijing 100191, China.
E-mail: wangyf@bjmu.edu.cn
**Correspondence to: Qiu-Jin Qian, M.D., Ph.D., Peking University Sixth Hospital, 51, Huayuan Bei Road, Haidian District, Beijing 100191, China.
E-mail: qianqiujin@bjmu.edu.cn

Article first published online in Wiley Online Library (wileyonlinelibrary.com)
DOI 10.1002/ajmg.b.32273

such as cell adhesion, cell division, neuron migration and synaptic plasticity [Yang et al., 2013].

There are higher rates of ADHD in males compared with females. Boys tend to be more hyperactive [Adler et al., 2008], while girls are usually more inattentive [Cuffe et al., 2005]. It has been hypothesized that this sex difference might be related to genes on the X chromosome, and our group have previously reported the association between two genes on X chromosome with ADHD including synaptophysin gene (*SYP*) and monoamine oxidase A (*MAOA*) [Guan et al., 2009; Liu et al., 2013].

SYP is localized to chromosome Xp11.23-p11.22, and is critical for the basic differentiation process of the neurons such as proliferation, fiber outgrowth and the formation of synapses. Researchers have suggested that the region of *SYP* may be associated with some other genetic diseases such Norrie disease [Sims et al., 1992], Wiskott–Aldrich syndrome [Cremin et al., 1993] as well as schizophrenia [Shen et al., 2012]. Studies have supported *SYP* as a candidate gene for ADHD. Two studies in Caucasians found that rs2293945 and rs5906754 were associated with ADHD-combined type (ADHD-C). Our group previously found that rs5906754 had a significant association with ADHD-inattentive subtype (ADHD-I) ($P = 0.048$) in a high density single-nucleotide polymorphism screen of 23 candidate genes [Guan et al., 2009]. Liu et al. also found the assocaiton of rs5906754 with ADHD-I ($P = 0.037$) in a larger sample, especially in male trios ($P = 0.015$); while in case-control studies, significant association was found for female ADHD ($P = 0.022$) [Liu et al., 2013].

MAOA maps to Xp11.23. Animal and human studies have revealed that *MAOA* gene may be related with externalizing behaviors, including impulsivity and aggression [Brunner et al., 1993; Cases et al., 1995; Manuck et al., 2000; Lawson et al., 2003], which are common symptoms in ADHD. With respect to gene polymorphisms, many studies have focused on the 30 bp upstream repeats (MAOA-uVNTR) which is a functional polymorphism in the promoter region of *MAOA*. Researches supported that the longer alleles (3.5R, 4R, and 5R) were more efficient in transcription than the shorter alleles (2R and 3R) [Deckert et al., 1999]. Association between this polymorphism and ADHD has been conflicting [Manor et al., 2002; Lawson et al., 2003; Domschke et al., 2005; Xu et al., 2007]. Brookes et al. found the association between SNPs of *MAOA* and ADHD-C subtype [Brookes et al., 2006] . While in our previous report, Guan et al. suggested an association between 12 SNPs of *MAOA* with ADHD, particularly with ADHD-I subtype [Guan et al., 2009].

In summary, our previous studies have suggested that genetic variations at *SYP* and *MAOA* may be associated with ADHD-I subtype, we set out in this study to test the hypothesis that the variants of *SYP* and *MAOA* would jointly affect the inattentiveness in ADHD.

MATERIALS AND METHODS

Subjects

Clinical samples. A total of 1,462 ADHD cases were recruited from child psychiatric clinics at the Peking University Sixth Hospital/Institute of Mental Health. From all ADHD cases, there were 177 female probands who, along with their parents, constituted trios to be analyzed for the family-based study. This work was approved by the Ethics Committee of Peking University Health Science Center.

Eligible cases in the study met inclusion criteria as follows: (1) met DSM-IV ADHD diagnostic, (2) age between 6 and 16 years, (3) had a full-scale IQ ≥ 70, and (4) both biological parents were of Chinese Han descent. Individuals with major neurological disorders, a diagnosis of schizophrenia, pervasive development disorder, epilepsy, mental retardation, or other brain disorders were excluded.

Control samples. A total of 807 unaffected controls were ascertained from healthy blood donors from the Blood Center of Peking University First Hospital, healthy volunteers at our Institute, and local elementary schools. All were of Chinese Han descent. The exclusion criteria for controls were ADHD, other major psychiatric disorders, family history of psychosis, severe physical diseases, and substance abuse (more details have been described in a previous publication of our group [Guan et al., 2009]).

Assessments and diagnosis. A clinical diagnosis was conducted by psychiatrists with the parents and their offspring together using the Clinical Diagnostic Interview Scale (CDIS) [Barkley, 1998]. The Chinese version of CDIS, which was translated by our group, showed excellent sensitivity (97.2%) and specificity (100%) [Yang et al., 2001; Yang et al., 2004]. The CDIS assessed the three DSM-IV subtypes of ADHD, including ADHD inattentive type (ADHD-I), ADHD hyperactive-impulsive type (ADHD-HI), and ADHD combined type (ADHD-C).

ADHD symptoms were evaluated using ADHD Rating Scale-IV (ADHD-RS-IV). It consisted of 18 items according to DSM-IV for ADHD. Each symptom was scored on how often it occurred (i.e., if "never" presented the symptom, rated as 0; if "occasionally," 1; "often," 2; and "always," 3. The score yielded by this instrument ranges from 0 (symptoms "never" occur) to 54 (all symptoms "always" occur) [Barkley et al., 2002].

SNP selection and genotyping. Previously, Liu et al. genotyped three SNPs of *SYP* (rs5906754/rs3817678/rs2293945) with strong linkage disequilibrium (LD) and rs5906754 indicated the smallest *P*-value, which was also in Guan's study [Guan et al., 2009; Liu et al., 2013]. Liu and Guan both included four SNPs of *MAOA* (rs5905859/rs3027400/rs2239448/rs1137070) and rs5905859 showed strong LD with another three SNPs and the smallest *P*-value [Guan et al., 2009; Liu et al., 2011]. Therefore, to maintain a proper power, given the relative small sample size and strong LD between SNPs of *MAOA* and *SYP* [Carlson et al., 2004], we judiciously chose these two sites. PCR conditions and quality control of rs5905859 and rs5906754 were shown as the reference [Liu et al., 2011; Liu et al., 2013]. The frequencies for rs5905859 and rs5906754 were shown in Table I.

Hardy–Weinberger equilibrium (HWE). We used the HAPLOVIEW version 4.0 to test HWE in females, and found no departure from HWE for two SNPs (all $P > 0.05$) for our data. Minor allele frequencies (MAFs) for two SNPs were 0.43 for rs5905859 and 0.36 for rs5906754, respectively.

Gene-gene interaction. Pedigree-based generalized multifactor dimensionality reduction method (PGMDR) was used to calculate the genetic interactions for family-based study [Lou et al., 2008], while generalized multifactor dimensionality reduction (GMDR) was used for case-control study [Lou et al., 2007].

TABLE I. Genotypic Frequencies of rs5905859 and rs5906754 in ADHD and Controls

	ADHD, n (%)			Control, n (%)		
	Male	Female	Total	Male	Female	Total
rs5905859						
AA	463 (38.1)	46 (18.7)	509 (34.8)	211 (41.6)	58 (19.3)	269 (33.3)
AC	—	113 (45.9)	113 (7.7)	—	142 (47.3)	142 (17.6)
CC	753 (61.9)	87 (35.4)	840 (57.5)	296 (58.4)	100 (33.3)	396 (49.1)
rs5906754						
CC	777 (63.9)	113 (45.9)	890 (60.9)	329 (64.9)	119 (39.7)	448 (55.5)
CT	—	114(46.3)	114 (7.8)	—	144 (48.0)	144 (17.8)
TT	439 (36.1)	19 (7.7)	458 (31.3)	178 (35.1)	37 (12.3)	215 (26.6)

ADHD, attention-deficit/hyperactivity disorder.

PGMDR and GMDR are score-based, nonparametric and model-free to linear or logistic regression method to detect and characterize nonlinear interactions among discrete genetic and environmental attributes which allowed for adjustment of covariates, handling of both continuous and dichotomous phenotypes and patterns of missing data [Lou et al., 2007; Lou et al., 2008].

We explored gene-gene interaction on ADHD presence using logistic regression score calculation, while linear score calculation for ADHD symptoms. Then we repeated above analyses by subgroup and gender. The significant interaction model was chosen according to the threshold of cross validation consistency (CVC) >8/10, prediction accuracy >50% and empirical *P*-value based on 1,000 permutation tests <0.05. Then we verified the significant interaction model from GMDR by analyses of covariance (ANCOVA) using SPSS 17.0.

RESULTS

Demographic and Clinical Features of the Sample

All the samples in our study have been used for earlier publication by Liu et al. [Liu et al., 2013] and 322 for [Guan et al. 2009]. To reduce the noise of missing data in GMDR, we just included subjects with complete genotype for both SNPs. In the present study, we covered 150 (82.4%) ADHD cases and 172 (93.5%) controls in Guan's study [Guan et al., 2009], while 1,462 ADHD (80.2% of [Liu et al. 2011] 86.9% of [Liu et al. 2013]) cases and 807 (84.3%) controls in Liu's research [Liu et al., 2011; Liu et al., 2013]. Many ADHD children met diagnostic criteria for other disorders: 31.9% had oppositional defiant disorder (ODD), 5.8% had conduct disorder (CD), 15.2% had tic disorder (TD), and 37.3% had learning disability (LD). Among these ADHD cases, there were 177 female probands who, along with their parents, constituted trios to be analyzed for family-based study. Demographic features of sample were shown in Tables II and III.

Family-Based Study

For X-chromosome genes, the current version of PGMDR is unable to do gene–gene interaction analysis for males, so we only analyzed in female ADHD trios. We firstly calculated the interaction effect of rs5905859 and rs5906745 on ADHD presence. Secondly, to explore the interaction effect on ADHD symptoms, we allowed age and ADHD subtype as covariates for adjustment in PGMDR for female ADHD trios. To reduce the heterogeneity caused by subtype, we further performed above analyses for three subtypes separately.

In this two-locus model (rs5905859-rs5906745), we found that the interplay had an effect on the presence for female ADHD (cross-validation consistency = 10/10, prediction accuracy = 58.82%, $P < 0.001$), but not for three subtypes. We did not detect significant interactions on ADHD RS-IV total scores, attentive scores or hyperactive/impulsive scores for each group (Table IV).

Case-Control Association

Logistic regression was used to assess the genotype distribution between ADHD or its three subtypes and controls. We transformed genotype into dummy variables, allowing homozygotes of minor allele as reference category. The results showed that there was no different genotype distribution of each SNP after controlling for potentially confounding factors including gender and age (Supplementary Table SI, data for dummy variables weren't shown, all $P > 0.05$). When we conducted gender specific analyses by controlling for age, there was still no significant association for genotypes but significant effect for age (data not shown).

TABLE II. Demographic and Clinical Characteristics of Female ADHD Trios for Family-Based Study

Group	N	Age (years) (mean ± SD)
ADHD	177	9.49 ± 2.41
ADHD-I	105	9.79 ± 2.32
ADHD-HI	7	7.80 ± 1.38
ADHD-C	65	9.17 ± 2.54

ADHD, attention-deficit/hyperactivity disorder.

TABLE III. Demographic and Clinical Characteristics of Subjects in the Case-Control Study

	All		Male		Female	
Group	N	Age (mean ± SD)	N	Age (mean ± SD)	N	Age (mean ± SD)
ADHD	1,462	10.15 ± 2.63	1,216	10.22 ± 2.63	246	9.84 ± 2.58
ADHD-I	750	10.76 ± 2.67	608	10.88 ± 2.68	142	10.23 ± 2.55
ADHD-HI	72	8.98 ± 2.48	64	9.18 ± 2.53	8	7.35 ± 1.11
ADHD-C	640	9.58 ± 2.41	544	9.59 ± 2.39	96	9.47 ± 2.55
Control	807	11.49[a]	507	18.00[a]	300	10.02[a]

ADHD, attention-deficit/hyperactivity disorder.
[a]aThe age of the control group was not normally distributed so the statistic presented is the median.

Thus we explored gene–gene interaction on ADHD presence adjusted for age and gender, and then analyzed by subtype and gender by GMDR. We evaluated potential interplay on ADHD RS-IV scores, allowing age, subtypes, and gender as covariates in ADHD cases, and then analyzed by subtypes and gender (Fig. 1).

We found that the interplay had a significant effect on ADHD RS-IV scores only in female ADHD-I (cross-validation consistency = 10/10, prediction accuracy = 56.91%, $P = 0.036$). We did not find interactions either on the presence or on ADHD RS-IV scores for ADHD, ADHD-HI, and ADHD-C group, even after sub-grouped by gender (Table V). For ADHD-HI, only males were analyzed because of the extremely small sample of females (n = 8).

According to the GMDR results, we divided female ADHD-I samples into low- and high-risk genotype combination group. When the subjects had AC genotypes of rs5905859 or TT of rs5906754 or AA of rs5905859 combined with CT of rs5906754 (light gray boxes shown as Fig. 2), we put them into the low-risk group, while others into the high-risk group. We carried on ANCOVA using ADHD RS-IV scores as dependent variables, age as covariate in female ADHD-I group. Compared to the low-risk group, the high-risk group showed a trend for having higher total symptom scores (27.88 ± 7.47 vs. 25.64 ± 8.07, $P = 0.089$).

DISCUSSIONS

The main finding from our analyses was that gene-gene interaction of *MAOA* rs5905859 and *SYP* rs5906754 may be a risk factor for ADHD and had influence on ADHD symptoms in Chinese Han female samples.

In family-based analyses, the two-locus model was found to be significantly associated with ADHD in female samples, while no significant interactions were found for analyses in subtypes. Although we failed to detect the association in case-control study, we did find the interaction of rs5905859 and rs5906754 was associated with ADHD symptoms measured by ADHD RS-IV scores in female ADHD-I. There was, a trend significant difference between high- and low-risk group (27.88 ± 7.47 vs. 25.64 ± 8.07, $P = 0.089$), in spite of the small sample size (n = 142). Guan et al. reported both rs5906754 and rs5905859 were associated with ADHD-I in 83 ADHD-I samples and 184 controls [Guan et al., 2009]. Liu et al. also found association of rs5906754 with ADHD-I in 570 trios, and

TABLE IV. rs5905859 by rs5906745 Interaction Models for Female ADHD, Female ADHD-I, Female ADHD-C by PGMDR

Group	Measure	Prediction accuracy (%)	Cross-validation consistency	*P*-value based on 1,000 permutations
Female ADHD	Presence	58.82	10	<0.001
	Attention score	49.80	10	0.463
	Hyperactive/impulsive score	40.96	10	0.967
	Total score	41.13	10	0.868
Female ADHD-I	Presence	59.07	10	0.377
	Attention score	41.89	10	1.000
	Hyperactive-impulsive score	44.34	10	0.986
	Total score	31.40	10	0.999
Female ADHD-C	Presence	57.26	10	0.172
	Attention score	56.20	10	0.423
	Hyperactive-impulsive score	46.25	10	0.802
	Total score	34.91	10	0.970

ADHD, attention-deficit/hyperactivity disorder. Empirical *P*-value <0.05 was indicated in bold.

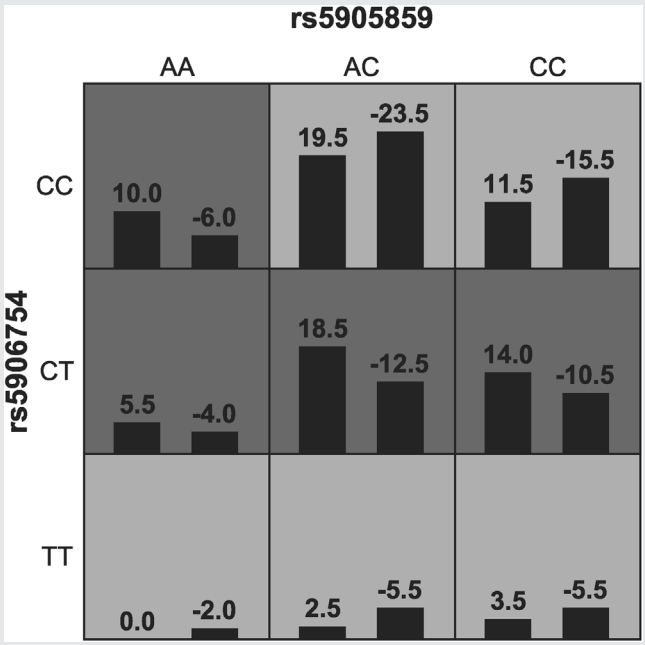

FIG. 1. The rs5905859 × rs5906754 interaction showed a significant association with ADHD presence ($P < 0.001$) in female ADHD trios by PGMDR. In each cell, the left bar represented a positive score, and the right bar a negative score. High-risk cells were indicated by dark shading and low-risk cells by light shading.

the association was also significant between female ADHD-I cases (n = 865) and female controls (n = 240) [Liu et al., 2013]. Consistent with our previous studies [Guan et al., 2009; Liu et al., 2013], rs5906754 and rs5905859 may act as independent candidate genes as well as components of gene–gene interaction in the etiology of ADHD especially of inattentive subtype. The result provided evidence for involvement of multiple gene variants of small effect size in ADHD [Faraone et al., 2005].

We also noted the inconsistency between family-based and case-control studies. In the family-based analyses, we found the interaction was significantly associated with ADHD in females but not in two subtypes (ADHD-I and ADHD-C); however, the significant interaction was only found in ADHD-I subtype from case-control analyses. The discrepancies in findings between family-based and case-control studies, which have been reported previously [Liu et al., 2013], may attribute to population stratification or the potential mismatch between case and control samples. Another inconsistency was the interaction effect on particular subtype. In our study, we found the interaction model was associated with ADHD in general and ADHD-I, but no significant association with ADHD-HI or ADHD-C subtypes. Our data implied the heterogeneity among three subtypes, which was pointed out by others that there were both shared and unique genetic influences among ADHD subtypes [Faraone et al., 1995; Carlson and Mann, 2000; Nikolas and Burt, 2010; Shang et al., 2011; Asherson and Gurling, 2012]. It may be worthwhile to explore subtypes separately in genetic studies of ADHD.

Another important finding of the current study was the differences between males and females. Despite we only analyzed in females samples in family-based study, interaction model in case-control study was found to be significantly associated with ADHD in females only, but not males. We are interested in X chromosome genes such as *MAOA* and *SYP* due to the biased ADHD prevalence between boys and girls [Gaub and Carlson, 1997; Ford et al., 2003] and their distinguished subtype distributions [Rhee et al., 1999; Carlson and Mann, 2000]. As gender difference of ADHD has been proposed from clinical, psychological, genetic, and animal studies, these findings raised the possibility that expression of X-linked genes might be associated with sex differences in vulnerability to ADHD [Davies, 2014]. One possible mechanism was X-linked genes might escape the process of X-inactivation [Davies, 2014]. Carrel et al. found about 15% of X-linked genes escaped inactivation, and these genes were found in different regions including Xp11, where *MAOA* and *SYP* were located [Carrel and Willard, 2005]. It was assumed that *MAOA* expression might partially escape X-inactivation in some tissues of human; however, the results of X-chromosome inactivation were inconclusive and where or when it escaped has not yet been identified [Trent and Davies, 2012]. Thus we anticipated genetic variation of *SYP* and *MAOA* might be involved in X-inactivation to some degree, and then showed gender difference in ADHD genetic model. Another possible explanation was that ADHD risk genes had sexually dimorphic effects, especially genes such as *MAOA*, which is located on X-chromosome, can directly affect sexual dimorphism independent of the influence of sex hormones [Skuse, 2006; Biederman et al., 2008]. On the other hand, hormones was proved to influence various serotonin functions in humans and animals, and may play an important role in the sex differences of psychiatric disorders such as autism (ASD) [Knickmeyer and Baron-Cohen, 2006; Wu et al., 2009], schizophrenia(SCZ), and mood disorders [Ozcan and Banoglu, 2003]. Studies suggested that disorders including autism and schizophrenia differed in incidence, symptomatology, and genetic mechanisms between males and females [Klein and Corwin, 2002; Shors, 2002; Levy et al., 2011; Sanders et al., 2011]. Our result provided promising insights into inconsistency between genders in ADHD genetic model.

In previous studies, *SYP* and *MAOA*, as independent factors, were reported to have an association with ADHD [Guan et al., 2009; Liu et al., 2013]. To our knowledge, this is the first report of genetic interaction between *SYP* and *MAOA* with ADHD and their prediction effect on ADHD symptoms. Genetic epidemiology has assembled convincing evidence that ADHD are substantially influenced by genetic factors which is highly complex, polygenic, and epistatic. For further exploration of biological function of interaction, *MAOA* and *SYP* may share some kind of functional relationship in neurotransmitters circulation. In pre-synaptic neurons, neurotransmitter release involves two partially overlapping cycles, the neurotransmitter cycle and the synaptic vesicles (SVs) cycle [Fon and Edwards, 2001]. The neurotransmitter cycle involves transmitter biosynthesis, storage, reuptake, and degradation. The SVs cycle involves targeting vesicles to the nerve terminal where docking, fusion, endocytosis, and recycling occur. MAO-A in the

TABLE V. rs5905859 by rs5906745 Interaction Models in Case-Control Study by GMDR

Group	Measure	Prediction accuracy (%)	Cross-validation consistency	*P*-value based on 1,000 permutations
ADHD	Presence	47.91	10	0.797
	Attention score	47.65	10	0.818
	Hyperactive-impulsive score	49.03	10	0.681
	Total score	47.11	10	0.888
Male ADHD	Presence	49.51	10	0.727
	Attention score	52.20	10	0.126
	Hyperactive-impulsive score	49.55	10	0.495
	Total score	49.74	10	0.495
Female ADHD	Presence	48.45	10	0.610
	Attention score	45.72	10	0.810
	Hyperactive-impulsive score	48.76	10	0.525
	Total score	47.84	10	0.641
ADHD-I	Presence	49.56	10	0.556
	Attention score	50.11	10	0.435
	Hyperactive-impulsive score	48.70	10	0.689
	Total score	50.31	10	0.496
Male ADHD-I	Presence	50.70	10	0.406
	Attention score	50.76	10	0.297
	Hyperactive-impulsive score	48.12	10	0.839
	Total score	49.16	10	0.558
Female ADHD-I	Presence	50.86	10	0.329
	Attention score	50.64	10	0.516
	Hyperactive-impulsive score	59.66	10	0.100
	Total score	56.91	10	0.036
ADHD-HI	Presence	47.87	10	0.597
	Attention score	50.54	10	0.500
	Hyperactive-impulsive score	53.94	10	0.139
	Total score	53.46	10	0.160
Male ADHD-HI	Presence	51.41	10	0.296
	Attention score	49.47	10	0.604
	Hyperactive-impulsive score	53.67	10	0.170
	Total score	52.33	10	0.255
ADHD-C	Presence	47.35	10	0.788
	Attention score	48.39	10	0.782
	Hyperactive-impulsive score	44.41	10	0.976
	Total score	44.69	10	0.979
Male ADHD-C	Presence	47.17	10	0.734
	Attention score	48.86	10	0.628
	Hyperactive-impulsive score	47.79	10	0.696
	Total score	46.52	10	0.825
Female ADHD-C	Presence	50.92	10	0.352
	Attention score	55.08	10	0.115
	Hyperactive-impulsive score	47.43	10	0.619
	Total score	47.65	10	0.625

ADHD, attention-deficit/hyperactivity disorder. Empirical *P*-value <0.05 was indicated in bold.

mitochondria plays an important role in the metabolism of monoamine neurotransmitter [Shih et al., 1999], while SYP participates in the formation of the synaptophysin/synaptobrevin (Syp/Syb, also named Syp/VAMP2) complex, which is mutually exclusive with the SNARE complex, and then potentially influences the fusion process [Valtorta et al., 2004]. In addition, synaptophysin is important to maintain the synaptic membrane integrity and promote the biogenesis of SV by its cholesterol-binding function [Thiele et al., 2000]. The role of these activities has remained unclear; however, genetic variants of *MAOA* and *SYP* might affect any of these steady steps and break balance in the concentration and signaling of neurotransmitter, which have been suggested to contribute to the development of ADHD [Scassellati et al., 2012].

There are several limitations needed to be acknowledged in our study. First, we only chose two SNPs of *MAOA* and *SYP* based on the current literature, and they only provided limited coverage of the

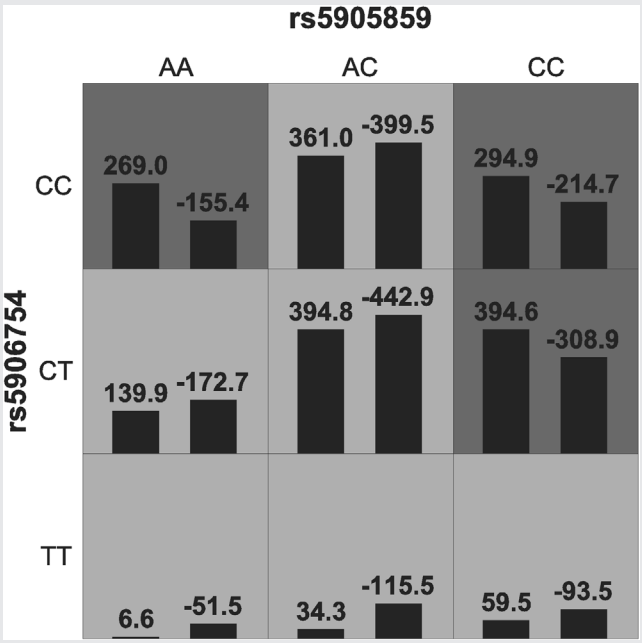

FIG. 2. The rs5905859 × rs5906754 interaction showed a significant association with ADHD RS-IV scores ($P = 0.036$) in female ADHD-I group after adjusted by age by GMDR. In each cell, the left bar represented a positive score, and the right bar a negative score. High-risk cells were indicated by dark shading and low-risk cells by light shading.

entire genes. Further exploring the interaction using GWAS or sequencing strategies may provide further understanding. Second, it is still unknown how many subjects are required to get a proper power for identifying gene–gene interactions in GMDR/PGMDR [Ritchie et al., 2003; Motsinger and Ritchie, 2006] and we only conducted analyses in females for family-based tests which was of relatively small sample size. Third, one of our main findings is for genetic interactions with female ADHD-I subtype in case-control study, but the sample size of female ADHD-I group was relatively small. This may be a common problem when we conduct analyses in subgroups. Fourth, cases and controls were not well matched with significant differences of gender and age. Although we have controlled these two confounding factors, well-matched samples will be better for future research to reduce the noise. In addition, the interaction model by GMDR/PGMDR was a statistic model and the biological significance remains unclear. Finally, we essentially used the same set of cases-control (probands) in both the family-based and case-control analyses, it would be more helpful to use another independent case-control sample to test the findings from the family-based analyses.

In conclusion, our study found a significant interaction effect of *MAOA* and *SYP* on female ADHD from family-based analyses, and the interaction model had an influence on ADHD symptoms in female ADHD-I from case-control study. These findings provide some evidence for multifactorial inheritance, subtype-specific, and gender-specific in pathophysiology of ADHD.

ACKNOWLEDGMENTS

The authors would like to thank the patients and their family members who participate in this study. We thank all the colleagues and students who helped collect the data. This study was partly funded by the National Basic Research Development Program of China (Grant number: 973 program 2014CB846104), the National Natural Sciences Foundation of China (Grant number: 81071109; 81301171), and the Program for New Century Excellent Talents in University (Grant number: NCET-11-0013).

REFERENCES

Adler LA, Faraone SV, Spencer TJ, Michelson D, Reimherr FW, Glatt SJ, Marchant BK, Biederman J. 2008. The reliability and validity of self- and investigator ratings of ADHD in adults. J Atten Disord 11(6):711–719.

Asherson P, Gurling H. 2012. Quantitative and molecular genetics of ADHD. Curr Top Behav Neurosci 9:239–272.

Barkley RA. 1998. Attention-deficit hyperactivity disorder: A clinical workbook. 2nd edition. New York: Guilford pp 39–55.

Barkley RA, Fischer M, Smallish L, Fletcher K. 2002. The persistence of attention-deficit/hyperactivity disorder into young adulthood as a function of reporting source and definition of disorder. Am Psychol Assoc 111:279–289.

Biederman J, Kim JW, Doyle AE, Mick E, Fagerness J, Smoller JW, Faraone SV. 2008. Sexually dimorphic effects of four genes (COMT, SLC6A2, MAOA, SLC6A4) in genetic associations of ADHD: A preliminary study. Am J Med Genet B Neuropsychiatr Genet 147B(8):1511–1518.

Brookes K, Xu X, Chen W, Zhou K, Neale B, Lowe N, Anney R, Franke B, Gill M, Ebstein R, et al. 2006. 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 11-(10):934–953.

Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA. 1993. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. Science 262(5133): 578–580.

Carlson CL, Mann M. 2000. Attention-deficit/hyperactivity disorder, predominantly inattentive subtype. Child Adolesc Psychiatr Clin N Am 9(3):499–510.

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. 2004. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 74(1):106–120.

Carrel L, Willard HF. 2005. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434(7031):400–404.

Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC, et al. 1995. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 268(5218):1763–1766.

Cremin SM, Greer WL, Bodok-Nutzati R, Schwartz M, Peacocke M, Siminovitch KA. 1993. Linkage of Wiskott-Aldrich syndrome with three marker loci, DXS426, SYP and TFE3, which map to the Xp11.3-p11.22 region. Hum Genet 92(3):250–253.

Cuffe SP, Moore CG, McKeown RE. 2005. Prevalence and correlates of ADHD symptoms in the national health interview survey. J Atten Disord 9(2):392–401.

Davies W. 2014. Sex differences in attention deficit hyperactivity disorder: Candidate genetic and endocrine mechanisms. Front Neuroendocrinol 35(3):341–346.

Deckert J, Catalano M, Syagailo YV, Bosi M, Okladnova O, Di Bella D, Nothen MM, Maffei P, Franke P, Fritze J, et al. 1999. Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum Mol Genet 8(4):621–624.

Domschke K, Sheehan K, Lowe N, Kirley A, Mullins C, O'Sullivan R, Freitag C, Becker T, Conroy J, Fitzgerald M, et al. 2005. Association analysis of the monoamine oxidase A and B genes with attention deficit hyperactivity disorder (ADHD) in an Irish sample: Preferential transmission of the MAO-A 941G allele to affected children. Am J Med Genet B Neuropsychiatr Genet 134B(1):110–114.

Faraone SV, Biederman J, Chen WJ, Milberger S, Warburton R, Tsuang MT. 1995. Genetic heterogeneity in attention-deficit hyperactivity disorder (ADHD): Gender, psychiatric comorbidity, and maternal ADHD. J Abnorm Psychol 104(2):334–345.

Faraone SV, Mick E. 2010. Molecular genetics of attention deficit hyperactivity disorder. Psychiatr Clin North Am 33(1):159–180.

Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P. 2005. Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry 57(11):1313–1323.

Fon EA, Edwards RH. 2001. Molecular mechanisms of neurotransmitter release. Muscle Nerve 24(5):581–601.

Ford T, Goodman R, Meltzer H. 2003. The British child and adolescent mental health survey 1999: The prevalence of DSM-IV disorders. J Am Acad Child Adolesc Psychiatry 42(10):1203–1211.

Gaub M, Carlson CL. 1997. Gender differences in ADHD: A meta-analysis and critical review. J Am Acad Child Adolesc Psychiatry 36(8):1036–1045.

Guan L, Wang B, Chen Y, Yang L, Li J, Qian Q, Wang Z, Faraone SV, Wang Y. 2009. 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 14(5):546–554.

Klein LC, Corwin EJ. 2002. Seeing the unexpected: How sex differences in stress responses may provide a new perspective on the manifestation of psychiatric disorders. Curr Psychiatry Rep 4(6):441–448.

Knickmeyer RC, Baron-Cohen S. 2006. Fetal testosterone and sex differences in typical social development and in autism. J Child Neurol 21(10):825–845.

Lawson DC, Turic D, Langley K, Pay HM, Govan CF, Norton N, Hamshere ML, Owen MJ, O'Donovan MC, Thapar A. 2003. Association analysis of monoamine oxidase A and attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 116B(1):84–89.

Levy D, Ronemus M, Yamrom B, Lee YH, Leotta A, Kendall J, Marks S, Lakshmi B, Pai D, Ye K, et al. 2011. Rare de novo and transmitted copy-number variation in autistic spectrum disorders. Neuron 70(5):886–897.

Liu L, Chen Y, Li H, Qian Q, Yang L, Glatt SJ, Faraone SV, Wang Y. 2013. Association between SYP with attention-deficit/hyperactivity disorder in Chinese Han subjects: Differences among subtypes and genders. Psychiatry Res 210(1):308–314.

Liu L, Guan LL, Chen Y, Ji N, Li HM, Li ZH, Qian QJ, Yang L, Glatt SJ, Faraone SV, et al. 2011. Association analyses of MAOA in Chinese Han subjects with attention-deficit/hyperactivity disorder: Family-based association test, case-control study, and quantitative traits of impulsivity. Am J Med Genet B Neuropsychiatr Genet 156B(6):737–748.

Lou XY, Chen GB, Yan L, Ma JZ, Mangold JE, Zhu J, Elston RC, Li MD. 2008. A combinatorial approach to detecting gene-gene and gene-environment interactions in family studies. Am J Hum Genet 83(4):457–467.

Lou XY, Chen GB, Yan L, Ma JZ, Zhu J, Elston RC, Li MD. 2007. A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. Am J Hum Genet 80(6):1125–1137.

Manor I, Tyano S, Mel E, Eisenberg J, Bachner-Melman R, Kotler M, Ebstein RP. 2002. Family-based and association studies of monoamine oxidase A and attention deficit hyperactivity disorder (ADHD): Preferential transmission of the long promoter-region repeat and its association with impaired performance on a continuous performance test (TOVA). Mol Psychiatry 7(6):626–632.

Manuck SB, Flory JD, Ferrell RE, Mann JJ, Muldoon MF. 2000. A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity. Psychiatry Res 95(1):9–23.

Motsinger AA, Ritchie MD. 2006. Multifactor dimensionality reduction: An analysis strategy for modelling and detecting gene–gene interactions in human genetics and pharmacogenomics studies. Hum Genomics 2(5):318–328.

Neale BM, Lasky-Su J, Anney R, Franke B, Zhou K, Maller JB, Vasquez AA, Asherson P, Chen W, Banaschewski T, et al. 2008. Genome-wide association scan of attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 147B(8):1337–1344.

Neale BM, Medland S, Ripke S, Anney RJ, Asherson P, Buitelaar J, Franke B, Gill M, Kent L, Holmans P, et al. 2010a. Case-control genome-wide association study of attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 49(9):906–920.

Neale BM, Medland SE, Ripke S, Asherson P, Franke B, Lesch KP, Faraone SV, Nguyen TT, Schafer H, Holmans P, et al. 2010b. Meta-analysis of genome-wide association studies of attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 49(9):884–897.

Nikolas MA, Burt SA. 2010. Genetic and environmental influences on ADHD symptom dimensions of inattention and hyperactivity: A meta-analysis. J Abnorm Psychol 119(1):1–17.

Ozcan ME, Banoglu R. 2003. Gonadal hormones in schizophrenia and mood disorders. Eur Arch Psychiatry Clin Neurosci 253(4):193–196.

Rhee SH, Waldman ID, Hay DA, Levy F. 1999. Sex differences in genetic and environmental influences on DSM-III-R attention-deficit/hyperactivity disorder. J Abnorm Psychol 108(1):24–41.

Ritchie MD, Hahn LW, Moore JH. 2003. Power of multifactor dimensionality reduction for detecting gene-gene interactions in the presence of genotyping error, missing data, phenocopy, and genetic heterogeneity. Genet Epidemiol 24(2):150–157.

Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, Chu SH, Moreau MP, Gupta AR, Thomson SA, et al. 2011. Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70(5):863–885.

Scassellati C, Bonvicini C, Faraone SV, Gennarelli M. 2012. Biomarkers and attention-deficit/hyperactivity disorder: A systematic review and meta-analyses. J Am Acad Child Adolesc Psychiatry 51(10):1003–1019.

Shang CY, Gau SS, Liu CM, Hwu HG. 2011. Association between the dopamine transporter gene and the inattentive subtype of attention deficit hyperactivity disorder in Taiwan. Prog Neuropsychopharmacol Biol Psychiatry 35(2):421–428.

Shen YC, Tsai HM, Ruan JW, Liao YC, Chen SF, Chen CH. 2012. Genetic and functional analyses of the gene encoding synaptophysin in schizophrenia. Schizophr Res 137(1–3):14–19.

Shih JC, Chen K, Ridd MJ. 1999. Monoamine oxidase: From genes to behavior. Annu Rev Neurosci 22:197–217.

Shors TJ. 2002. Opposite effects of stressful experience on memory formation in males versus females. Dialogues Clin Neurosci 4(2):139–147.

Sims KB, Lebo RV, Benson G, Shalish C, Schuback D, Chen ZY, Bruns G, Craig IW, Golbus MS, Breakefield XO. 1992. The Norrie disease gene maps to a 150kb region on chromosome Xp11.3. Hum Mol Genet 1(2):83–89.

Skuse DH. 2006. Sexual dimorphism in cognition and behaviour: The role of X-linked genes. Eur J Endocrinol 155(1):S99–S106.

Thiele C, Hannah MJ, Fahrenholz F, Huttner WB. 2000. Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. Nat Cell Biol 2(1):42–49.

Trent S, Davies W. 2012. The influence of sex-linked genetic mechanisms on attention and impulsivity. Biol Psychol 89(1):1–13.

Valtorta F, Pennuto M, Bonanomi D, Benfenati F. 2004. Synaptophysin: Leading actor or walk-on role in synaptic vesicle exocytosis?. Bioessays 26(4):445–453.

Wu JB, Chen K, Li Y, Lau YF, Shih JC. 2009. Regulation of monoamine oxidase A by the SRY gene on the Y chromosome. Faseb J 23(11): 4029–4038.

Xu X, Brookes K, Chen CK, Huang YS, Wu YY, Asherson P. 2007. Association study between the monoamine oxidase A gene and attention deficit hyperactivity disorder in Taiwanese samples. BMC Psychiatry 7:10.

Yang L, Neale BM, Liu L, Lee SH, Wray NR, Ji N, Li H, Qian Q, Wang D, Li J, et al. 2013. Polygenic transmission and complex neuro developmental network for attention deficit hyperactivity disorder: Genome-wide association study of both common and rare variants. Am J Med Genet B Neuropsychiatr Genet 162B(5):419–430.

Yang L, Wang Y, Qian Q, Gu BM. 2001. Primary exploration of the clinical subtypes of attention deficit hyperactivity disorder in Chinese children. Chin J Psychiatry 34(4):204–207.

Yang L, Wang YF, Qian QJ, Biederman J, Faraone SV. 2004. DSM-IV subtypes of ADHD in a Chinese outpatient sample. J Am Acad Child Adolesc Psychiatry 43(3):248–250.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.