Association between \( SYP \) with attention-deficit/hyperactivity disorder in Chinese Han subjects: Differences among subtypes and genders

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**A B S T R A C T**

Dysfunction of neurotransmitters has been suggested to be involved in the etiology of attention-deficit/hyperactivity disorder (ADHD). Hence, genes encoding proteins involved in the vesicular release process of those neurotransmitters are attractive candidates in ADHD genetics. One of these genes is \( SYP \), which encodes synaptophysin, a protein known to participate in regulating neurotransmitter release and synaptic plasticity. Several studies have reported an association between \( SYP \) and ADHD, but more work is needed to refine the association. In the present study, we attempt to investigate their association in Chinese Han subjects by family-based and case-control studies. Transmission disequilibrium tests (TDTs) in 1112 trios found significant association between \( SYP \) and the predominantly inattentive subtype (ADHD-I), especially for males with ADHD-I, both from single nucleotide polymorphism (SNP) and haplotypic analyses. Chi-square tests in 1682 ADHD probands and 957 comparison subjects indicated possible association of \( SYP \) with female ADHD and female ADHD-I. However, the associated alleles and haplotypes between males and females were reversed. In conclusion, our results suggested that \( SYP \) may be primarily associated with ADHD-I and its genetic mechanism may be gender-specific. Thus, it is necessary to take subtype and gender into account in ADHD genetic studies.

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1. Introduction

The etiology of attention-deficit/hyperactivity disorder (ADHD) has been studied for several years, but remains unclear. Genetic factors have been suggested to be important for the neuropathology of ADHD as its relatively high heritability of approximately 0.76 (Faraone and Mick, 2010).

In the past two decades, geneticists have focused on several neurotransmitter systems to explore the possible genetic variants leading to the cause of ADHD, including dopamine (DA), norepinephrine (NE), serotonin (5-HT) pathways (Li et al., 2005, 2006, 2007; Faraone and Mick, 2010; Wu et al., 2012) among others. Although abundant evidence from neurobiologic, neuropharmacologic, and animal studies has revealed the important role of these neurotransmitters (Scassellati et al., 2012), the existing genetic association studies of these pathways have failed to provide consistent evidence of a relationship to ADHD susceptibility (Gizer et al., 2009). A consensus has formed that the dysfunction of a single neurotransmitter system may not be sufficient to cause ADHD, whereas several neurotransmitters and/or their interactions may each play a role. As such, genes encoding proteins that are involved in the more generalized vesicular release process of neurotransmitters have become attractive targets. In recent years, genes encoding synaptic vesicle (SV) proteins have been studied, including \( \text{VAMP2} \) (encoding vesicle-associated membrane protein, also called synaptobrevin), \( \text{SNAP25} \) (encoding synaptosomal-associated protein of 25 kDa), \( \text{STX1A} \) (encoding syntaxin), \( \text{SYT1} \) (encoding synaptotagmin), \( \text{SLC18A2} \) (encoding vesicle monoamine transporter, also named solute carrier family 18, member 2) and \( \text{SYN} \) (encoding synaptophysin).

Synaptophysin (Syp) was the first SV protein to be isolated and cloned. It is a 38 kD transmembrane protein functioning in conjunction with SNARES (soluble N-ethylmaleimide-sensitive factor-attachment protein receptors). Although the precise function of synaptophysin is still unclear, promising evidence shows that it indeed participates in regulating neurotransmitter release and synaptic plasticity (Alder et al., 1992, 1995). Synaptophysin

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\(^{1}\) These authors contributed equally in this work.
combines with synaptobrevin to prevent the composition of the fusion core complex to regulate the function of SNARE (Valtorta et al., 2004).

The gene encoding synaptophysin (SYP) maps to Xp11.23-p11.22. Several studies have indicated that the region where SYP is located was associated with several different genetic diseases such as Norrie disease (Sims et al., 1992), Wiskott–Aldrich syndrome (Cremine et al., 1993) and some psychiatric diseases such as schizophrenia (Shen et al., 2012). Evidence from different studies has suggested that some genetic variants of SYP may be risk factors for ADHD. In the study of Brookes et al. (2005), they screened and analyzed 61 single nucleotide polymorphisms (SNPs) of genes regulating vesicular release of neurotransmitters in 180 ADHD-Combined type (ADHD-C) probands and 180 comparison subjects. After several stages of analyses, only rs2293945 of SYP, which maps to the gene’s 5′-untranslated region (5′UTR), was associated with ADHD in both case-control and transmission-disequilibrium test (TDT) analyses. In another study by Brookes (2006), rs5906754 of SYP was associated with ADHD-C in 776 probands. Besides these two studies in Caucasians, our previous study utilizing a high density single-nucleotide polymorphism screen of 23 candidate genes in 182 ADHD children and 184 healthy controls of Chinese Han descent indicated an association between the SNP rs5906754 of SYP with ADHD but only for the inattentive subtype (ADHD-I) (Guan et al., 2009).

The present study attempted to investigate the association between three SNPs of SYP with ADHD, including rs3817678, which has been studied for schizophrenia (Wei and Hemmings, 2006; Shen et al., 2012) and the two above-mentioned SNPs previously implicated in ADHD. We utilized a larger sample of Chinese Han subjects than we previously examined (Guan et al., 2009) in both family-based and case-control analyses.

2. Methods

2.1. Subjects

This study was approved by the Ethics Committee of Peking University Health Science Center. We recruited ADHD cases from child psychiatric clinics of Peking University Institute of Mental Health. All cases met the DSM-IV diagnostic criteria of ADHD based on a semi-structured interview by psychiatrists using the Clinical Diagnostic Interview Scale (CDIS) (Barkley, 1998). The mandarin version of CDIS, which was developed by our group (Yang et al., 2001, 2004) shows good sensitivity (97.2%) and specificity (100%). The test-retest reliability Pearson correlation coefficient is 0.89 and the inter-rater reliability kappa coefficient is 0.74 (P<0.01). The CDIS assesses the three DSM-IV subtypes of ADHD: ADHD inattentive type (ADHD-I), ADHD hyperactive-impulsive type (ADHD-HI), and ADHD combined type (ADHD-C).

All probands had to meet the following criteria: (1) meet DSM-IV criteria for ADHD, (2) be between 6 and 16 years old, (3) have a full scale IQ > 70 according to the Chinese-Wechsler Intelligence Scale for Children (Gong and Cai, 1993), (4) be drug-naïve with respect to ADHD therapies, and (5) be of Chinese Han descent. All cases with major neurological disorders, a diagnosis of schizophrenia, pervasive development disorder, epilepsy, mental retardation or other brain disorders were excluded. (More details have been described by (Liu et al., 2011).)

The recruit of typically developing comparison subjects was from local elementary schools, healthy blood donors from the Blood Center of the First Hospital, Peking University, and from healthy volunteers at our institute. All were of Chinese Han descent. The exclusion criteria were: ADHD, other major psychiatric disorders, family history of psychosis, severe physical diseases and substance abuse (see more details in Guan et al. (2009)).

2.2. SNP selection and genotyping

We included three SNPs in the present study. Fig. 1 shows the locations of these three SNPs. rs3817678 has been studied in schizophrenia (Wei and Hemmings, 2006; Shen et al., 2012). Rs2293945 was associated with ADHD in Caucasian subjects (Brookes et al., 2005), while rs5906754 was associated with ADHD both in Caucasian (Brookes et al., 2006) and Chinese Han subjects (Guan et al., 2009), but for different subtypes.

Peripheral blood samples were collected from each subject, followed by extracting genomic DNA using standard protocols (Omega Bio-tek Inc., Doraville, GA, USA). All SNP genotyping was completed using a Taqman allele genotyping assay (Livak, 1999) on an ABI 7900 HT instrument (Applied Biosystems, Foster City, California, USA), following the standard protocol provided. PCR cycling conditions consisted of 95 °C for 10 min, 40 cycles of 92 °C for 15 s, and 60 °C for 1 min. For quality control, first, two to four negative test controls were confirmed as having no genotype called in every 384-well plate. Second, 3% of the samples were randomly selected and genotyped as duplicates across 384-well plates, indicating the concordance of 100%. Finally, call rates for SNPs ranged from 98.6% to 99.5%.

2.3. Statistics

We used HAPLOVIEW version 4.0 to test for Hardy–Weinberg equilibrium (HWE) only in females, and found no departures from HWE for any of the three SNPs (all P > 0.05) both in case and control groups. Minor allele frequencies (MAFs) for all SNPs were ≤0.3. The linkage disequilibrium (LD) map for SYP was also generated, showing the strong LD of these three SNPs (Fig. 1). For association analyses, we used HAPLOVIEW version 4.0 (Barrett et al., 2005) to conduct analyses of both single SNPs and their constituent haplotypes, with TDTs for the family-based association study and chi-square tests for the case-control study. The accepted level of nominal significance was 0.05 for all analyses. To correct for multiple testing biases at the gene-wide level, we performed a total of 10,000 permutations to empirically estimate significance levels. Since SYP is located on Chr. X, we also conduct analyses in different sex groups. In addition, to reduce the heterogeneity, we further conducted above analyses in three different ADHD subtypes: ADHD-C, ADHD-I, and ADHD-HI which is the hyperactive/impulsive subtype.

Fig. 1. Linkage disequilibrium (LD) plot of SYP determined by Haploview software, displaying LD values in D′ (left) and R² (right). Three SNP markers code for the haplotype block and were sorted correspondingly: rs3817678, rs2293945, and rs5906754.
3. Results

A total of 1682 ADHD cases were included in the final analyses (1404 males, 278 females; mean age 10.2 ± 2.6 years), including 865 ADHD inattentive type (ADHD-I), 84 ADHD hyperactive-impulsive type (ADHD-HI) and 733 ADHD combined type (ADHD-C) subjects. From all ADHD cases, there were 1112 probands who, along with their parents, constituted trios to be included for the family-based association study (938 males, 174 females; mean age 10.0 ± 2.6 years), including 570 ADHD-I trios, 62 ADHD-HI trios and 480 ADHD-C trios. In the control group, 957 males and 72 females were analyzed due to the extremely small sample of HI trios, even after sub-grouped by gender (for ADHD-HI, only No signification (Table 1).

3.1. Family-based association

For the entire sample of ADHD, no biased transmission of any allele of any of the three SNPs was observed. When the samples were sub-grouped by gender, there was still no significant association (Table 1).

To reduce the possible heterogeneity caused by subtypes, we further repeated above analyses in the three subtypes separately. No significant association was found for either ADHD-C or ADHD-HI trios, even after sub-grouped by gender (for ADHD-HI, only males were analyzed due to the extremely small sample of females) (Table 1).

We found biased transmission of several alleles of SYP in the ADHD-I trios. We observed over-transmission of the T allele of rs5906754 (P=0.0250, after permutation 0.0377) and the T allele of rs2293945 (P=0.0528, after permutation 0.0794) also showed a tendency for over-transmission (Table 2), though these did not attain statistical significance after permutation. Repeated gender-specific analyses also indicated similar results in male ADHD-I trios as were seen in the analysis of the entire ADHD-I trios, with even stronger evidence of association (Table 2). However, no significant association was found for female ADHD-I trios (Table 2).

3.2. Case-control study

The chi-square tests did not show any association between any allele of any SNP for the entire ADHD sample (Supplementary Table 2). However, further gender-specific analyses indicated that the G allele of rs3817678 (P=0.0374, after permutation 0.0478), the C allele of rs2293945 (P=0.0426, after permutation 0.0522) and the C allele of rs5906754 (P=0.0215, after permutation 0.0217) showed higher frequency in female ADHD probands than female controls (Table 3). When we repeated the above analyses for the three subtypes separately, we found similar results for female ADHD-I probands as were seen in the analysis of the entire female ADHD sample (Table 3). No significant association was found for the male-specific analyses or its subtypes (Supplementary Table 2).

In addition, the results of haplotypic analyses supported the above findings from single-SNP analyses. The frequency of the GCC haplotype in female ADHD probands was higher than that in controls (P=4.541, 0.0331, after permutation 0.0647); the frequency of the ATT haplotype was lower in female ADHD probands than controls (P=4.946, P=0.0262, after permutation 0.0308) (Supplementary Table 3).

Table 1

<table>
<thead>
<tr>
<th>SNP</th>
<th>ADHD</th>
<th>ADHD-HI</th>
<th>ADHD-C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (1112)</td>
<td>Male (938)</td>
<td>Female (174)</td>
</tr>
<tr>
<td>rs3817678</td>
<td>0.3500</td>
<td>0.2207</td>
<td>0.6419</td>
</tr>
<tr>
<td>rs2293945</td>
<td>0.3061</td>
<td>0.1541</td>
<td>0.4913</td>
</tr>
<tr>
<td>rs5906754</td>
<td>0.3678</td>
<td>0.1931</td>
<td>0.4913</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>ADHD</th>
<th>ADHD-HI</th>
<th>ADHD-C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (62)</td>
<td>Male (55)</td>
<td>Female (63)</td>
</tr>
<tr>
<td>rs3817678</td>
<td>0.3173</td>
<td>0.2207</td>
<td>0.3469</td>
</tr>
<tr>
<td>rs2293945</td>
<td>0.4328</td>
<td>0.2207</td>
<td>0.3914</td>
</tr>
<tr>
<td>rs5906754</td>
<td>0.3173</td>
<td>0.1444</td>
<td>0.1468</td>
</tr>
</tbody>
</table>

Abbreviations: TDT, transmission disequilibrium tests; ADHD, attention-deficit/hyperactivity disorder; SNP, single-nucleotide polymorphism. Results for ADHD-I trios were listed separately in Table 2. For ADHD-HI trios, no biased transmission of any allele of any SNP for the entire ADHD sample (Supplementary Table 2). However, further gender-specific analyses indicated that the G allele of rs3817678 (P=0.0374, after permutation 0.0478), the C allele of rs2293945 (P=0.0426, after permutation 0.0522) and the C allele of rs5906754 (P=0.0215, after permutation 0.0217) showed higher frequency in female ADHD probands than female controls (Table 3). When we repeated the above analyses for the three subtypes separately, we found similar results for female ADHD-I probands as were seen in the analysis of the entire female ADHD sample (Table 3). No significant association was found for the male-specific analyses or its subtypes (Supplementary Table 2).

In addition, the results of haplotypic analyses supported the above findings from single-SNP analyses. The frequency of the GCC haplotype in female ADHD probands was higher than that in controls (P=4.541, 0.0331, after permutation 0.0647); the frequency of the ATT haplotype was lower in female ADHD probands than controls (P=4.946, P=0.0262, after permutation 0.0308) (Supplementary Table 3).

Table 2

<table>
<thead>
<tr>
<th>SNP</th>
<th>Male ADHD-I trios (n=570)</th>
<th>Female ADHD-I trios (n=104)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T:NT</td>
<td>χ²</td>
</tr>
<tr>
<td>rs3817678</td>
<td>A</td>
<td>135:107</td>
</tr>
<tr>
<td>rs2293945</td>
<td>T</td>
<td>135:105</td>
</tr>
<tr>
<td>rs5906754</td>
<td>T</td>
<td>132:98</td>
</tr>
</tbody>
</table>

Abbreviations: TDT, transmission disequilibrium tests; ADHD, attention-deficit/hyperactivity disorder; SNP, single-nucleotide polymorphism; T, transmitted; NT, non-transmitted.
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Table 3
Chi-square tests of case-control study for single SNPs in female ADHD, female ADHD-I and female ADHD-C.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>Female ADHD (n=278)</th>
<th>Female ADHD-I (n=162)</th>
<th>Female ADHD-C (n=107)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case, control freq</td>
<td>Nominal $\chi^2$</td>
<td>$p$</td>
<td>Empirical $P^a$</td>
</tr>
<tr>
<td>rs3817678</td>
<td>G</td>
<td>0.684, 0.627</td>
<td>4.331</td>
<td>0.0374</td>
</tr>
<tr>
<td>rs2293945</td>
<td>C</td>
<td>0.683, 0.627</td>
<td>4.189</td>
<td>0.0426</td>
</tr>
<tr>
<td>rs5906754</td>
<td>C</td>
<td>0.686, 0.623</td>
<td>5.284</td>
<td>0.0215</td>
</tr>
</tbody>
</table>

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; SNP, single-nucleotide polymorphism.

a Females ADHD-II were not analyzed due to the small sample size (n=9).

b Empirical $P$ from HAPLOVIEW assessed the gene-wide significance value estimated on the basis of 10,000 permutations.

Table 4
Summary of the significant associations in the present study both from TDT tests for family-based association study and chi-square tests for case-control study.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Associated phenotype</th>
<th>Over-transmitted allele</th>
<th>Nominal $P$</th>
<th>Empirical $P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3817678</td>
<td>Male ADHD-I</td>
<td>A</td>
<td>0.0378</td>
<td>0.0537</td>
</tr>
<tr>
<td>rs2293945</td>
<td>Male ADHD-I</td>
<td>T</td>
<td>0.0528</td>
<td>0.0794</td>
</tr>
<tr>
<td>rs5906754</td>
<td>Male ADHD-I</td>
<td>T</td>
<td>0.0216</td>
<td>0.0294</td>
</tr>
</tbody>
</table>

Haplotype

<table>
<thead>
<tr>
<th>GCC</th>
<th>Associated phenotype</th>
<th>Over ↑/under ↓</th>
<th>Nominal $P$</th>
<th>Empirical $P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT</td>
<td>Male ADHD-I</td>
<td>↑</td>
<td>0.0265</td>
<td>0.0292</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Associated phenotype</th>
<th>Higher allele frequency</th>
<th>Nominal $P$</th>
<th>Empirical $P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3817678</td>
<td>Female ADHD</td>
<td>G</td>
<td>0.0374</td>
<td>0.0478</td>
</tr>
<tr>
<td>rs2293945</td>
<td>Female ADHD</td>
<td>C</td>
<td>0.0426</td>
<td>0.0522</td>
</tr>
<tr>
<td>rs5906754</td>
<td>Female ADHD-I</td>
<td>C</td>
<td>0.0215</td>
<td>0.0217</td>
</tr>
</tbody>
</table>

Haplotype

<table>
<thead>
<tr>
<th>GCC</th>
<th>Higher ↑/Lower ↓</th>
<th>Nominal $P$</th>
<th>Empirical $P^a$</th>
</tr>
</thead>
</table>

4. Discussion

In the present study, we investigated three SNPs previously implicated in ADHD or other neuropsychiatric disorders for association with ADHD in children of Chinese Han descent. Consistent with our previous smaller study (182 ADHD cases, 184 controls) (Guan et al., 2009), we still did not find any association between SYT and ADHD in general. However, after dividing the whole sample by gender and subtype, we found that SYT may be associated with ADHD-I from both family-based analyses and case-control studies. However, the associated risk variant for two genders is strikingly distinct (see Table 4 for a summary of the significant associations). We also noticed that results from family-based and case-control studies did not verify with each other completely. We will discuss these intriguing findings respectively below.

The most significant association was found for SYT and ADHD inattentive subtype (ADHD-I). The family-based association analyses found a significant association only for ADHD-I. Male-specific and haplotypic analyses also supported this finding. This is consistent with what we reported in our previous study, which only analyzed one SNP (rs5906754) in 83 ADHD-I cases and 184 controls (Guan et al., 2009). Although the present case-control study did not completely replicate the results of the family-based study, it suggested a possible association between SYT with female ADHD-I. Taken together our present and previous findings, we speculate that SYT may be involved in the etiology of ADHD inattentive subtype. This subtype-specific association also has been pointed out by others (Zhang et al., 2005; Roman et al., 2006; Guan et al., 2009; Shang et al., 2011; Liu et al., 2011; Sengupta et al., 2012), which is consistent with reports of heterogeneity among subtypes (Farace et al., 1995, 2000; Carlson and Mann, 2000; Todd et al., 2001; Nigg et al., 2005; Diamond, 2005; Nikola and Burt, 2010; Grizzenko et al., 2010; Asherson and Gurling, 2012). Our data suggest that the heterogeneity between different subtypes may extend to associations of candidate genes with ADHD. Refining phenotypes by subtyping, assuming large-enough sample sizes, may help us to build more accurate models of condition-specific genetic influences on susceptibility to ADHD. An important phenomenon that we could not omit is the failure of complete verification between family-based and case-control studies. From TDT tests, we showed significant association of SYT with ADHD-I and ADHD-C.
with ADHD-I that was only retained for males. Expectant replication did not appear in case-control analyses instead of significant findings for female ADHD and its ADHD-I subtype. In our previous study, we also have reported some inconsistent results between these two strategies (Liu et al., 2011). There may be some potential stratification artifacts which may add noise to case-control analyses and then lead to either false positive or false negative results. Although TDT tests could control for these factors and make its results more credible, we should note that only trios with parents of heterozygous genotype would be used in final analyses. On the one hand, it will reduce the sample size and the statistic power to prevent our detecting of truly existed minor genetic affects, which may be related to the negative findings for female ADHD trios. On the other hand, the reduced sample size could also possibly lead to false positive findings. Hence, we should view these results cautiously. Further expansion of sample size and replication in other independent subjects may help us to illustrate this discrepancy.

We also note the inconsistency between our current findings and previous reports. Brookes et al. (2005) tested 19 SNPs of SYP by DNA-pooling, and found suggestive evidence of association of rs2293945 in both case-control and TDT analyses. Another study by Brookes et al. (2006) only included rs5906754 and also indicated suggestive association. However, all subjects referred in these two studies were ADHD-C subtype probands. Yet, in our present study, we did not find any evidence of association for ADHD-C, but only for ADHD-I. One reason for these discrepant findings may be the influence of ancestry. The subtype composition of ADHD in Chinese of Han descent is different from that reported in Caucasians. The most common subtype in Caucasians is ADHD-C, while in Chinese it is ADHD-I (~51% reported (Yang et al., 2004) and in the present study). Secondly, numerous studies have suggested that there is a difference in genetic patterns among different ancestral populations. Gelernter et al. (1997, 1999) has compared alleles and haplotype frequencies of SLC6A4 in different populations and found significant global genetic variation. Studies of ADR2A in Chinese Han (Wang et al., 2006) and Korean subjects (Cho et al., 2008) have shown risk alleles for ADHD opposite to those reported in studies of Caucasian population (Park et al., 2005).

Another interesting finding from this study was the differences between males and females. In males, we found that the A allele of rs3817678, the T allele of rs2293945 and the T allele of rs5906754 were the risk alleles. In addition, the ATT haplotype was the risk haplotype for male ADHD-I probands, while the GCC appeared to be protective. On the contrary, although we did not find any association in females from TDT tests, case-control studies revealed that the G allele of rs3817678, the C allele of rs2293945, the C allele of rs5906754 and the GCC haplotype were risk factors for female ADHD and/or female ADHD-I, while the ATT haplotype was protective. Gender-specific phenomena have been previously reported in ADHD genetic studies (Steinhausen, 2009; Sengupta et al., 2012). The higher prevalence of ADHD in males reported in epidemiologic and clinical studies suggests that the disorder has some gender-specificity. Our previous studies have reported some gender-specific associations for ADHD (Qian et al., 2003; Liu et al., 2011) as have other groups (Guimaraes et al., 2007; Rommelse et al., 2008; Biederman et al., 2008; Steinhausen, 2009). The study by Rommelse et al. (2008) found that the same haplotype, comprising three SNPs of MAOA was associated with poorer motor control in ADHD boys, but with better working memory in ADHD girls. Taken together, these results suggest that gender may have moderate genetic effects for ADHD, making it difficult to find the true risk variants for the disorder, especially when the associated orientation is completely opposite across genders as seen in our current study. Another possible reason for the different results between males and females may be that different risk variants in males and females are in linkage disequilibrium with the same risk variant for ADHD. Nevertheless, before firm conclusions can be reached, more female ADHD probands should be collected for further exploration.

Overall, our results suggest a potential role of the SYP gene in the pathophysiology of ADHD. Abundant evidence has indicated that synaptophysin may be involved in multiple aspects of the release process of neurotransmitters, including exocytosis, endocytosis and biogenesis of synaptic vesicle (Valtorta et al., 2004). Based on the existing literature, we speculate that the most possible mechanism of SYP involved in the etiology of ADHD is the potential influence of its genetic variants on the formation of the synaptophysin/synaptobrevin (Syp/Syb, also named Syp/VAMP2) complex, which is mutually exclusive with the SNARE complex (Valtorta et al., 2004). Among ADHD probands, risk variants may enhance the combination of synaptophysin and synaptobrevin, which will prevent the dissociation of the Syp/VAMP2 complex. Then, the SNARE complex formation will be inhibited and cannot play its important role in the exocytotic membrane fusion process efficiently. And finally the neurotransmitter release will be influenced and decrease. However, further genetic studies for the gene–gene interaction of SYP and VAMP2, and functional studies for the action mode of Syp/VAMP2 complex may help to illustrate the precise role of SYP in the etiology of ADHD. In addition, synaptophysin is important to maintain the synaptic membrane integrity and promote the biogenesis of synaptic vesicles by its cholesterol-binding function (Thiele et al., 2000). Any genetic variants affecting its cholesterol-binding ability will influence the synaptic circulation and the release of neurotransmitters associated with ADHD. Finally, neuroimaging studies showed that ADHD probands may be characterized by a delay in cortical maturation relative to normal comparison subjects, especially in prefrontal regions (Shaw et al., 2007). Neurobiology research suggests that this delay of development may be caused by synaptic pruning, and synaptophysin has been widely used as a proxy for synaptic density (Webster et al., 2011). The risk genetic variants of SYP may have effect on the synthesis of synaptophysin by influencing alternative splicing and/or expression, which may lead to the disturbance of synaptophysin. All above mentioned possible mechanisms may ultimately lead to harmful synaptic alterations in the whole brain or specific regions and finally cause neuropsychiatric symptoms and disorders. However, there is a long process from genetic variants to protein level and phenotypes. Hence, more molecular genetic and neurobiological studies are needed to explore the explicit mechanism and promote our understanding of the role of SYP in the etiology of ADHD and other psychiatric disorders.

Our results must be considered in the context of several limitations. First, we only used three SNPs based on evidence from existing reports. These SNPs do not provide complete coverage of SYP and may miss important regions. Deeper exploration by GWAS or sequencing strategies may be helpful for us to detect more influential functional variants directly influencing risk for ADHD. In addition, sequencing of Chr. X, where SYP is located, may also be worthwhile, since previous reports have suggested the importance of this chromosome in ADHD’s etiology (Liu et al., 2011; Guan et al., 2009; Li et al., 2008). Second, sample collection should be continued to expand the sample size. In the current study, the sample size for ADHD-I was relatively small especially for females. In light of the high heterogeneity of ADHD, a larger sample size could allow us to refine the phenotype more systematically. Finally, the significance values indicated in our present study are not very robust, which underlines the necessity of replication studies.

In conclusion, our present study did not find a significant association between SYP and ADHD; however, after refining the
phenotype into gender and symptomatic subtypes, significant associations were observed, particularly for the ADHD-I subtype. In addition, our gender-specific analyses suggested different genetic associations for males and females. These findings underscore the value of considering subtype and gender in future genetic studies of ADHD.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.psychres.2013.04.029.

References