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ORIGINAL PAPER

STX1A gene variations contribute to the susceptibility of children attention-deficit/hyperactivity disorder: a case–control association study

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Abstract

It was presumed syntaxin-1A (STX1A) might relate to the pathophysiology of attention-deficit/hyperactivity disorder (ADHD), but the results were inconsistent. The present study aims to confirm whether the *STX1A* gene is involved in the susceptibility of children ADHD. We genotyped three single nucleotide polymorphisms (SNPs) of *STX1A* gene using Sequenom MassARRAY technology. A case–control study was performed among Chinese Han population including 754 cases and 772 controls from two different provinces. The Conners Parent Symptom Questionnaire and Integrated Visual and Auditory Continuous Performance Test were used to assess ADHD clinical symptoms. We found for the first time that rs3793243 GG genotype carriers had a lower risk of ADHD compared with AA genotype (OR 0.564, 95% confidence interval (CI) 0.406–0.692, $P=0.001$), and rs875342 was also associated with children ADHD (OR 1.806, 95% CI 1.349–2.591, $P=0.001$). In addition, the two positive SNPs were also significantly associated with the clinical characteristics of ADHD. Expression quantitative trait loci analysis indicated that rs3793243 might mediate *STX1A* gene expression. Using a case–control study to explore the association between *STX1A* gene and children ADHD in Chinese Han population, our results suggest *STX1A* genetic variants might contribute to the susceptibility of children ADHD.

Keywords Attention-deficit/hyperactivity disorder · *STX1A* gene · Association study · Genetic susceptibility

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder in children and adolescents of school age. Its core symptoms are persistent age-inappropriate inattention, hyperactivity, and impulsivity, often accompanied by learning difficulty, conduct disorder, and maladjustment [1]. According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) diagnostic criteria, ADHD is divided into three clinical subtypes: predominantly inattentive type (ADHD-I), predominantly hyperactive–impulsive type (ADHD-HI), and combined type (ADHD-C). A meta-analysis review estimated a prevalence rate of children ADHD was 5.9–7.1% worldwide [2], as of 2015, approximately 51 million children and adolescents were suffering from ADHD [3], and there was 3–9 times more frequently in boys compared with girls [4, 5]. Worse still, the symptoms of 30–50% patients will continue to reach adulthood [6].

As many other psychiatric disorders as, ADHD is a complex multi-factor disease with unknown etiology [7], which is generally recognized that ADHD is the result of interaction of gene, environment, and social factors [8]. Family, twin, and adoption studies suggested that genetic factor was an important risk etiology for ADHD, with an average heritability of up to 76% [9]. The molecular genetics of ADHD was based on the neurobiochemical hypothesis that

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the abnormal regulation of neurotransmitter metabolic pathways may be the basis of the pathogenesis of ADHD [10]. It was presumed that the abnormal release of neurotransmitter might involve the pathophysiology of ADHD [11]. The fusion of synaptic vesicles and presynaptic plasma membrane is the essence of neurotransmitter release, while this fusion is closely related to soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptors (SNARE) protein [12]. Therefore, SNARE protein may implicate with ADHD.

The primary proteins of SNARE are syntaxin-1A (STX1A), synaptosomal-associated protein of 25 kDa (SNAP-25), and the vesicle-associated membrane protein (VAMP, also called synaptobrevin), in which syntaxin-1A and SNAP-25 are classified as t-SNAREs, because they are located in the target membrane, while VAMP as v-SNARE anchored in the vesicle membrane [13–15]. The above three proteins constitute a ternary complex, also known as core complex, composed of a four-helical bundle which is essential for vesicular trafficking, docking, fusion, and neurotransmitter exocytosis [16]. The fusion of synaptic vesicles and presynaptic plasma membrane is mediated by SNARE protein as follows: Syntaxin-1A is initially not combined with VAMP or SNAP-25, and the formation of ternary complex is the begin of membrane fusion. Further zippering of the parallel helices induced by calcium ions makes the vesicles contact with plasma membrane. Calcium ions bind to the two calcium-binding domains of synaptotagmin (a regulatory protein, located in synaptic vesicle), allowing Syntaxin-1A contacts with synaptotagmin, and then, the energy provided by SNARE complex can promote the complete fusion of synaptic vesicles and presynaptic plasma membrane, and finally, the neurotransmitters are released into the synaptic cleft [12] (the biochemical process is shown in Fig. 1).

With regard to the association between SNARE protein genes and ADHD, most of the current researches

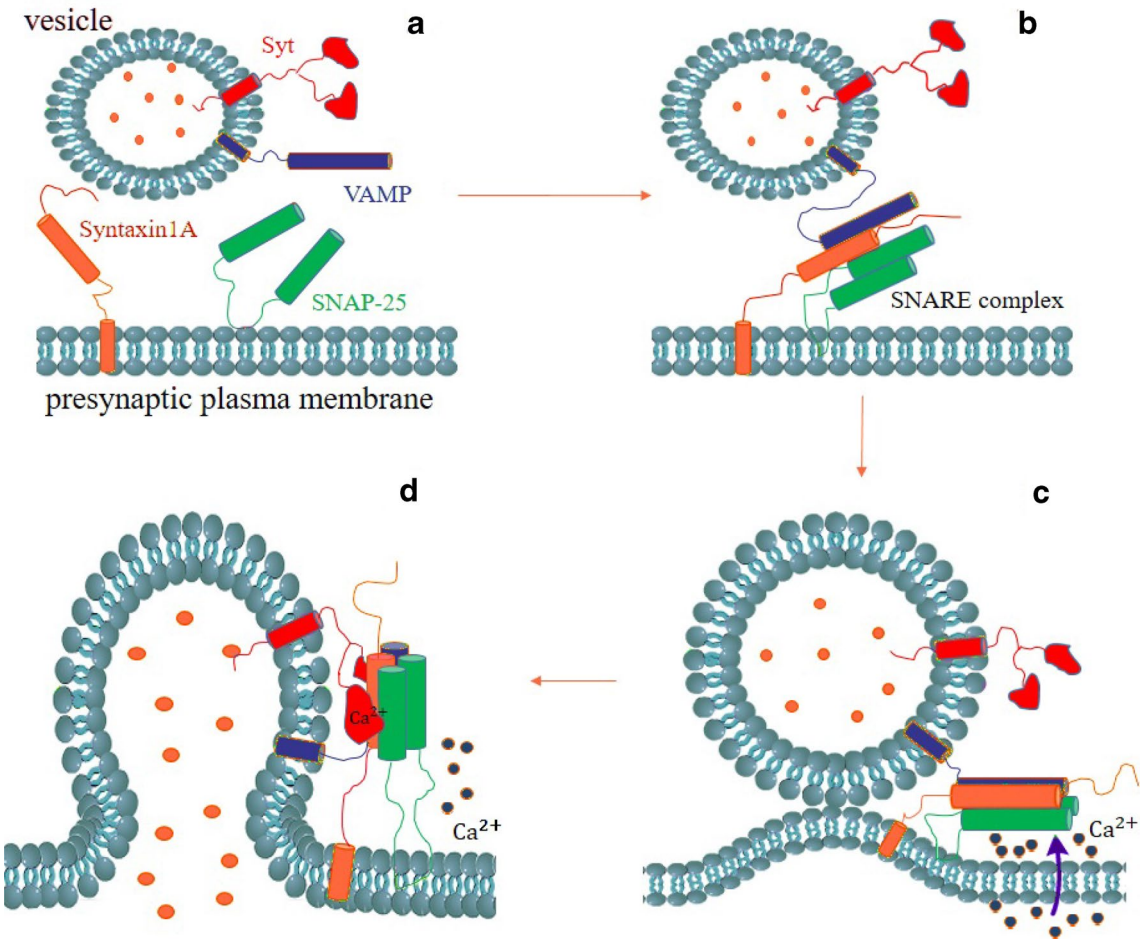

Fig. 1 Fusion of synaptic vesicles and presynaptic plasma membrane mediated by SNARE proteins. **a** Syntaxin-1A is initially not combined with VAMP or SNAP-25. **b** Formation of ternary complex is the begin of membrane fusion. **c** Further zippering of the parallel helices induced by Ca^{2+}makes the vesicles contact with plasma membrane. **d** Ca^{2+} binds to the two calcium-binding domains (red irregular polygon) of Syt (synaptotagmin), the energy provided by SNARE complex can promote the complete fusion, and finally, the neurotransmitters are released into the synaptic cleft

concentrated upon the *SNAP-25* gene, both animal experiments and candidate gene studies have shown that *SNAP-25* gene polymorphism was associated with ADHD in children [17–24]. However, *STX1A*, which belongs to t-SNAREs with *SNAP-25*, was rarely mentioned in the literature, and there was no positive report on *VAMP*; consequently, we aimed to explore *STX1A* gene.

STX1A gene is located at the Chromosome 7 long arm q11.23, 73.11–73.13 Mb area, and spanning 20.6 kb with 10 exons, and encodes syntaxin-1A (the structure of this gene is shown in Fig. 2). Syntaxin-1A is a specific protein of the nervous system that is involved in the docking of synaptic vesicles and the presynaptic membrane. Syntaxin-1A binds synaptotagmin in a calcium-dependent fashion, which is a key molecule in ion channel regulation and synaptic exocytosis. As a key protein for neuronal exocytosis, syntaxin-1A affect the release of serotonin (5-HT) in the brain and the neurotransmission of GABAergic, and finally leading to ADHD.

Though early genome-wide association studies (GWAS) have had limited success in identifying associations at the critical significance level ($P \leq 5 \times 10^{-8}$) [25–28], pathway, and gene set analyses of GWAS data implicated pathways involved in the regulation of neurotransmitter release contributing to the etiology of ADHD [29]. Accordingly, as an important regulator gene for neurotransmitter release, *STX1A* gene has its value for ADHD. For the moment, studies on the association between *STX1A* gene polymorphism and ADHD showed inconsistent results. A study by Gao et al. suggested that *STX1A* SNP rs875342 had a nominal association with children ADHD in Chinese Han population, but the results were only applicable in male [30]. The other studies in Caucasian population have not reached the same conclusion [31–33]. To get a relatively more reliable conclusion, we used a case–control study with 754 cases and 772 controls from two different centers to explore the association between *STX1A* gene and children ADHD in Chinese Han population (male and female were both included).

Materials and methods

Participants

This study was a case–control study from multicenter. In Hunan province, 346 newly diagnosed ADHD children and adolescents were included from the Children's Hospital of Hunan Province from January 2015 to June 2017; meanwhile, 359 controls were healthy children for physical examination in the same hospital during the same period. The sample of Hubei province including 406 ADHD cases and 415 healthy controls enrolled from Wuhan Medical and Health Center for Women and Children at the same time. Finally, 754 cases and 772 controls were included in our study. The inclusion criteria of the cases were as follows: (1) the Swanson, Nolan, and Pelham Questionnaire (SNAP-IV) score was higher than 1.5 scores by more than two experienced psychiatrists; (2) age 6–18; (3) the intelligence test of Chinese-Wechsler Intelligence Scale for Children was more than 70 points [34]; (4) new cases without any treatment; and (5) children with neurological disorders, pervasive developmental disorders, bipolar disorders, seizure disorders, or psychotic disorders were excluded. The standard of controls were as follows: (1) children with an SNAP-IV scale of 0–1; (2) age 6–18; (3) the intelligence test of Chinese-Wechsler Intelligence Scale for Children was more than 70 points; (4) no major family events in the near future; (5) there was no history of ADHD or chronic somatic infection, and no other family history of psychiatric disorders; and (6) no psychoactive drugs have been taken in the past two weeks.

The Ethics Committees of Tongji Medical College of Huazhong University of Science and Technology approved this study. The guardians or parents of the children who participated in this study were informed of what study they participation in, and signed informed consent to obtain the participants' biological samples with the voluntary cooperation of the parties.

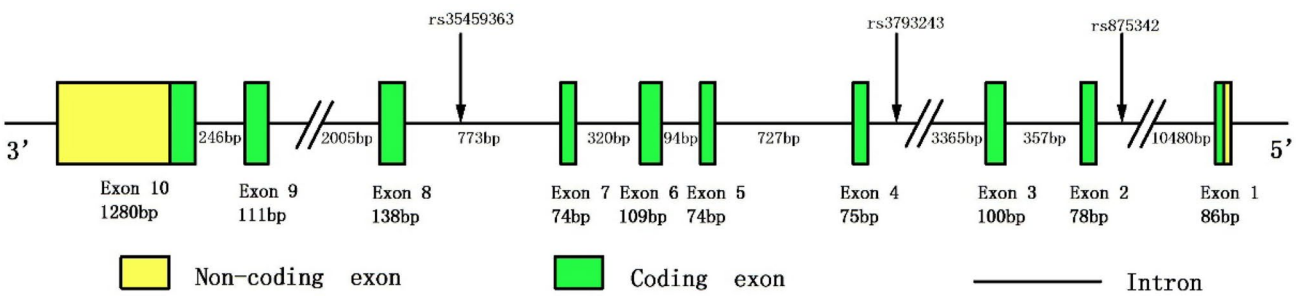

Fig. 2 Human *STX1A* gene structure and location of selected SNPs

Clinical information collection

Intelligence quotient (IQ) evaluation

The Chinese Wechsler Intelligence Scale for Children (C-WISC) was used to evaluate children's IQ. Based on the commonly used Wechsler Intelligence Scale, C-WISC combined with Chinese cultural background, which is more suitable for Chinese children's IQ evaluation.

SNAP-IV ADHD screening scale

The SNAP-IV ADHD screening scale was based on the DSM-IV diagnostic criteria, and the SNAP-IV questionnaire was designed to determine whether children have symptoms of ADHD, their severity, and the degree of damage to them. Clinicians score between 0 and 3 depending on the answers given by parents, guardians, or teachers. When the score is difficult to choose, the lower score is chosen. Finally, the scores of each question are added together with the total score of all questions, and then, the final score is obtained. The higher the score, the more serious the symptoms are.

Conners Parent Symptom Questionnaire (PSQ)

A Chinese version of the Conners Parent Symptom Questionnaire (PSQ) was used to measure all participants' ADHD symptoms [35, 36]. The questionnaire contains six subscales, while our questionnaire focused on three ADHD symptom subscale scores: hyperactivity/impulsivity, hyperactivity index, and total score. The hyperactivity/impulsivity score was used to implicate both hyperactivity and impulsivity behaviors of ADHD children, while the hyperactivity index score was mainly used to reflect the hyperactivity behavior.

Integrated visual and auditory continuous performance test (IVA-CPT)

IVA-CPT is a software system developed by an American brain science authority—Braintrain, which was dedicated to testing ADHD. The system includes visual and auditory stimuli, 22 primitive quotients, and 6 comprehensive quotients were used to evaluate the response control and attention level of the research subjects through the corresponding calculation. It is currently one of the important auxiliary diagnostic methods to determine whether children suffer from ADHD.

Candidate SNPs' selection

First, we used a bioinformatics approach to select SNPs. The SNPs were selected based on SNP genotype information downloaded from 1000 Genomes CHS (Southern Han Chinese) database with the criteria of linkage disequilibrium of $r^2 \geq 0.8$ and the minor allele frequencies (MAF) of the examined SNPs > 0.1. At the same time, we placed the tag SNPs into an integrated bioinformatics tool "SNPinfo" (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm) and HaploReg v4.1 (https://archive.broadinstitute.org/mammals/haploreg/haploreg.php) database to select the potential functional SNPs. Second, multiple comparison burden should be considered: more SNPs' mean heavier burden. Finally, three loci of rs3793243, rs35459363, and rs875342 were selected, and the basic characteristics of the three SNPs are shown in Supplementary Table 1 and Fig. 2.

DNA extraction and genotyping

According to the manufacturer's instructions, genomic DNA was extracted from 2 ml of peripheral blood sample using the Relax Gene Blood DNA System DP319-02 (Tiangen, Beijing, China). Based on the manufacturer's iPLEX Application Guide, the selected SNPs were genotyped using MassARRAY technology (Sequenom Inc., Dan Diego, CA, USA) which is currently the world's leading technology platform for qualitative and quantitative analyses of nucleic acids in both stages. The primers were designed using the Assay Design 3.0 software supplied by Sequenom. The iPLEX™ reaction products were dispensed onto a 384-well SpectroChip, and they were processed and analyzed in a Compact Mass Spectrometer using the Mass ARRAY Workstation 4.0 software (Sequenom Inc., San Diego, CA, USA).

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) for genotypes in all participants was assessed by a goodness-of-fit χ^2 test. The Pearson χ^2 test or t test was used to compare the general demographic characteristics and the distribution characteristics of the candidate SNPs. The associations of ADHD clinical features scores with SNPs were explored by ANOVA analysis. The association of candidate SNPs with ADHD was analyzed by multivariate logistic regression model after adjusting for age, gender, and site, which were estimated by odds ratios (ORs) and the 95% confidence intervals (95% CIs). In the logistic regression model, different genetic models have different coding methods. Using rs3793243 as an example, we let $X = 0$ for wild-type homozygote (AA) and $X = 1$ for heterozygote (AG) and variant homozygote (GG) in the dominant model. Then, we let $X = 0$ for GG genotype and $X = 1$ for AA and AG genotypes in the recessive model. As for additive model, we let $X = 0$ for AA genotype, $X = 1$ for AG genotype, and $X = 2$ for GG genotype. P values < 0.05 were considered statistically significant on the base of two-sided test. Multiple comparison results were corrected by Bonferroni adjustment [37]. The testing level of Bonferroni

adjustment is $\alpha' = \alpha/n$, and n is the number of comparisons. Above-mentioned statistical analyses were conducted with the IBM SPSS v21.0 software (Inc., Chicago, IL, USA).

Expression quantitative trait loci (e-QTL) are genetic loci that control the expression of target gene, which use the mRNA expression level as a quantitative trait based on traditional QTL method. The e-QTL analysis can specifically affect the genetic variation of one or more gene expression levels, which makes it an effective and feasible strategy to assess the biological mechanism of SNPs in non-coding regions. To detect the effects of positive SNPs on gene expression levels, we used the BRAINEAC database (https://caprica.genetics.kcl.ac.uk/BRAINEAC/) to carry out e-QTL analysis. The data of this database came from MRC Sudden Death Brain Bank in Edinburgh, UK and the Sun Health Research Institute, including 10 brain regions (temporal cortex, frontal cortex, occipital cortex, hippocampus, medulla, putamen, substantia nigra, thalamus, intralobular white matter, and cerebellum).

Power analysis

Our discovery based on a case–control study, using Power V3.0 to estimate the statistical power [38]. At the test level of $\alpha = 0.05$ with MAF = 0.300 in CHS, the power to detect an OR of 1.5 was 0.975 with 752 cases and 774 controls.

Results

General characteristics of participants

The general characteristics of participants are shown in Table 1. Participants were 1526 children aged 6–18 years with an average age of 8.29 ± 1.92 years including 752 ADHD children (boys: girls = 2.96, average 8.43 ± 1.75 years) and 774 healthy controls (boys: girls = 2.39, average 8.16 ± 2.08 years). There was no significant difference in age ($P = 0.061$), sex ($P = 0.181$), BMI ($P = 0.153$), and intelligence quotient (IQ, $P = 0.399$) between case and control groups. Among ADHD children, inattention type (ADHD-I), hyperactive/impulsive type (ADHD-HI), and combined type (ADHD-C) were 44.3%, 20.9%, and 34.8%, respectively. The scores of SNAP-IV scale, PSQ, and IVA-CPT indicated ADHD case group was significantly higher than control group.

Distribution of candidate SNPs

As shown in Table 2, the call rates of all SNPs were higher than 95%, and the genotype distributions of rs3793243 and rs875342 were statistically different between the case and

Table 1 Demographic and clinical characteristics of the sample

Characteristics	Case ($n = 752$)	Control ($n = 774$)	t or χ^2	P
Age (mean ± SD)	8.43 ± 1.75	8.16 ± 2.08	1.885	0.061
BMI (mean ± SD)	16.49 ± 3.14	17.23 ± 9.10	− 1.433	0.153
IQ score (mean ± SD)	98.04 ± 13.88	96.84 ± 13.29	0.927	0.399
Gender				
Male	562	546	1.653	0.181
Female	190	228		
SNAP-IV score (mean ± SD)				
Attention-deficit score	16.65 ± 4.76	5.82 ± 3.56	35.631	**< 0.001**
Hyperactive/impulsive score	16.04 ± 4.96	5.25 ± 3.31	33.680	**< 0.001**
Average score	1.79 ± 0.46	0.61 ± 0.26	42.627	**< 0.001**
PSQ score (mean ± SD)				
Impulsive/hyperactive score	1.29 ± 0.58	0.87 ± 0.42	11.097	**< 0.001**
Hyperactive index	1.32 ± 0.44	0.90 ± 0.28	15.196	**< 0.001**
Total score	41.36 ± 17.34	30.94 ± 11.63	12.472	**< 0.001**
IVA-CPT (mean ± SD)				
FRCQ	79.05 ± 19.22	113.35 ± 14.03	− 26.941	**< 0.001**
FAQ	66.18 ± 19.58	112.33 ± 21.22	− 28.474	**< 0.001**
VRT	660.48 ± 104.71	761.47 ± 148.91	− 10.963	**< 0.001**
ART	701.39 ± 129.73	611.46 ± 146.23	12.354	**< 0.001**
HI	2.20 ± 0.92	0.74 ± 0.46	20.784	**< 0.001**

The significant results were in bold

FRCQ full-scale response control quotient, *FAQ* full-scale attention quotient, *VRT* visual response time, *ART* auditory response time, *HI* hyperactive index

Table 2 Distribution of candidate SNPs in participants

SNP	Call rate (%)	Case			Control			χ^2	P*	MAF	HWE-χ^2	P
		HW	HT	HV	HW	HT	HV					
rs3793243	100.0	239	398	114	211	398	165	10.720	**0.005**	0.444	1.647	0.199
rs35459363	97.5	312	336	104	271	360	81	5.482	0.065	0.364	0.979	0.322
rs875342	98.5	376	288	84	408	300	47	11.969	**0.003**	0.283	0.526	0.468

The signifcant results are shown in bold

HW wild-type homozygote, *HT* heterozygote, *HV* variant homozygote, *P** the significant level was corrected with the formula of $\alpha' = \alpha/3 = 0.017$ according to the Bonferroni method, *MAF* minor allele frequency

control groups after the Bonferroni adjustment. All SNPs accorded with HWE ($P > 0.05$).

Association analysis between candidate SNPs and the ADHD risk

Table 3 shows the summary of a genetic model analysis for the association of candidate SNPs and ADHD risk; after Bonferroni adjustment, the test level is $\alpha' = \alpha/3/5 = 0.003$. All the results were adjusted by age, gender, and site. The results indicated that rs3793243 was associated with ADHD risk under the recessive model (OR 0.668, 95% CI 0.498–0.836, $P = 0.001$) and the additive model (OR 0.792, 95% CI 0.674–0.912, $P = 0.001$). No association was found between rs35459363 and ADHD risk. We also discovered a nominal association of rs875342 and ADHD risk in the additive model ($P = 0.022$). Besides, rs3793243 GG genotype carriers had a lower risk of ADHD compared with AA genotype (OR 0.564, 95% CI 0.406–0.692, $P < 0.001$), and rs875342 AA genotype carriers had a high risk against GG genotype (OR 1.806, 95% CI 1.349–2.591, $P = 0.001$).

Association analysis between positive SNPs and ADHD clinical characteristics

Aforementioned positive SNPs, we included all participants to analysis their associations with ADHD clinical characteristics (as shown in Table 4). The rs3793243 was significantly correlated with the scores of SNAP-IV, and there was an association between rs875342 and SNAP-IV and IVA-CPT scores. After Bonferroni adjustment, the specific performance as follows: the rs3793243 showed significant associations with attention-deficit score and total score in the SNAP-IV scale ($P = 0.001$, respectively). The results indicated that AA wild type homozygote showed more obvious attention deficit behaviors than GG variant homozygote. The rs875342 also associated with attention-deficit score and total score in the SNAP-IV scale ($P < 0.001$, respectively), which means that AA variant homozygote expressed more attention deficit behaviors compared with GG wild-type homozygote. Furthermore, in the full-scale response control quotient (FRCQ) and full-scale attention quotient (FAQ) of IVA-CPT, the rs875342 was equally significant. AA variant homozygote hold lower attention quotient and response control quotient ($P < 0.001$, respectively).

e-QTL analysis

According to the e-QTL data from the BRAINEAC database, we found that the rs3793243 affected *STX1A* gene expression in the intralobular white matter (WHMT) and medulla (MEDU) (*P* values were 0.0058 and 0.0057, respectively), with the A allele showing lower mRNA levels compared to G allele, as shown in Fig. 3.

Discussion

To the best of our knowledge, Gao et al. were the first to discovery the association between *STX1A* gene and children ADHD in Chinese Han population [30]. In this study, a case–control study was used to repeatedly verify the association in Chinese Han population. We found for the first time that *STX1A* SNP rs3793243 was associated with ADHD, and the risk of ADHD in rs3793243 GG genotype variant homozygotes was 56.4% compared with AA genotype wild homozygotes. While the result of rs875342 was consistent with Gao et al. In addition, the two SNPs were also significantly associated with the clinical characteristics of ADHD.

The association between *STX1A* gene and ADHD was first reported in adult ADHD. In 2013, Sánchez-Mora et al. found that the *STX1A* gene was associated with adult ADHD [$P = 0.0041$, OR 1.28 (1.08–1.51)], followed by Kenar and Olgiati et al. [33, 39, 40]. As for children ADHD, early in 2005, Brookes et al. had explored the association of *STX1A* gene with ADHD in the Caucasian population, but the results were negative [31]. The above Sánchez-Mora et al. reported the same results in children ADHD. However, in 2015, Gao et al. found that *STX1A* SNP rs875342 was nominally associated with children ADHD [$P = 0.004$, OR 1.32 (1.09–1.60)] using a case–control study in a Chinese Han population with 1404 male patients and 617 male controls

Table 3 Association between candidate SNPs and risk of ADHD

SNP	Allele		HT vs. HW(12 vs. 11)		HV vs. HW(22 vs. 11)		Dominant(11 vs. 12+22)		Recessive(22 vs. 11+12)		Additive(11 vs. 12 vs. 22)	
	1[a]	2[b]	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**
rs3793243	A	G	0.903 (0.707, 1.087)	0.215	**0.564 (0.406, 0.692)**	**< 0.001**	0.819 (0.655, 0.967)	**0.028**	**0.668 (0.498, 0.836)**	**0.001**	**0.792 (0.674, 0.912)**	**0.001**
rs35459363	G	A	0.926 (0.639, 1.333)	0.536	1.466 (0.887, 2.650)	0.181	1.128 (0.751, 1.554)	0.754	1.484 (0.841, 2.678)	0.082	1.129 (0.893, 1.425)	0.351
rs875342	G	A	1.023 (0.852, 1.303)	0.702	**1.806 (1.349, 2.591)**	**0.001**	1.072 (0.966, 1.317)	0.228	1.118 (0.902, 1.376)	0.286	1.215 (1.036, 1.412)	**0.022**

The significant results are shown in bold

[a] major allele, [b] minor allele, *OR* odd ratio, *CI* confidence interval, *P** were adjusted for age, gender and site, the significant level was $\alpha' = \alpha/3/5 = 0.003$ according to the Bonferroni method

[30], which was consistent with our research. Furthermore, we found the association between *STX1A* SNP rs3793243 and children ADHD for the first time, where was not discovered by Gao et al. In view of the inconsistent results, furthermore, we need to increase the sample size for more stages. For the phenomenon that the association between *STX1A* gene and children ADHD only occurred in the Chinese Han population but not observed in Caucasians may be related to different races. Different ethnicities have different genetic models. In addition, we compared our results of the associated SNPs of *STX1A* gene with the readily public available GWAS results—Ricopili (https://data.broadinstitute.org/mpg/ricopili/)—from the Ricopili database; we found that the smallest *P* value of *STX1A* gene was 1.80×10^{-2} that not reach the corrected significant level as many other significant candidate genes as [41]. However, the participants in the Ricopili database were European origin, while our research was based on Chinese Han population, so we will increase the sample size from more sites to verify our results in future research. Though there was no SNP of *STX1A* gene passed the critical significant level, a recent ADHD-GWAS paper by Demontis et al. suggested genes that implicated neurodevelopmental processes were likely to be relevant to ADHD [42], which indicated *STX1A* gene that involved in axonal growth [43] and neurite sprouting [44] might be related to ADHD.

From the physiological functions, animal experiments, and pharmacological studies of *STX1A* gene, the association of this gene with ADHD is reasonable.

The *STX1A* gene encodes syntaxin-1A protein, which is a member of the syntaxin superfamily. Syntaxin-1A contains a single C-terminal transmembrane domain (known as H3) that is involved in binding other SNAREs and an N-terminal regulatory domain (Habc) [45], which together with SNAP-25 and VAMP constitute a SNARE complex that regulates vesicle fusion and neurotransmitter exocytosis [16]. Besides, syntaxin-1A is involved in axonal growth and neurite sprouting, and appears to be related to the Brain-Derived Neurotrophic Factor (BDNF) secretion: a hormone that regulates the development and plasticity of neurons [46].

In animal models, by targeting disruption of *STX1A* produced gene knockout mice, Fujiwara et al. reported not only the long-term potentiation in hippocampal slices was significantly decreased, but also the consolidation of conditioned fear memory by weak training was impaired from *STX1A*(−/−) mice [47]; these evidences indicated that *STX1A* gene may be related to synaptic plasticity. They also found that *STX1A*(−/−) and *STX1A*(+/−) mice showed abnormal behaviors similar to those observed in human neuropsychiatric patients. In the latent inhibition (LI) test, compared with wild type mice, *STX1A*(−/−) and *STX1A*(+/−) mice were significantly attenuated on the inhibition ratio. The deficit in the LI test appears to be closely involved in the

Table 4 Association between candidate SNPs and ADHD clinical characteristics

Clinical characteristics	rs3793243			*F*	*P**	rs875342			*F*	*P**
	HW	HT	HV			HW	HT	HV		
SNAP-IV score (mean ± SD)										
Attention-deficit score	11.39 ± 6.73	11.53 ± 7.13	9.75 ± 6.00	7.364	**0.001**	10.93 ± 6.82	11.01 ± 6.92	13.94 ± 6.01	11.169	**< 0.001**
Hyperactive/impulsive	10.17 ± 6.25	10.73 ± 6.83	9.37 ± 6.62	4.509	0.011	10.37 ± 6.71	10.10 ± 6.58	11.97 ± 6.35	4.144	0.016
Average score	1.20 ± 0.67	1.24 ± 0.72	1.06 ± 0.64	6.574	**0.001**	1.18 ± 0.68	1.17 ± 0.70	1.44 ± 0.65	8.295	**< 0.001**
PSQ score (mean ± SD)										
Impulsive/hyperactive score	1.12 ± 0.59	1.05 ± 0.55	1.05 ± 0.54	2.452	0.086	1.07 ± 0.57	1.05 ± 0.54	1.16 ± 0.60	2.021	0.133
Hyperactive index	1.15 ± 0.44	1.07 ± 0.43	1.06 ± 0.42	5.702	0.003	1.08 ± 0.45	1.09 ± 0.40	1.19 ± 0.48	3.310	0.037
Total score	35.88 ± 16.75	36.01 ± 17.29	34.94 ± 15.11	0.433	0.649	35.41 ± 16.30	35.90 ± 17.04	37.97 ± 19.06	1.274	0.280
IVA-CPT (mean ± SD)										
FRCQ	95.75 ± 24.41	97.09 ± 24.17	97.17 ± 26.86	0.470	0.625	97.73 ± 24.40	96.94 ± 24.72	88.10 ± 25.12	8.473	**< 0.001**
FAQ	87.55 ± 31.92	89.94 ± 30.52	93.02 ± 31.72	2.674	0.069	91.88 ± 30.16	89.42 ± 32.03	77.00 ± 29.95	12.736	**< 0.001**
VRT	726.22 ± 151.38	740.38 ± 147.10	740.21 ± 142.83	1.453	0.234	737.81 ± 142.86	733.60 ± 154.37	727.72 ± 143.16	0.321	0.725
ART	645.23 ± 160.56	659.19 ± 150.46	650.81 ± 134.03	1.290	0.275	658.09 ± 142.68	646.39 ± 157.25	653.37 ± 169.84	1.011	0.364
HI	1.79 ± 1.21	1.78 ± 1.22	1.61 ± 1.19	0.678	0.508	1.74 ± 1.22	1.79 ± 1.20	1.75 ± 1.23	0.135	0.874

The signifcant results are shown in bold

*P** the significant level was corrected with the formula of $\alpha' = \alpha/2/11 = 0.002$ according to the Bonferroni method

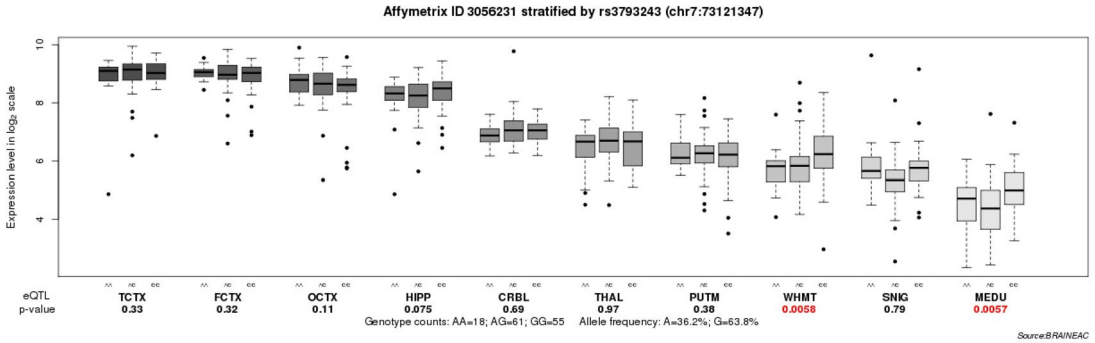

Fig. 3 Association of rs3793243 and *STX1A* expression in human brain tissues in BRAINEAC database

deficit in attentional processes or behavioral control; consequently, deletion of *STX1A* gene may be related to neuropsychological changes. At the same time, the LI test is mainly used to measure selective attention; in other words, *STX1A* gene knockout mice may be related to attention deficit. Besides LI attenuation, in the social interaction test, *STX1A* gene knockout mice showed abnormal behaviors as well as in the novel object exploration test that aimed to measure cognition were impaired [48]. These results indicated that the deletion of *STX1A* gene might lead to cognition deficit.

In ADHD pharmacology, multitier studies have shown that the genetic variation of SNARE complex-related genes was involved in the efficacy of ADHD [49]. For animal model of pharmacology, Barakauskas et al. observed that haloperidol treatment would increase the level of syntaxin-1A in the striatum region of rats [50]. In a recent pharmacogenetic study, the authors evaluated four SNPs of *STX1A* (rs2228607), *VAMP2* (26bp Ins/Del) and *SYT1* (rs1880867 and rs2251214) whether they were effective to immediate-release methylphenidate (IR-MPH) treatment, and the results showed that *SYT1*-rs2251214 was associated with the categorical short-term response to IR-MPH, indicating that SNARE complex-related genes were associated with the treatment of MPH [51].

As a key protein of neurotransmitter release, syntaxin-1A encoded by *STX1A* gene is directly involved in the release of 5-HT, and alters its subcellular localization and expression [52]. Previous study indicated chronic deficit of 5-HT at the synapse could trigger ADHD symptoms [53]. In an animal experiment, glutamate was used to stimulate hypothalamic or hippocampal slices of mice to induce 5-HT release from the presynaptic membrane, simultaneous analyzing the dose–response relationship. The authors observed that the dose–response curve of *STX1A*(−/−) mouse was shifted to the right, which meant that the effect of glutamate stimulation on 5-HT release in *STX1A*(−/−) mice was significantly lower than in wild-type mice, that is to say, *STX1A* gene knockout decreases the release of 5-HT [54]. Accordingly, one of the mechanisms of *STX1A* gene associated with ADHD may be that the low expression of *STX1A* gene decreases 5-HT release, which in turn causes ADHD-related symptoms.

In addition to the regulation of 5-HT, *STX1A* gene also takes part in glutamate transport and the conversion of glutamate to gamma-aminobutyric acid (GABA), which makes *STX1A* an important part of GABAergic system. GABA is an important inhibitory neurotransmitter in the central nervous system. Glutamate transporter mediates the reuptake of neurotransmitters, which can remove GABA from the synaptic cleft and store it again at the end of the presynaptic membrane; thus, it is crucial for maintaining synaptic excitability and avoiding nerve damage. Meanwhile, a study indicated that as a key regulator of the internalization of glutamate transporters, syntaxin-1A was also an intrinsic enhancer to glutamate transporters endocytic sorting [55]. As mentioned above, the *STX1A* gene may cause ADHD by affecting the expression of glutamate transporters.

Furthermore, the results of e-QTL analysis suggested that the rs3793243 variation was associated with the *STX1A* mRNA expression. The dangerous allele A showed a lower mRNA level in the WHMT and MEDU, indicating that the SNP in our study may affect the expression of *STX1A* gene then affecting the release of 5-HT in the brain and the neurotransmission of GABAergic, and ultimately leading to ADHD.

In summary, using a case–control study, this research repeatedly found that *STX1A* genetic variation was associated with children ADHD in Chinese Han population, and we first discovered that *STX1A* rs3793243 genetic variation was associated with children ADHD; however, this result needs to further functional studies to confirm. Besides, there are some limitations in our research. First, the sample size was not very large, and future researches should increase the sample size for more stages. Second, we only selected three important potential functional SNPs, so the follow-up needs to increase other loci for gene sequencing.

Conclusions

Using a case–control study to explore the association between *STX1A* gene and children ADHD in Chinese Han population, our results suggest that *STX1A* genetic variants might contribute to the susceptibility of children ADHD. Future studies with larger sample sizes are needed to confirm these results and further functional researches are warranted to identify the mechanism of how potential functional SNPs play roles in ADHD.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interests.

Informed consent Written informed consent was obtained from all participants. The Ethics Committees of Tongji Medical College of Huazhong University of Science and Technology approved this study.

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