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The Association of SNAP25 Gene Polymorphisms in Attention Deficit/Hyperactivity Disorder: a Systematic Review and Meta-Analysis

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Abstract Attention deficit/hyperactivity disorder (ADHD) is one of the most highly heritable psychiatric disorders in childhood. The risk gene mutation accounts for about 60 to 90 % cases. Synaptosomal-associated protein of 25 kDa (SNAP-25) is a presynaptic plasma membrane protein which is expressed highly and specifically in the neuronal cells. A number of evidences have suggested the role of SNAP-25 in the etiology of ADHD. Notably, the animal model of coloboma mouse mutant bears a ~2-cM deletion encompassing genes including *SNAP25* and displays spontaneous hyperkinetic behavior. Previous investigators have reported association between SNPs in *SNAP25* and ADHD, and controversial results were observed. In this study, we analyzed the possible association between six polymorphisms (rs3746544, rs363006, rs1051312, rs8636, rs362549, and rs362998) of *SNAP25* and ADHD in a pooled sample of ten family-based studies and four case–control studies by using meta-analysis. The combined analysis results were significant only for rs3746544 ($P = 0.010$) with mild association (odds ratio (OR) = 1.14). And, the meta-analysis data for rs8636, rs362549, and rs362998 are the first time to be reported; however, no positive association was detected. In conclusion, we report some evidence supporting the association of *SNAP25* to ADHD. Future research should emphasize genome-wide association studies in more specific subgroups and larger independent samples.

Keywords ADHD · SNAP-25 · Polymorphism · Association · Meta-analysis

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Introduction

Attention deficit/hyperactivity disorder (ADHD [MIM143465]) is a common psychiatric disorder [1, 2] in childhood worldwide that affects 6–7 % of school age children when diagnosed via the Diagnostic and Statistical Manual IV (DSM-IV) criteria [3]. Boys are diagnosed approximately threefold greater incidence than girls [4, 5]. It is typically characterized by significant problems of attention, hyperactivity, or acting impulsively that are not appropriate for a person's age [6]. Three different subtypes are recognized according to DSM-IV as follows: primarily inattentive (ADHD-I), primarily hyperactive/impulsive (ADHD-HI), and combined (ADHD-C), among them, the inattentive and combined subtypes are the most prevalent [7]. ADHD children are at high risk of negative long-term outcomes including academic underachievement and high accident rates and difficulty sustaining stable social relationships [8]. And, if they were left untreated, the effects tend to persist in adulthood [9, 10] with approximately 2.5 % of adults meeting diagnostic criteria for ADHD [11]. ADHD adults are at high risk of car accidents, divorce, substance misuse, and frequent job changes [12–14]. It is believed that both genetic factors and environmental factors and its interactions can contribute to this disorder [15]. Family, twin and adoption studies have indicated a strong genetic component in the susceptibility to ADHD, with an average estimated heritability (the proportion of phenotypic variance explained by genetic factors) of 0.76 [8].

Pathophysiology of ADHD

Animal and human studies have implicated the dysregulation of prefrontal cortical areas [8], basal ganglia, cerebellum, and temporal and parietal cortex [8, 16] in the pathophysiology of ADHD. And, this condition is complex and likely associated with functional impairments in some of the brain's neurotransmitter systems, particularly those involving dopamine and norepinephrine. To date, a large number of studies on different candidate genes for ADHD have been published (dopaminergic neurotransmission system: *DRD4*, *DAT1/SLC6A3*, *DRD5*, *COMT*, and *DBH*; noradrenergic neurotransmission system: *NET1/SLC6A2*, *ADRA2A*, and *ADRA2C*; serotonergic: *5-HTT/SLC6A4*, *HTR1B*, *HTR2A*, and *TPH2*; and central nervous system development pathway: *SNAP25* and *BDNF*) in the etiology of ADHD [17–20]. Alterations in the dopamine and norepinephrine pathways can impair the prefrontal cortex (PFC) function which is responsible for integrating cortical and subcortical inputs to execute essential cognitive functions such as attention, motivation, working memory planning, and decision making [21–24]. There may additionally be abnormalities of this disorder in serotoninergic and cholinergic pathways [25, 26]. And, the synaptosomal-associated protein 25 (SNAP-25) is interesting for its involvement in a number of processes including axonal growth, synaptic plasticity, and the vesicular release of neurotransmitters [27, 28].

In addition, the environmental factors such as prenatal nicotine [29], alcohol [30] or lead exposure, and low birth weighting [31] are believed to play a lesser role in the pathogenesis of ADHD.

Structure of SNAP-25

Synaptosomal-associated protein 25 gene (*SNAP25* [MIM 600322]) is located at chromosome 20p11.2 [32] in humans. Differential splicing of *SNAP25* results in the expression of two transcripts, SNAP25a and SNAP25b [33, 34]. These splice variants differ by only 9 out of 206 amino acids, a result of differential usage of two alternative exon 5 sequences (exon 5a/5b). SNAP-25 has been identified in contributing two α-helix motifs to the N-ethylmalemide-sensitive factor attachment protein receptor (SNARE) complex [35], SN1 and SN2, as revealed from the crystal structure of the four-α-helix domain complex [36]. The C-terminus of SNAP-25 SN2 is known to be the target of botulinum neurotoxins A and E (BoNT/A and BoNT/E), which block the release of neurotransmitters in vivo [37–39]. Biochemical analyses have shown that SNAP-25 amino acids (AA) 7–83 and 141–204 are essential motifs that are spontaneously assembled into helical SNARE complexes with Syntaxin1 and synaptobrevin 2 motifs [40]. SNAP-25 mutations introduced to the C terminal of the protein at AA positions 78, 81, and 202 resulted in a near elimination of exocytosis [40].

Functions of SNAP-25

Regulating Neural Signal Transmission: Role as a SNARE Protein (Fig. 1)

SNAP-25 is a presynaptic membrane bound protein which is anchored to the cytosolic surface of membranes via palmitoyl side chains located in the central region of the molecule [41, 42]. Together with synaptobrevin/VAMP and syntaxin/HPC-1, SNAP-25 constitutes the soluble SNARE protein core complex [43, 44] which is essential for docking and holding synaptic vesicles at the presynaptic membrane in preparation for Ca^{2+}-triggered neurotransmitter exocytosis [42, 45–47]. SNAP-25 has been identified in contributing two α-helices to the SNARE complex, one located around the center and another at the C-terminal end of the SNARE bundle [48, 49]. At the presynaptic plasma membrane site wherein SNAP-25 located primarily, on contact, SNARE protein complex is initiated amino-terminally and proceeds

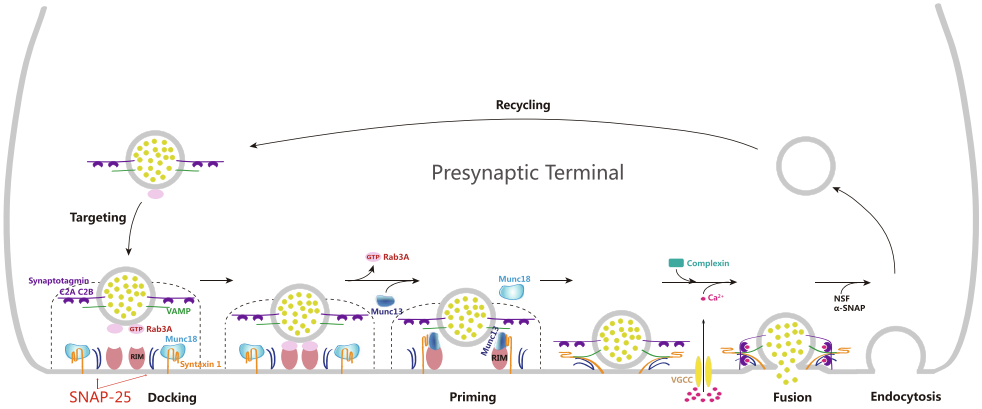

Fig. 1 The function of SNAP-25 in the neurotransmitter release

toward the C terminus in a zipper-like fashion, thus pulling the synaptic vesicle and the presynaptic membranes together [40, 50]. Recently, it has been discovered that the interaction with the central SNARE motifs of SNAP-25 is essential for vesicle docking, priming, and fast fusion-triggering exocytosis. As to the C-terminal binding interface, it only plays a subsidiary role in triggering but is required for the full size of the readily releasable pool [49].

Regulating Neural Signal Transmission: Role as a Cellular Calcium Modulator (Fig. 1)

Calcium channel activity, as a kind of second messenger, has an immediate impact on synaptic activity [51]. SNAP-25 interacts with different types of voltage-gated calcium channels (VGCCs) [52], including N-type [53], P/Q-type [54, 55], and L-type [56, 57], inhibiting their function and thus reducing neuronal calcium responsiveness to depolarization [58–60]. It is notable that the different neuropsychiatric alterations where SNAP-25 has been involved are characterized by a dysregulation of calcium homeostasis [61, 62]. Different levels of SNAP-25 expression in excitatory versus inhibitory neurons may profoundly modulate neuronal responses to synaptic stimuli in a dose-dependent manner, and therefore, SNAP-25 is involved in the regulation of neuronal excitability [58]. SNAP-25 has been discovered to be a target of protein kinase C (PKC) on its residue serine in position 187 (Ser187), and PKC phosphorylation of SNAP-25 at Ser187 was found to be crucial for the negative regulation of VGCCs [59]. For the reason that Ser187 phosphorylation is transiently induced by neuronal activity [59], it is suggested that SNAP-25 provides a negative feedback mechanism for controlling neuronal excitability. It is also possible that the effects of reducing endogenous SNAP-25 expression have a greater impact on VGCC regulation than on the function of the protein as a SNARE [63, 64].

Axonal Growth and Synaptic Plasticity

Evidence derived from organisms ranging from reptiles such as geckos to humans indicates that SNAP-25 can promote outgrowth and elongation of neuritis [65, 66]. A high level of SNAP-25 expression in the adult brain was found to contribute to nerve terminal plasticity [43]. Upregulating and downregulating SNAP-25 by combined lentiviral packaging and siRNA, the result that SNAP-25 is specific for neural remodeling has been obtained [67]. Findings that selective inhibition of SNAP-25 expression imposed restrictions on neurite outgrowth have been reported [27, 68–70]. In addition, SNAP25 is associated with neuronal maturation and synaptogenesis during development [71] and also affects the expression of receptors like NMDARs in the plasma membrane [72, 73]. These findings indicate that SNAP25 may be involved in a mechanism relevant to axonal growth and synaptic plasticity.

SNAP-25 and ADHD

According to the functions of SNAP-25, it is possible that any variation in SNAP-25 which located primarily and specifically in axons and nerve terminals [67, 68] might interfere in the susceptibility of ADHD by influencing the release of neurotransmitters [74–76] and establishing neural circuits during central nervous system (CNS) development [77].

SNAP25 and ADHD Mouse Model

The coloboma mouse mutant, heterozygous for a ~2-cM deletion of chromosome 2 (Cm) that encompasses the *SNAP-25* gene and therefore exhibiting 50 % reduction of SNAP-25 expression [78], is considered an animal model of ADHD, as it displays certain hallmarks of ADHD including mainly locomotor hyperactivity [79] as well as inattention and impulsivity [80]. Furthermore, the

hyperactive phenotype of coloboma mouse has been shown to be ameliorated by the psychostimulant d-amphetamine or with a transgenic insertion [81–83]. Animal studies have also shown involvements of SNAP-25 in neurotransmitter systems like dopamine and norepinephrine pathways [84, 85]. A significant reduction of Ca^{2+}-dependent dopamine release from the dorsal striatum region but not ventral striatum [82, 86] and an increase of up to 40 % in noradrenaline within the striatum and the nucleus accumbens [87] of the coloboma (Cm/+) mouse have been reported. Additionally, an enhanced calcium response through L-type channels has been described in the SHR model of ADHD, where a reduction of SNAP-25 also occurs [88]. Consequently, SNAP-25 mediates neurotransmitter release like acetylcholine [89, 90] and plays essential roles in neurotransmitter release at different steps. These findings represent therefore that the gene *SNAP25* could play a part in several heritable neurocognitive and behavioral abnormalities [91].

The Genetic Variation of SNAP25 and ADHD

Using a transmission disequilibrium test (TDT), Barr et al. [92] found a trend of excess transmission of the C allele of rs1051312 in Canadian and Brophy et al. [93] reported preferential transmission of allele T of rs1051312 in Irish ADHD cases. Significant association between rs3746544 (1065 T>G) and ADHD was reported in Chinese [94] and Colombian [95] populations in case–control studies, while negative results were reported in Irish [21, 93], Indian [96], Canadian [92, 97], US Caucasian [97], and UK Caucasian [98] populations. Feng et al. [97] examined 12 SNPs in two independent samples of ADHD families and found significant over-transmission of the rs66039806-C, rs362549-A, rs362987-A, and the rs362998-C alleles which located in introns 2, 4 and 4 and exon 6, respectively, in the Canadian sample, but not in the southern California sample. When they tested Canadian sample with quantitative analysis for hyperactivity and inattention ADHD subtypes, they found associations for both of behavioral ADHD subtypes with *SNAP25*. Mill [98] and Kim [99] reported significant association with additional SNPs rs363006 (intron 7) and rs3787283 (intron 7), respectively. Nevertheless, several studies [100, 101] have yielded negative results for the association of ADHD with the polymorphisms mentioned above.

In our study, we carry out a comprehensive meta-analysis to summarize the associations of the reported polymorphisms of SNAP-25 gene (rs3746544, rs363006, rs1051312, rs8636, rs362549, and rs362998) (Fig. 2) with childhood ADHD.

Methods

Study Sample Identification and Inclusion/Exclusion Criteria

We used a three-stage approach to identify relevant studies for meta-analysis. Firstly, we conducted searches of four databases—PubMed, Web of Science, Elsevier, and Google Scholar—to identify an initial set of articles. These potentially relevant reports were published in a definite time from June 1996 to February 2016. The search terms used to query the databases included "ADHD" or "attention deficit hyperactivity disorder" and related terms such as "ADD," "attention deficit," "inattention," and "hyperactivity." Each of these terms was combined with "Synaptosomal-associated protein 25" or "SNAP25" to conduct searches. Then, we searched reference lists of relevant review and original published studies identified in the first stage to identify additional studies that might have been missed in stage 1. Finally, to determine which studies would be included in the meta-analysis for a given polymorphism, a series of inclusion and exclusion criteria were applied as follows: (1) studies evaluating the association between SNAP-25 gene and ADHD were included, and other pharmacological, functional, biochemical, or animal model studies were excluded; (2) family-based studies employing haplotype-based haplotype relative risk (HHRR) procedure or transmission disequilibrium test (TDT) and case–control studies were included; (3) studies including SNPs (rs3746544, rs1051312 et al.) of SNAP-25 gene and containing useful original data which was able to calculate the odds ratio (OR) were included; (4) each included study was required to report data from an independent sample; (5) only studies that used children samples (age range from 7 to 16) were included, and studies using adult samples were excluded from the present investigation; (6) all ADHD probands or cases used in each included study met DSM-IV/III criteria; and (7) all subjects used in each included study had an intelligence quotient (IQ) test score above 70 and were free of other neurological disorders.

Meta-Analytic Methods

Heterogeneity in effect sizes across studies was assessed using the Q-statistic [102], and its magnitude was quantified using I^2 [103], which is an index that describes the proportion of total variation in study effect size. The heterogeneity was considered significant when $P<0.10$ for Q-statistic and qualified by I^2. Mild heterogeneity might account for less than 30 % of the variability in point estimates, notable heterogeneity more than 50 %,

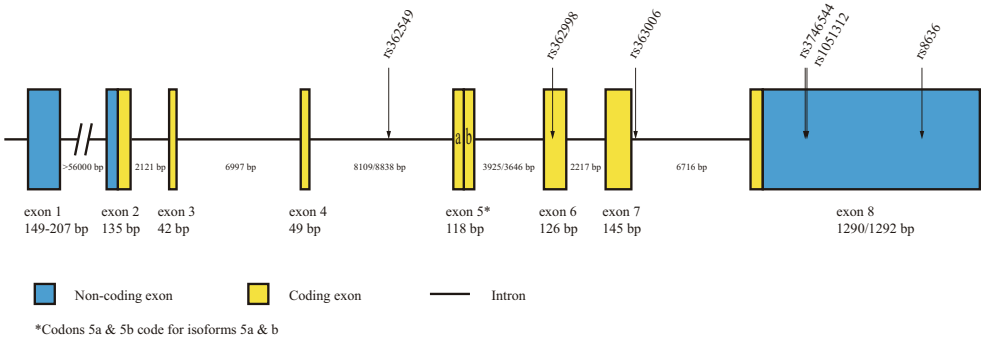

Fig. 2 Human SNAP-25 gene structure and location of SNAP-25 polymorphisms. *Codons 5a and 5b code for isoforms 5a and b

and moderate heterogeneity between them [103]. For TDT studies, ORs were estimated from the number of transmissions versus non-transmissions of the designated "high-risk" allele to ADHD cases from heterozygous parents. For case–control studies, ORs were estimated by contrasting the ratio of counts of the "high-risk" versus "low-risk" alleles in ADHD cases versus non-disordered controls. We calculated the pooled ORs with 95 % CIs and drew forest plots. A fixed effect model (FEM) was applied if the heterogeneity is not statistically significant ($P<0.05$); otherwise, a random effect model (REM) was adopted [104]. Funnel plot, Egger's linear regression model, and Begg's rank correlation test were applied to evaluate the evidence for publication bias, and no publication bias exists when $P>0.05$. Sensitivity analyses were performed if the heterogeneity was moderate and notable to estimate the sources of heterogeneity. The meta-analysis was performed by the metafor package (version 1.9-5; http://cran.r-project.org/web/packages/metafor/index.html) (Nicodemus KK 2008 [105]) in R (version 3.1.2; http://www.r-project.org/).

Results

A total of 14 studies were finally included after the meta-analysis literature selection, and the flow chart is shown in Supplementary Fig. 1. Ten were TDT designs, three were case–control designs, and one study adopted both methods. When one study employed two association analysis methods, data from the method that presented the largest dataset were included according to the finding that estimates of association were equivalent in aggregate across methods with observed differences most likely due to uncertainty in the estimates resulting from small sample sizes [106]. As a result, data from these 14 studies that analyzed six common variants within the *SNAP25* gene was applied for meta-analytical procedures (Supplementary Fig. 2) and the descriptive characteristics of the studies are shown in Tables 1 and 2. Meta-analytic results for associations between candidate gene polymorphisms in the *SNAP25* gene and childhood ADHD and general information about the SNPs are shown in Table 3.

For the meta-analysis of the association between childhood ADHD and rs3746544, eight TDT studies and four case–control studies were identified. Moderate heterogeneity in effect sizes across studies was observed (Q-statistic $\chi^2=19.3528$, $P=0.055$, $I^2=41.56$ %). The pooled results (Fig. 3a) indicated a significant and modest association between ADHD and the "T" allele (fixed effects: OR = 1.14, 95 % CI 1.03–1.26, $P=0.010$). For sensitivity analysis, three of the pooled ORs changed qualitatively after excluding one single study each time. It suggested that the results of this meta-analysis were not stable (Supplementary Table 1). The funnel plot was generally symmetrical, showing no evidence of publication bias (Supplementary Fig. 3a). And, Egger's test ($P=0.109$) and Begg's test ($P=0.153$) also suggested that publication bias was not significant.

The meta-analysis for rs363006 (Fig. 3c) included six TDT studies. The heterogeneity in effect sizes across studies was mild (Q-statistic $\chi^2=5.9374$, $P=0.3124$, $I^2=0.00$ %). The pooled results were non-significant (fixed effects: OR = 1.04, 95 % CI 0.87–1.25, $P=0.667$). And, no significant publication bias existed (Egger's test: $P=0.514$; Begg's test: $P=0.272$).

As to the meta-analysis for rs1051312 (Fig. 3b), seven TDT studies and two case–control studies were included. The pooled results did not support the association with ADHD (random effects: OR = 0.96, 95 % CI 0.79–1.17, $P=0.688$). Notable heterogeneity in effect sizes across studies was observed (Q-statistic $\chi^2=16.207$, $P=0.040$, $I^2=50.78$ %). Subsequently, sensitivity analysis was performed and the pooled ORs did not change qualitatively after excluding one single study each time. It suggested that the results of this meta-analysis were stable (Supplementary Table 1).

Table 1 Descriptive characteristics of the included studies in transmission disequilibrium test design

Site	Study	Ethnicity\country	Samples size[a]	Allele	T	NT	*P* value	Odds radio
rs3756544	Barr CL, 2000	Caucasian\Canada	97	T 0.663	48	47	0.918	1.02 (0.68, 1.53)
	Brophy K, 2002	Caucasian\Ireland	69	T	35	33	0.808	1.06 (0.66, 1.71)
	Kustanovich V, 2003	Caucasian\USA	113	T	117	92	0.084	1.27 (0.97, 1.67)
	Mill J, 2004	Caucasian\UK	188	T	68	56	0.281	1.21 (0.85, 1.73)
	Feng Y, 2005 (1)	Caucasian\USA	99	T 0.601	36	35	0.906	1.03 (0.65, 1.64)
	Feng Y, 2005 (2)	Caucasian\Canada	186	T 0.673	79	82	0.813	0.96 (0.71, 1.31)
	Kim JW, 2007	Caucasian\USA	229	T	125	104	0.165	1.20 (0.93, 1.56)
	Hawi Z, 2013	Caucasian\Ireland, Australia, UK	339	T	143	162	0.277	0.88 (0.70, 1.11)
rs1051312	Barr CL, 2000	Caucasian\Canada	97	T 0.772	32	48	0.074	0.67 (0.43, 1.04)
	Brophy K, 2002	Caucasian\Ireland	69	T	34	16	*0.011*	2.12 (1.17, 3.85)
	Kustanovich V, 2003	Caucasian\USA	113	T	106	119	0.386	0.89 (0.69, 1.16)
	Mill J, 2004	Caucasian\UK	188	T	43	31	0.163	1.39 (0.87, 2.20)
	Feng Y, 2005 (1)	Caucasian\USA	99	T 0.786	18	25	0.286	0.72 (0.39, 1.32)
	Feng Y, 2005 (2)	Caucasian\Canada	186	T 0.779	72	71	0.933	1.01 (0.73, 1.41)
	Kim JW, 2007	Caucasian\USA	229	T	111	111	1.000	1.00 (0.77, 1.30)
rs363006	Mill J, 2004	Caucasian\UK	188	G	48	29	*0.030*	1.66 (1.04, 2.62)
	Feng Y, 2005 (1)	Caucasian\USA	99	G 0.850	14	20	0.391	0.70 (0.35, 1.39)
	Feng Y, 2005 (2)	Caucasian\Canada	186	G 0.825	56	57	0.925	0.98 (0.68, 1.42)
	Kim JW, 2007	Caucasian\USA	229	G	72	69	0.801	1.04 (0.75, 1.45)
	Renner TJ, 2008	Caucasian\Germany	111	C	37	38	0.908	0.97 (0.62, 1.53)
	Zhang H, 2011	Asian\China	102	G	18	22	0.527	0.82 (0.44, 1.53)
rs8636	Feng Y, 2005 (1)	Caucasian\USA	99	T 0.392	34	34	1.000	1.00 (0.62, 1.61)
	Feng Y, 2005 (2)	Caucasian\Canada	186	T 0.337	82	80	0.875	1.02 (0.75, 1.39)
	Hawi Z, 2013	Caucasian\Ireland, Australia, UK	339	T	174	149	0.164	1.17 (0.94, 1.45)
rs362549	Feng Y, 2005 (1)	Caucasian\USA	99	A 0.509	23	32	0.225	0.72 (0.42, 1.23)
	Feng Y, 2005 (2)	Caucasian\Canada	186	A 0.503	108	74	*0.012*	1.46 (1.09, 1.96)
	Kim JW, 2007	Caucasian\USA	229	A	137	126	0.498	1.09 (0.85, 1.38)
	Zhang H, 2011	Asian\China	102	A	47	45	0.835	1.04 (0.39, 1.57)
rs362998	Feng Y, 2005 (1)	Caucasian\USA	99	C 0.943	6	9	0.606	0.67 (0.24, 1.87)
	Feng Y, 2005 (2)	Caucasian\Canada	186	C 0.940	28	13	*0.019*	2.15 (1.12, 4.16)
	Kim JW, 2007	Caucasian\USA	229	C	34	28	0.446	1.21 (0.74, 2.00)
	Hawi Z, 2013	Caucasian\Ireland, Australia, UK	339	C	40	32	0.346	1.25 (0.79, 1.99)

Italicized text indicates significant result at $P < 0.05$. All reported *P* values are one tailed

T transmitted, *NT* not transmitted, *OR* odds radio

[a] The sample size was counted in a unit of ADHD families or ADHD probands

The funnel plot (Supplementary Fig. 3b) was substantially symmetrical, with Egger's test ($P = 0.763$) and Begg's test ($P = 0.920$) results shown no evidence of publication bias.

Each of the meta-analyses for rs8636 (Fig. 3d), rs362549 (Fig. 3e), and rs362998 (Fig. 3f) included four studies which is the low threshold for meta-analysis procedure. The pooled results did not indicate any association with ADHD (fixed effects: OR = 1.09, 95 % CI 0.93–1.27, $P = 0.289$; OR = 1.14, 95 % CI 0.97–1.34, $P = 0.122$; OR = 1.31, 95 % CI 0.98–1.75, $P = 0.069$, respectively). The heterogeneity (*Q*-statistic $\chi^2 = 0.831$, $P = 0.842$, $I^2 = 0.00$ %; *Q*-statistic $\chi^2 = 5.849$, $P = 0.119$, $I^2 = 47.61$ %; *Q*-statistic $\chi^2 = 3.965$, $P = 0.265$, $I^2 = 0.00$ %, respectively) in effect sizes across studies was mild for rs8636 and rs362998 and moderate for rs362549. We did sensitivity analysis for rs362549, and the pooled ORs did not change qualitatively after excluding one single study each time, suggesting that the results of this meta-analysis were stable (Supplementary Table 1). Egger's test ($P = 0.100$; $P = 0.425$; $P = 0.728$, respectively) and Begg's test

Table 2 Descriptive characteristics of the included studies in case–control design

Site	Study	Ethnicity\country	Samples size	Allele	Case	Control	*P* value	Odds radio
rs3756544	Choi TK, 2007	Mongoloid\Korea	95\102	T	152	154	0.283	1.30 (0.81, 2.09)
				G	38	50		
	Gao XP, 2009	Asian\China	138\119	T	232	177	*0.007*	1.82 (1.18, 2.81)
				G	44	61		
	Sarkar K, 2012	Caucasoid\India	150\100	T	225	148	0.801	1.05 (0.70, 1.59)
				G	75	52		
	Galvez JM, 2014	Caucasian\Colombia	73\152	T	118	201	*0.001*	2.16 (1.34, 3.47)
				G	28	103		
rs1051312	Sarkar K, 2012	Caucasoid\India	150\100	T	267	178	1.000	1.00 (0.56, 1.77)
				C	33	22		
	Galvez JM, 2014	Caucasian\Colombia	73\152	T	105	243	0.057	0.64 (0.41, 1.02)
				C	41	61		
rs8636	Sarkar K, 2012	Caucasoid\India	150\100	T	78	52	1.000	1.00 (0.67, 1.50)
				C	222	148		

Italicized text indicates significant result at $P < 0.05$. All reported *P* values are one tailed

($P = 0.750$; $P = 0.750$; $P = 0.750$, respectively) results did not show any evidence of publication bias.

Discussion

Imaging studies have suggested the contribution of a wider range of dysfunctions of neural networks to the diversity of ADHD symptoms [23]. Based on the effect of psychostimulants used in the pharmacological treatment of ADHD [107, 108], such as methylphenidate or amphetamines, dysfunctions in neuroplasticity mechanisms and synapses have been postulated to be involved in the pathogenesis of ADHD [109–111]. Specifically, the lowered expression of SNAP-25 in regions that are critical for attention and inhibition, such as inferior frontal gyrus (IFG), may ultimately decrease the efficiency of neurotransmitter release and synaptic function, impair behavior and cognition, and confer risk to ADHD [21]. Furthermore, the physiological functions of SNAP-25 in docking and fusion of synaptic vesicles in presynaptic neurons, as well as in axonal growth and synaptic plasticity, also make *SNAP25* an important candidate gene for ADHD.

In the present study, we investigated the association of six SNPs within *SNAP25* with childhood ADHD and did find some evidences to support the association. In a latest meta-analysis, Gizer and colleagues [20] evaluated four previously studied SNPs in the 3′-UTR and introns of *SNAP25* in association with ADHD and found only one significant association (a pooled OR of 1.15 for the T allele of rs3746544 was found, including data from seven studies). When we extract data from previous studies for a pooled analysis, also only significant mild association between rs3746544 and childhood ADHD was observed. These results are in accordance with Gizer's [20] and the previous meta-analyses [112, 113]. Although the association between 3′-UTR rs3746544 polymorphism and childhood ADHD existed moderate heterogeneity in effect sizes across studies in our meta-analysis, it still generated the even increased positive evidence for this association (OR 1.21, 95 % CI 1.08–1.35) when the study by Hawi et al. [21] according to the sensitivity analysis was removed.

In contrast to this positive result, non-significant combined results between the rest of five SNPs (rs1051312-T, rs363006-G, rs8636-T, rs362549-A, and rs362998-C) and childhood ADHD were observed, which are also uniform with the meta-analysis by Gizer [20]. And, to our knowledge, the meta-analysis results for rs8636, rs362549, and rs362998 are the first time to be reported. It is worthwhile to note that the association between rs1051312 and rs362549 polymorphisms and childhood ADHD existed notable and moderate heterogeneity in effect sizes across studies, respectively. However, when further sensitivity analyses were performed, none of the results changed qualitatively after excluding one single study each time. This indicated that our negative meta-analysis results were stable.

Heterogeneity in effect sizes across studies characterized several of the reviewed markers, including some that showed significant evidence for association and others that did not. This highlights the need for future studies that examine differences in methodological aspects and sample characteristics that can explain such heterogeneity and point to ways of maximizing associations.

Table 3 Meta-analytic results for associations between candidate gene polymorphisms in the SNAP25 gene and childhood ADHD and general information about the SNPs

Polymorphism	Location	Major/minor allele[a]	MAF	Studies (TDT/CC)	Meta-analysis model (fixed/random)	Results		Q-statistics		Begg's rank correlation test Kendall's tau (*P* value)	Egger's linear regression model *t* (*P* value)
						OR (95 % CI)	Z score (*P* value)	X^2 (*P* value)	I^2 (%)		
rs3746544	3′-UTR	T/G	0.29	12 (8/4)	Fixed	1.14 (1.03, 1.26)	2.562 (0.010)	19.353 (*0.055*)	41.56	0.333 (0.153)	1.759 (0.109)
rs363006	Intron 7	G/A	0.16	6 (6/0)	Fixed	1.04 (0.87, 1.25)	0.431 (0.667)	5.937 (0.312)	0.00	−0.467 (0.272)	−0.716 (0.514)
rs1051312	3′-UTR	T/C	0.15	9 (7/2)	Random	0.96 (0.79, 1.17)	−0.401 (0.688)	16.207 (**0.040**)	50.78	0.056 (0.920)	0.314 (0.763)
rs8636	3′-UTR	C/T	0.27	4 (3/1)	Fixed	1.09 (0.93, 1.27)	1.061 (0.289)	0.831 (0.842)	0.00	−0.333 (0.750)	−2.919 (0.100)
rs362549	Intron 4	G/A	0.46	4 (4/0)	Fixed	1.14 (0.97, 1.34)	1.546 (0.122)	5.849 (0.119)	47.61	−0.333 (0.750)	−0.993 (0.425)
rs362998	Exon 6	C/T	0.14	4 (4/0)	Fixed	1.31 (0.98, 1.75)	1.821 (*0.069*)	3.965 (0.265)	0.00	−0.333 (0.750)	−0.400 (0.728)

Bold text indicates significant result at $P < 0.05$. Italics indicate a trend toward significant at $P < 0.10$. All reported *P* values are one tailed. I^2 describes the proportion of total variation in study effect size due to heterogeneity

MAF minor allele frequency, *OR* odds radio

[a] The information is derived from NCBI's dbSNP

All of the associations that we meta-analyzed and reviewed herein were for single polymorphisms in genes, but multiple polymorphisms and/or their interactions were also observed. For example, strong linkage disequilibriums (LDs) between rs3746544-rs8636 in subjects from USA and Canada [97], British [114], and Indian [96] as well as LD between rs1051312-rs8636 [96, 97] and rs3746544-rs1051312 [96] were reported. Other SNPs in *SNAP25* which were not presented in our study like rs1889189 and rs362569 were also reported to have a LD in Dutch [115]. Such samples both from single site and multi-sites studies will facilitate the detection of replicable associations and be more accurate estimation of the magnitude of risk conferred [116]. Thus, it is possible that the SNPs that we estimated might have allele-dependent functional effects and probably in LD with genetic variations (located in protein-coding or regulatory regions) of functional relevance.

The sample included in our meta-analysis is obviously insufficient compared with the large samples in the genetic studies on other psychiatric diseases (the meta-analysis for schizophrenia is provided with approximately 3500 or even more participants on average [117]). This can be partly explained by the use of family-based approaches like the TDT adopted by most of our included studies, which have the inherent disadvantage of the effective samples being substantially smaller than the initial samples [118].

Several of the papers reported associations in the stratified analysis. For example, a trend toward sex-dependent transmission of alleles from parents to ADHD probands has been reported earlier for the T alleles of rs3746544 [119] and rs1051312 [93], and in both of these investigations, the over-transmission was paternal. In addition, associations for both of behavioral ADHD subtypes with *SNAP25* were reported in a Canadian sample [97]. Due to the lack of data, we did not do the further stratified analysis, but it is expected that future meta-analysis will do more detailed and stratified analysis.

Depending on the position and flanking sequence in the gene, SNPs may have varied functional effects on protein sequence, transcriptional regulation, RNA splicing, or microRNA (miRNA) binding. According to a web server SNPinfo (http://www.niehs.nih.gov/snpinfo) [120], rs3746544 located in 3′-UTR is to be predicted as a binding site of miRNAs (miRanda) which are ~22-nucleotide-long endogenous non-coding RNA regulators of gene activity at the post-transcriptional level [121, 122]. This is also consistent with our meta-analysis and supports the association between *SNAP25* and ADHD. However, the direct evidence is still missing.

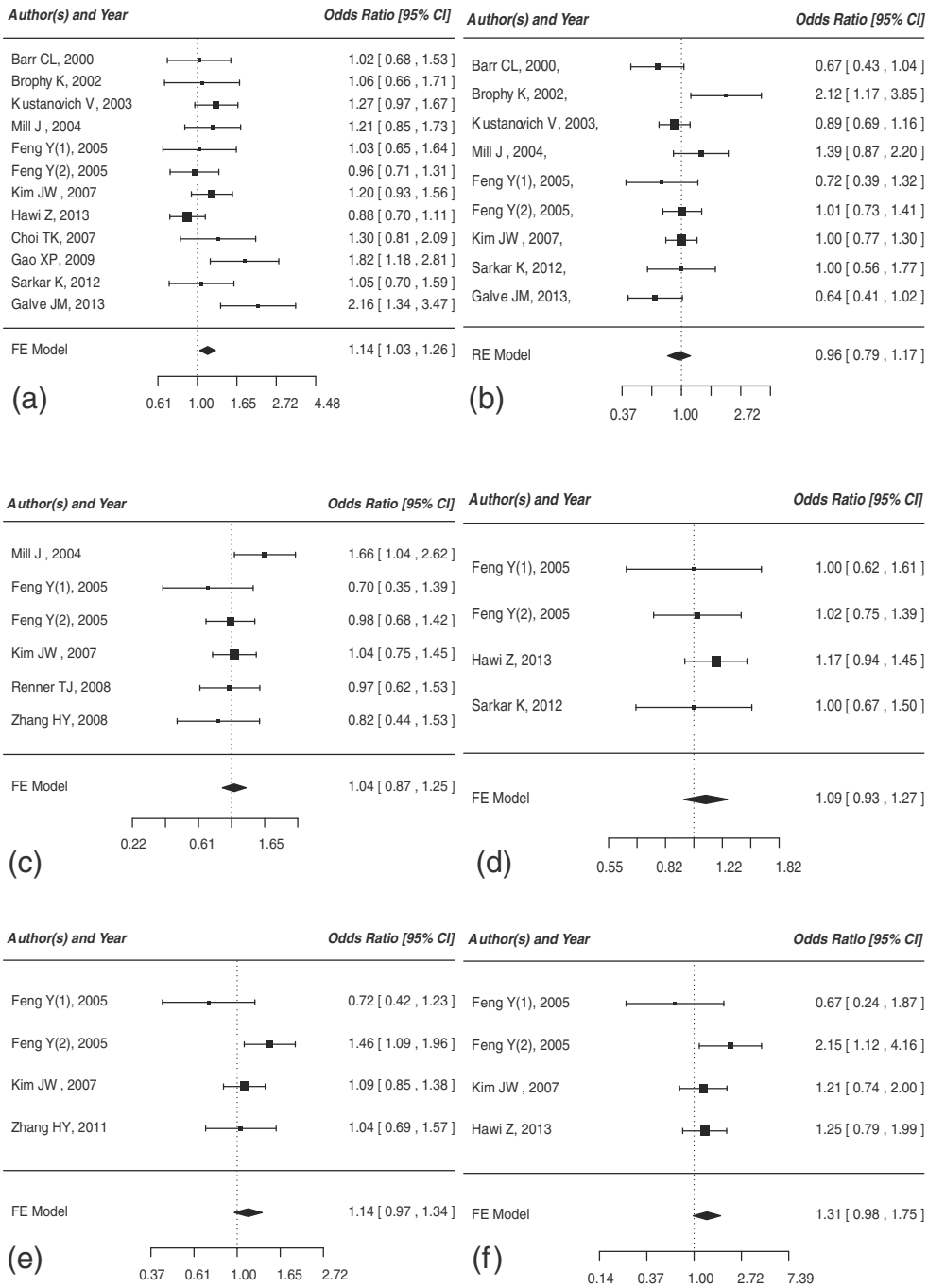

Fig. 3 Forest plot OR and pooled OR from the meta-analysis of ADHD and SNAP-25 SNPs. **a** The single study and pooled ORs for the association between ADHD and rs3746544 (pooled OR = 1.14, 95 % CI 1.03–1.26, $P = 0.010$; Q-statistic $\chi^2 = 19.3528$, $P = 0.055$, $I^2 = 41.56$ %). **b** The single study and pooled ORs for the association between ADHD and rs1051312 (pooled OR = 0.96, 95 % CI 0.79–1.17, $P = 0.688$; Q-statistic $\chi^2 = 16.207$, $P = 0.040$, $I^2 = 50.78$ %). **c** The single study and pooled ORs for the association between ADHD and rs363006 (pooled OR = 1.04, 95 % CI 0.87–1.25, $P = 0.667$; Q-statistic $\chi^2 = 5.937$, $P = 0.312$, $I^2 = 0.00$ %). **d** The single study and pooled ORs for the association between ADHD and rs8636 (pooled OR = 1.09, 95 % CI 0.93–1.27, $P = 0.289$; Q-statistic $\chi^2 = 0.831$, $P = 0.842$, $I^2 = 0.00$ %). **e** The single study and pooled ORs for the association between ADHD and rs362549 (pooled OR = 1.14, 95 % CI 0.97–1.34, $P = 0.122$; Q-statistic $\chi^2 = 5.849$, $P = 0.119$, $I^2 = 47.61$ %). **f** The single study and pooled ORs for the association between ADHD and rs362998 (pooled OR = 1.31, 95 % CI 0.98–1.75, $P = 0.069$; Q-statistic $\chi^2 = 3.965$, $P = 0.265$, $I^2 = 0.00$ %)

In conclusion, we did provide modest support for one of the first reported markers (rs3746544, MnlI) of *SNAP25* with ADHD, but we were not able to confirm the association of variants of *SNAP25* with ADHD. In order to explore more effective and direct evidence, further extensive animal experiments and pharmacological studies and larger and more various and detailed genome-wide association studies are crucial and conclusive in light of the impact of *SNAP25* on disease processes.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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