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Genetic analysis for cognitive flexibility in trail-making test in attention deficit hyperactivity disorder patients from single nucleotide polymorphism, gene to pathway level

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Abstract

Objectives: Investigation of the genetic basis of endophenotype and analysis the pathways with multiple genes of small effects might increase the understanding of the genetic basis of attention deficit hyperactivity disorder (ADHD). Here we aimed to explore the genetic basis of cognitive flexibility in ADHD at SNP, gene and pathway level.

Methods: The Trail-Making Test (TMT) was used to test the cognitive flexibility of 788 ADHD patients. A genome-wide association analysis of cognitive flexibility was conducted for 644,166 single nucleotide polymorphisms (SNPs).

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Statement of interest

Dr. Faraone is a consultant to Genomind. The other authors declare no conflict of interest.

Supplementary Materials

Supplementary Table 1 SNPs with *P*<e-5 for the association analysis of cognitive flexibility.
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Supplementary Figure 1 Gene expression of *DLGAP1* in different tissues (from GTEx(Consortium 2015)).
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Supplementary Figure 3 Gene expression of *CADPS2* in different tissues (from GTEx(Consortium 2015)).
Supplementary Figure 4 *CADPS2* related network from STRING database(Franceschini and others 2013).

Results: The top SNP rs2049161 (P = 5.08e-7) involved gene *DLGAP1* and the top gene *CADPS2* in the gene-based analysis obtained much literature evidence to be associated with psychiatric disorders. Gene expression and network analysis showed their contribution to cognition function. The interval enrichment analysis highlighted potential contribution of 'adenylate cyclase activity' and *ADCY2* to cognitive flexibility. Candidate pathway-based analysis for all SNPs found glutamate system, neurite outgrowth and noradrenergic system related pathways were significantly associated with cognitive flexibility (FDR < 0.05), among which the neurite outgrowth pathway was also associated with ADHD symptoms.

Conclusions: This study provides evidence for the genes and pathways associated with cognitive flexibility and facilitate the uncovering of the genetic basis of ADHD.

Keywords

attention deficit hyperactivity disorder; cognitive flexibility; Trail-Making Test; genome-wide association study; genetics

Introduction

Attention deficit hyperactivity disorder (ADHD) is a common, early-onset and enduring neurodevelopmental disorder (Faraone and others 2015). The exploration of genes associated with ADHD has become a priority because of its high heritability of about 0.76 (Biederman and Faraone 2005). However, discovering genetic risk variants for ADHD has been difficult since it is clinically heterogeneous. Hence, endophenotypes have been introduced to reduce phenotypic heterogeneity and facilitate gene discovery. Executive functions (EF) are candidate endophenotypes for ADHD (Crosbie and others 2008; Gau and Shang 2010a). Cognitive flexibility, as one of main components of executive function, is the ability to regulate behavior to the demands of a changing environment (Armbruster and others 2012). It was reported that cognitive flexibility deficits are found in 25% to 35% of children with ADHD (Frazier and others 2004; Willcutt and others 2005). In our previous work, we have found ADHD children have cognitive flexibility deficits compared with control groups (Huang and others 2016). In addition, such deficits occur in a number of psychiatric disorders, including schizophrenia, obsessive-compulsive disorder, autism spectrum disorders and major depression disorder (Ebmeier and others 2006; Francazio and Flessner 2015; Pooragha and others 2013; Thoma and others 2007). Each of these disorders has been shown to be comorbid with ADHD or to show some degree of genetic overlap with ADHD. So, understanding the genetic basis of cognitive flexibility might benefit uncovering of the mechanism of ADHD and related psychiatric disorders (Flint and Munafo 2007; Gottesman and Gould 2003).

Twin studies have estimated a heritability of about 50% for general cognitive ability (Polderman and others 2006). Candidate gene association studies of cognitive flexibility have focused on several important neurotransmitter systems, including dopamine, serotonin, norepinephrine and acetylcholine transmitter systems (Logue and Gould 2014). For example, an association of *COMT* polymorphisms and cognitive flexibility has been reported for schizophrenia (Barnett and others 2007) and bipolar disorder I (Soeiro-de-Souza and others 2012). Wishart et al. found that there was a *COMT-ANKK1* interaction

associated with cognitive flexibility as measured by the Trail-Making Test (Wishart and others 2011). There has been no genome-wide association study (GWAS) for cognitive flexibility.

Many psychiatric disorders have been reported to be affected by a combination of genetic variants or pathways (Network and others 2015). Bralten et al. explored the association of candidate genetic pathways with ADHD symptoms (Bralten and others 2013). Their report indicated that pathway-based association analysis improved the power of genetic analysis to investigate biological risk factors related to ADHD. Thus, thorough gene level and pathway level analyses for cognitive flexibility might provide new insights for the understanding of its genetic basis. In this study, we report the first genome-wide association study for cognitive flexibility in ADHD. Then we performed gene level analysis and an interval enrichment analysis for candidate SNPs. Furthermore, we selected 11 candidate genetic pathways and tested the association of these pathways with cognitive flexibility by analyzing all SNPs. We also compared the cognitive flexibility associated pathways with the pathways associated with ADHD symptoms. Our results should facilitate the understanding of the genetic basis of cognitive flexibility in ADHD.

Materials and Methods

Participants

All subjects were Han Chinese recruited from the child psychiatric clinics of Peking University Sixth Hospital. Patients met the diagnostic criteria for ADHD defined by the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV). The diagnosis was first suggested clinically by senior child psychiatrists, then confirmed using semi-structural interview (Clinical Diagnostic Interview Scale, CDIS) (Yang and others 2004). Those with major neurological disease, such as epilepsy, schizophrenia, pervasive development disorder and mental retardation (IQ <70), were excluded (Yang and others 2013b). IQ was assessed using the Chinese version of the Wechsler Intelligence Scale for Children (C-WISC), third edition. Among all patients, only one subject was using drug when doing the test. The drug was washed out for at least one month before the patient was recruited. Totally, 788 ADHD patients were recruited in this study. The mean age is 10.11 (SD = 2.35) years, mean IQ is 104.71 (SD = 14.7), 85.3% samples were male. According to the symptom dimensions described in DSM-5, the patients could be classified into three presentations. Those who had six or more symptom items in the inattention dimension but not the hyperactive-impulsive dimension were ADHD-inattentive presentation, those who had six or more symptom items in the hyperactive-impulsive dimension but not inattentive dimension were ADHD-hyperactive/impulsive presentation, whereas those who had six or more symptom items in both dimensions were ADHD-combined presentation. In our sample, the patients are either ADHD-inattentive presentation (37%) or ADHD-combined presentation (63%). In addition, we collected three dimensional symptoms for 1026 ADHD patients according to the Clinical Diagnostic Interview Scale (Barkley 2006). The three dimensional symptoms included hyperactivity-impulsivity symptom (CDISHI), Inattention symptom (CDISatt) and total symptom (CDISall). This study was approved by the Institutional Review Board of Peking University Health Science Center. All the parents signed a written informed consent.

Cognitive flexibility test

All the 788 ADHD patients finished the Trail-Making Test (TMT) to test their cognitive flexibility. The test consisted of two sections (A and B). In section A, with numbers from 1 to 25 randomly scattered in the page, the subject was asked to connect these numbers sequentially as quickly as possible. In section B, the subject was asked to connect numbers and letters alternately (*i.e.*, 1-->A-->2-->B-->3-->C, … L-->13). When the subject made an error, the investigator pointed out immediately before continuing the test. The time on section A T_A mainly indicates motor speed and visuoperceptual abilities, while the time on section B T_B was sensitive to working memory and cognitive flexibility. The shifting time T_B - T_A, which was highly related to T_B (r = 0.94), minimizes visuoperceptual and working memory demands, providing a relatively pure indicator of executive control (Chaytor and others 2006; Kortte and others 2002; Sanchez-Cubillo and others 2009). So, T_B - T_A was the main item to assess cognitive flexibility (Anderson 2001).

Genotyping, quality control and association analysis

Genomic DNA was extracted from peripheral blood using Omega DNA extraction Kit (Omega Bio-tek Inc., Doraville, GA). The genotyping was performed by Affymetrix 6.0 array at CapitalBio Ltd. (Beijing) using the standard Affymetrix protocol (Yang and others 2013a). After mapping the single nucleotide polymorphism (SNP) probes to SNPs with rs numbers, 653,428 SNPs remained. The individuals with per-individual autosomal heterozygosity >5 s.d. away from the mean, without age or IQ information, with a per-individual call rate <95% or with relatives with a genome identity PI_HAT ≥ 0.185 were further excluded. Then, principal component analysis (PCA) was conducted for the remaining samples using the independent SNPs with low linkage disequilibrium (LD) (pair wise r^2< 0.05) using EIGENSOFT4.2 software (Patterson and others 2006; Price and others 2006). Only the eigenvector 1 was significant in the Tracy-Widom test and thus was used as a covariate in the subsequent statistical analysis. For quality control at SNP level, SNPs with per-SNP call rate < 98%, Hardy-Weinberg equilibrium test P < 0.001, or minor allele frequency (MAF) < 1% were excluded. After quality control, 763 ADHD patients with 644,166 SNPs were remained for the association analysis.

We used MACH-admix 1.0 (Liu and others 2013) to impute non-genotyped SNPs, using the ASN data (286 individuals) from the 1000 Genomes Project Integrated Phase 1 Release (Abecasis and others 2012) as the reference panel. Imputed SNPs with a squared correlation between imputed and true genotypes (rsq) <0.6 or SNPs with minor allele frequency <0.01 were removed. The association between SNPs and shifting time was conducted using the additive linear regression model by PLINK (Purcell and others 2007) version 1.0.7 with age, IQ, sex and eigenvector 1 of PCA as covariates. Max(T) permutation with 10000 permutations were conducted using –mperm 10000 by PLINK, which obtained the emperical P-value. The gene-based association analysis was conducted using GATES in KGG (Li and others 2011), which used the extended Simes test to calculate association statistics. RefGene (version hg19) was used for the analysis. SNPs within 5kb upstream and 5kb downstream were mapped to each gene. The calculation of LD (linkage disequilibrium) between SNPs used the plink format genotype data. Multiple testing corrections were calculated using the Benjamini-Hochberg method.

Interval enrichment analysis for potential candidate SNPs

We put the SNPs with *P*<e-4 from the GWAS results for cognitive flexibility into the --show-tags command in PLINK to get independent intervals (--tag-r2 0.2, --tag-kb 1000). Then the resulting intervals were input into INRICH software (Lee and others 2012). All gene sets from Gene ontology, KEGG and MSigDB provided at the INRICH website were analyzed separately. The window for the mapping of genes was set as 20kb. For details about the algorithm, please refer to the original paper (Lee and others 2012). Briefly, for each gene set, the software counted the number of association intervals that contained at least one gene in the gene set, then evaluated the probability of observing the number of intersecting intervals by chance alone using a permutation procedure, which is the empirical *P*-value. Furthermore, the empirical *P*-value was further corrected by using bootstrapping.

In addition, to exploring potential candidate interval enriched enhancer regions in different tissues/cell types, we downloaded the dense peak regions of chromatin state 7, which are enhancers, for 127 tissues/cell types from the Roadmap Epigenome project website (Roadmap Epigenomics 2015). We grouped the peak regions in each tissue/cell type as one set (as pathway) and each peak region as one feature (as gene), and then used INRICH (Lee and others 2012) to do enrichment analysis to test if these intervals are enriched in some particular tissue/cell type. Since only the peak regions were regarded as the enhancer region rather than the flanking of peak region, the window for the mapping is set as 0.

Candidate genetic pathway selection

First, based on the review of genetic studies of ADHD (Li and others 2014) and cognitive flexibility (Logue and Gould 2014), we selected the dopaminergic, noradrenergic, serotonergic, cholinergic and glutamatergic neurotransmission systems. We got genes for these pathways from published literature: dopaminergic system related genes were from (Ribases and others 2012), noradrenergic system related genes were from (Hawi and others 2013), serotoninergic system related genes were from (Ribases and others 2009). For the cholinergic system, mainly the five receptor subtypes of the muscarinic system were included (Carruthers and others 2015). The glutamatergic system consists of two groups, metabotropic and ionotropic receptors. The 24 genes were extracted from articles exploring the association between glutamate and bipolar disorder or schizophrenia (Cherlyn and others 2010). Although the GRID family receptors do not form ion channels when expressed in transfected cells like other ionotropic receptors in glutamate system, it also had been classified as one of the ionotropic glutamate receptor family according to sequence homology (Yamazaki and others 1992). So we also included it in our study. Besides the neurotransmitter related pathways, neurotrophic factor and neurodevelopment related pathways have been hypothesized to be associated with ADHD. Neurotrophic factors and their receptors related genes (Ribases and others 2008) were also included into our analysis. In addition to the genes from literature, Bralten et al. selected genes for dopamine/Norepinephrine pathways, the serotonin pathway and neurite outgrowth from the Ingenuity Pathway Analysis (IPA) database to test their association with ADHD symptoms (Bralten and others 2013). We also included these three pathways in our analysis. Since Bralten et al.'s study didn't include the cholinergic and glutamatergic systems, we included the cholinergic synapse pathway and glutamatergic synapse pathway from KEGG (Kanehisa

and others 2008). Totally, 11 candidate pathways were analyzed. The gene list and data sources of all the pathways are shown in Supplementary Table 2.

Pathway-based analysis

An improved gene-set enrichment analysis (*i*-GSEA) algorithm (Zhang and others 2010) was used for the candidate genetic pathways to do pathway-based analysis. The key procedures of *i*-GSEA are as follows: (1) the lowest *P*-value from the SNPs of a gene is utilized to represent the gene; (2) for each pathway, the enrichment score (ES, a Kolmogorov-Smirnov like statistics with weight) reflects the trend for genes of the pathway to be more strongly associated with the phenotype than other genes. It is calculated based on a ranked gene list; (3) the significant proportion ratio, which emphasizes the relative proportion of significant genes (defined as genes mapped with at least1 SNP with $P < 0.05$), is multiplied by the ES to obtain the significance proportion based ES (SPES); and (4) SNP label permutation and normalization are performed to generate the null distribution of SPES and correct gene variation (i.e. different genes with different number of SNPs mapped will result in identification of gene sets containing genes with more SNPs mapped, instead of genes with functional correlation) and gene set variation (i.e. different gene sets contain different number of genes). The analysis was performed using the online web server *i*-GSEA4GWAS v2 (Zhang and others 2014). All SNPs with *P*-value from the GWAS association result were input into the web server. SNPs within the 5kb upstream and 5kb downstream were mapped to each gene. The selected 11 candidate pathways were used as the gene set search space. The minimum gene number for each pathway was set to 5 and the maximum was set to 200 (the default). Pathways/gene sets with FDR < 0.05 were regarded as being associated with cognitive flexibility.

Results

SNP and gene level results

After quality control, genome-wide association study for the T_B-T_A time from the Trail-Making Test was conducted in 763 ADHD patients for 644,166 SNPs. The SNP level association results did not identify genome-wide significant result ($P < 5e-8$). The SNPs with $P < e-5$ were shown in Supplementary Table 1. All of them had small empirical *P*-value (<5e-5) during 100000 permutation. The loci with the smallest *P*-values were rs2049161 ($P = 5.007e-07$) and rs16946051 ($P = 5.147e-07$), which are in high LD ($r^2 > 0.8$) and were within gene *DLGAP1*. The second top loci included rs6466819 ($P = 1.638e-06$) and rs6962249 ($P = 1.916e-06$), which were within gene *CADPS2*. The top results of the association analysis result for the SNPs after imputation were in high LD with the above loci. In the gene level association analysis, the top 15 genes with $P < 5e-4$ are shown in Table 1. No gene passed the multiple testing corrections. The top gene is *CADPS2*. *DLGAP1* is the 7th gene in Table 1.

Expression data in GTEx (Consortium 2015) showed gene *DLGAP1* was mainly expressed in brain, in which, frontal cortex has the highest expression level (Supplementary Figure 1). We further constructed the network connected by *DLGAP1* by using the STRING database (Franceschini and others 2013) (http://www.string-db.org) as shown in Supplementary

Figure 2. Several known important interactions, such as *DLGAP1* – *SHANK1*, *DLGAP1* – *KCNA4* and *DLGAP1* – *DLG1*, were included in the network. Gene *CADPS2* is mainly expressed in cerebellar and frontal cortex in brain (as shown in Supplementary Figure 3). In addition, *CADPS2* also interacted with several well-known psychiatric disorder related genes, including *BDNF*, *DRD2* and *NRG3* (as shown in Supplementary Figure 4).

Interval enrichment analysis result for potential candidate SNPs

We conducted interval enrichment analysis for the potential candidate SNPs with $P <$ e-4 using INRICH. Among the 82 SNPs, 53 independent intervals were obtained and were further input into INRICH. Firstly, three gene set files (KEGG, GO, MSigDB) were analyzed separately. No gene set passed the multiple test correction. The top one or two results for the three gene set files were shown in Table 2. The 'adenylate cyclase activity' pathway was the top result for GO and the second top result for MSigDB. More interestingly, *ADCY2* was involved in three of these four pathways. Furthermore, to explore these intervals enriched tissues and cell types, we conducted enrichment analysis for these intervals on the peak region of enhancers in different tissues and cell types. Three tissues were nominally significant although none passed the multiple test correction (Table 2). The three tissues/cell types were 'H9 Derived Neuron Cultured Cells', 'Brain Anterior Caudate' and 'Right Atrium', among which, two of them were related with neural system, indicating these potential candidate intervals for cognitive flexibility were enriched in the enhancer region regulating gene expression in neural system related tissues/cell types.

Cognitive flexibility associated pathways

As shown in Supplementary Table 2, we selected 11 candidate genetic pathways for the pathway-based analysis. Among the 644,166 SNPs from the GWAS association analysis of cognitive flexibility, after inputting these SNPs in *i*-GSEA4GWAS v2, 444,179 SNPs mapped to 17,324 genes. After the calculation of enrichment scores and permutation tests for each pathway, as shown in Table 3, five pathways achieved FDR < 0.05, which were regarded as significantly associated with cognitive flexibility. The pathways included 'glutamate/glutamine system (literature)', 'neurite outgrowth genes (IPA)', 'noradrenergic system (literature)', 'glutamatergic synapse pathway (KEGG)' and 'neurotrophic factors and their receptors (literature)'. For all significant pathways, most of the genes were mapped by GWAS SNPs, and more than half of the selected genes were significant (mapped with at least one of the top 5% of all SNPs) (Table 3).

Association of the pathways with ADHD symptoms

We also collected three ADHD symptom scores (inattention symptom, hyperactive-impulsive symptom and total symptom) for the samples who finished the TMT. Totally, 745 samples had both ADHD symptom scores and TMT score. Spearman correlation analysis showed the correlations were not significant. This suggested the TMT performance is not dependent on the ADHD severity in our sample, whereas it might reflect impaired cognition across the patients. Furthermore, we investigated the association of cognitive flexibility related pathways with different ADHD symptoms. The same pathway-based analyses were conducted for the GWAS SNPs list of hyperactivity-impulsivity symptom (CDISHI), inattention symptom (CDISatt) and total symptoms (CDISall) (as shown in

Supplementary Table 3, 4, and 5). The comparison of cognitive flexibility associated pathways with the pathway-based analysis result of ADHD symptoms traits was shown in Table 3. Among the five pathways, four were associated with total ADHD symptoms, in which, 'neurite outgrowth genes' is the only one associated with all ADHD symptom traits. Among the most investigated neurotransmitter systems (dopamine, norepinephrine, serotonin, cholinergic) by previous pathway studies, only the noradrenergic system was significantly associated with cognitive flexibility. It was also associated with inattentive ADHD symptoms and total symptoms.

Discussion

By using a case-only genome-wide association study designed for TMT shifting time, we explored the genetic basis of cognitive flexibility in ADHD patients at the SNP, gene and pathway levels. We identified two potential loci in gene *DLGAP1* and *CADPS2*. Interval enrichment analysis for potential candidate SNPs with $P<$ e-4 highlighted the 'adenylate cyclase activity' pathway and *ADCY2* gene. Hypothesis-based pathway association analysis identified five significant pathways. Several of them were also shared with ADHD symptoms. These results were supported by previous literature findings in psychiatric disorders.

The top result related gene *DLGAP1* encodes the protein guanylate kinase-associated protein (GKAP). It interacts with PSD95 protein, which is encoded by *DLG4* and has been reported to be a prediction of cognitive deficits (Sultana and others 2009; Whitfield and others 2014). Such interaction also contributes to the synaptic plasticity in obsessive-compulsive disorder (OCD) (Kim and others 1997; Welch and others 2007). In addition, *DLGAP1* is also reported to be associated with schizophrenia, major depression disorder and Alzheimer's disease (Bertram and others 2008; Li and others 2013; Mathias and others 2016). All these psychiatric disorders were reported to have impaired cognitive flexibility (Ebmeier and others 2006; Francazio and Flessner 2015; Pooragha and others 2013; Thoma and others 2007). So, *DLGAP1* may be associated with these mental disorders through the underlying impaired cognitive flexibility. The top result in the gene-level analysis *CADPS2* is a member of the CAPS/CADPS protein family. It is widely expressed in the brain, especially the cerebellum, and is involved in monoamine and neurotrophin neurotransmission (Sadakata and others 2017). *CADPS2* mediates BDNF release in neurons as it was reported that mice with the deficiency of *CADPS2* expressed less BDNF (Sadakata and others 2007). It is also reported to be associated with several psychiatric disorders, such as schizophrenia, intellectual disability, autism spectrum disorder and Alzheimer's disease (Bonora and others 2014; Hattori and others 2011; Velez and others 2013). Interval enrichment analysis for potential candidate SNPs with $P<$e-4 highlighted another candidate gene *ADCY2*. This gene codes an important enzyme involved in cyclic adenosine monophosphate cAMP signaling. *ADCY2* has been reported to be a risk locus for bipolar disorder in a GWAS (Muhleisen and others 2014). Given that impairment of cognitive flexibility is a cross-disorder phenotype, the association of *DLGAP1*, *CADPS2* and *ADCY2* with cognitive flexibility deserves further validation in a replication study.

We selected 11 pathways to conduct hypothesis based candidate pathway association analysis. The analysis implied the important roles of glutamate system, noradrenergic system and neurite outgrowth pathways in the etiology of cognitive flexibility. Glutamate, a major excitatory neurotransmitter in the brain, is related to several processes associated with ADHD, such as brain development, regulation of neuronal activity, bidirectional modulation of dopamine signaling, and synaptic plasticity (Lesch and others 2013; Mukherjee and Manahan-Vaughan 2013). Prior GWASs have identified several glutamate receptor/transporter polymorphisms associated with ADHD (Lesch and others 2013). There are also studies reporting increased glutamate levels in PFC, anterior cingulate cortex (ACC), and striatum in ADHD patients (Spencer and others 2014). The noradrenergic system was associated with cognitive flexibility and also with inattentive and total ADHD symptoms. This was consistent with a previous study, which reported that when noradrenergic activity level was low in medial prefrontal cortex, cognitive flexibility and attention were impaired (McGaughy and others 2008). After using the selective noradrenergic reuptake inhibitor drug, atomoxetine, cognitive flexibility and attention improved in children with ADHD (Gau and Shang 2010b). Emerging data also suggest that gene variants from the noradrenaline system may also explain individual differences in the ability to sustain attention (Barnes and others 2011). The pathway neurite outgrowth related genes confirmed previous findings that ADHD is a neurodevelopmental disorder. Bralten et al.'s finding showed neurite outgrowth significantly contributed to the hyperactive/impulsive symptom domain of ADHD (Bralten and others 2013). In addition, Bonvicini et al. also found an association between the neurite outgrowth network and adult ADHD (Bonvicini and others 2017). Notably, both methylphenidate and amphetamine stimulated neurite outgrowth and modulated the expression or function of genes or proteins implicated in neurite outgrowth (Lipton and others 2008; Park and others 2004). These evidence validated the significance of the pathway-based analysis result.

There are several limitations of this study. First, the sample size in this study is relatively small, which may be one of the reasons that there was no significant SNP and gene after multiple corrections. Another possible reason for the difficulty of genetic discovery for cognitive flexibility is its university and diversity (Dajani and Uddin 2015). Cognitive flexibility itself also comprised inhibition and working memory (Diamond 2013). It was not as closer as inhibition to the genetic basis of ADHD. In addition, the pathway-based analysis was for candidate pathways but all pathways. Also, only cases were included in this study. It is difficult to tell the pathway we identified was associated with ADHD or only cognitive flexibility. A replication with larger sample size and also control samples would facilitate discovery for the genetic mechanism of cognitive flexibility and ADHD.

In conclusion, we reported the first GWAS for cognitive flexibility in ADHD. Gene level association analyses and candidate pathway analyses detected possible associations with cognitive flexibility. Shared pathways with ADHD symptoms suggest some shared etiology between ADHD and impairments in cognitive flexibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Genes with nominal *P*<5e-4 in the gene level analysis.

Symbol	Nominal*P*	Corrected*P*	Chr.	Best SNP	IsGATESKeySNP	SNP *P*-value
CADPS2	0.0000293	0.425341424	7	rs6466819	Y	1.64E-06
GMCL1	0.0000373	0.425341424	2	rs4241261	N	6.53E-06
TRIM15	0.00017	0.61680506	6	rs1318638	Y	5.71E-05
ANXA4	0.00018	0.61680506	2	rs1531025	Y	7.41E-06
ALPK1	0.0002	0.61680506	4	rs1859142	Y	2.99E-06
TRIM26	0.0002	0.61680506	6	rs10947058	Y	3.12E-05
DLGAP1	0.00023	0.61680506	18	rs2049161	N	5.01E-07
GPR132	0.00035	0.61680506	14	rs7147669	Y	0.000158
MAP3K11	0.00036	0.61680506	11	rs1151488	Y	9.61E-05
GLB1L2	0.00037	0.61680506	11	rs1258852	Y	2.94E-05
SOX4	0.00038	0.61680506	6	rs9466124	Y	0.000384
WDR43	0.0004	0.61680506	2	rs6547889	Y	0.000118
TRIM10	0.00041	0.61680506	6	rs1318638	Y	5.71E-05
MXD1	0.00046	0.61680506	2	rs11126251	Y	5.87E-05
SKINT1L	0.00048	0.61680506	1	rs17468972	Y	5.97E-05

Table 2

Interval enrichment analysis result for the potential candidate SNPs (*P*<e-4).

	T_Size	Int_No	Empirical_P	Corrected_P	Target	Related Genes
KEGG	81	2	0.0211958	0.544491	Progesterone-mediated oocyte maturation	*ADCY2, SPDYA*
GO	12	2	0.00159968	0.469306	GO:0004016 adenylate cyclase activity	*ADCY2, ADCY10*
MSigDB	10	2	0.00019996	0.24995	Grandvaux IRF3 targets DN	*ANXA4, SORL1*
MSigDB	11	2	0.0009998	0.558488	REACTOME: adenylate cyclase activating pathway	*ADCY2, ADCY10*
	T_Size	**Int_No**	**Empirical_P**	**Corrected_P**	**Target**	**Tissue/cell type**
Enhancers	97241	35	0.0317936	0.581284	E010-7_Enh	H9 Derived Neuron Cultured Cells
Enhancers	98243	31	0.0339932	0.59948	E068-7_Enh	Brain Anterior Caudate
Enhancers	96605	30	0.0425915	0.646871	E104-7_Enh	Right Atrium

Table 3

Candidate pathways associated with cognitive flexibility.

Pathway	Description	Significant genes/Selected genes/All genes	*P*-value	FDR	Association with symptom traits		
					CDISHI	CDISatt	CDISall
set5 glutamatergic	glutamate/glutamine system (literature)	19/24/26	0.001	0.001	N.A.	N.A.	0.0054
pathway3 neurite	neurite outgrowth genes (IPA)	36/43/45	0.001	0.001	0.044	0.0033	0.002
set2 noradrenergic	noradrenergic system (literature)	7/9/14	0.001	0.0027	N.A.	0.0035	0.0073
pathway5 glutamatergic	glutamatergic synapse pathway (KEGG)	23/35/39	0.007	0.0205	N.A.	N.A.	0.004
set6 neurotrophic	neurotrophic factors and their receptors (literature)	6/9/10	0.017	0.0366	N.A.	N.A.	N.A.

The FDR of the pathways in the pathway-based analysis for the GWAS SNPs list of three ADHD symptom traits (CDISHI: hyperactivity-impulsivity, CDISatt: inattention, CDISall: overall assessment) were also shown in the table if the FDR < 0.05. N.A. denotes the pathway-based analysis result was not significant (FDR > 0.05).