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Association of Gene Variations in Ionotropic Glutamate Receptor and Attention-Deficit/Hyperactivity Disorder in the Chinese Population: A Two-Stage Case–Control Study

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Abstract

Objective: The aim of this study was to comprehensively explore the relationship between genetic variations within GRIN2A, GRIN2B, GRIK1, GRIK4, GRID2, and ADHD. **Method:** Genotyping was performed with the Sequenom MassARRAY system in a two-stage case–control study. ADHD symptoms were assessed using the Swanson, Nolan, and Pelham version IV scale and the Integrated Visual and Auditory Continuous Performance Test. In silico analysis was performed with website resources. **Results:** GRID2 rs1385405 showed a significant association with ADHD risk in the codominant model (OR = 2.208, 95% CI = [1.387, 3.515]) in the first stage and in the codominant model (OR = 1.874, 95% CI = [1.225, 2.869]) and recessive model (OR = 1.906, 95% CI = [1.265, 2.873]) in the second stage and related to inattention and hyperactivity symptom. In addition, rs1385405 disturbed the activity of exonic splicing enhancer and mediated GRID2 gene expression in the frontal cortex. **Conclusion:** our data provided evidence for the participation of GRID2 variants in conferring the risk of ADHD. *(J. of Att. Dis. XXXX; XX(X) XX-XX)*

Keywords
ADHD, GRID2, gene-symptoms association analysis, frontal cortex

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common childhood-onset neurodevelopmental disorder characterized by developmentally inappropriate levels of inattention and hyperactivity or impulsivity. The worldwide prevalence of ADHD in children is 2.6%–4.5% (Polanczyk et al., 2015), and the overall prevalence among children and adolescents in China is 5.4%–7.2% (Wang et al., 2017). Without prompt treatment in the early stages, 50%–80% of cases may persist into adulthood (Asherson et al., 2016; Volkow & Swanson, 2013) or beyond (Michielsen et al., 2012).

ADHD is highly heritable and multifactorial and is generally considered to be the result of environmental and genetic interactions (Thapar & Cooper, 2016). Familial, twin, and adoption studies have demonstrated a strong genetic component in the pathogenesis of ADHD, with a heritability ranging from 60% to 90% (Gallo & Posner, 2016). Specific single dopaminergic, noradrenergic, and serotonergic candidate genes have been significantly associated with ADHD status (Faraone et al., 2005; Gizer et al., 2009). However, genome-wide association studies (GWASs) have not yielded many genome-wide significant findings. Recently, a GWAS meta-analysis of ADHD identified the first genome-wide significant risk loci; several of the loci are located in or near genes implicated in neurodevelopmental mental processes that are likely to be associated with ADHD, including SORCS3, DUSP6, and FOXP2 (Demontis et al., 2019).

Although most ADHD molecular genetic studies have focused on catecholamine dysregulation, the involvement of the glutamatergic system in the pathophysiology and treatment of ADHD has also been emphasized (Huang

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et al., 2019). The concentration of glutamate in the anterior cingulate cortex is positively correlated with the score of impulsive symptoms (Ende et al., 2016). Glutamate is the major excitatory neurotransmitter in the central nervous system and mediates the effects of adjacent neurons by binding to metabolic glutamate receptors (mGluRs) or ionotropic glutamate receptors (iGluRs). iGluRs are subdivided into N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate (KA), and orphan receptors, which are involved in important neurophysiological processes, such as synaptic transmission, neuromodulation, and synaptic plasticity. Moreover, iGluRs are essential for maintaining a balance between tonic and phasic dopaminergic neurotransmission, which may have important implications for disorders of dopamine dysregulation such as ADHD (Tye et al., 2013).

Candidate gene studies have indicated that genetic variants of GRIN2B and GRIN2A conferred an increased susceptibility to attentional impairment in ADHD patients (Kim et al., 2016). GWASs conducted in the European population have implied that GRIK1, GRIK4, and GRID2 might be involved in the susceptibility and related clinical symptoms of ADHD (Hinney et al., 2011; Lasky-Su et al., 2008; Stergiakouli et al., 2012). The glutamate gene set, including those that code for the iGluRs, has been implicated with the severity of hyperactivity/impulsivity (Naaijen et al., 2017). In addition, clinical pharmacology studies have shown that GRIN2B played an important role in the treatment of ADHD with methylphenidate (MPH; Kim et al., 2017), which was the most commonly prescribed first-line agent of ADHD. N-methyl-D-aspartate receptor (NMDAR) antagonist, memantine, might improve the symptoms of inattention and hyperactivity (Surman et al., 2013).

ADHD rat models have demonstrated dysfunction of iGluRs in the prefrontal cortex (Lehohla et al., 2004), which implies changes in the expression of iGluR genes, such as GRIN1, GRIA1, GRIN2C, GRIA2, and GRIN3A (DasBanerjee et al., 2008; Dela Peña et al., 2015; Diana et al., 2015). In addition, AMPA receptors (AMPARs) interact with dopamine receptor D4 (DRD4) to maintain regular glutamatergic transmission (Yuen et al., 2010, 2013), and the genetic variation of DRD4 has been widely recognized as an important mechanism in the risk of ADHD (Naumova et al., 2019).

Although an increasing number of researchers are shifting attention to the role of the glutamatergic system in the pathophysiological processes of ADHD, no relevant studies have explored the association between iGluRs and ADHD susceptibility in the Chinese population. Therefore, the aim of this study is to comprehensively explore the association of iGluR genetic polymorphisms with ADHD risk and clinical symptoms in a two-stage case–control study.

Materials and Methods

Study Participants

A total of 869 children with ADHD and 967 healthy control children were enrolled in the study using a two-stage case–control design. Based on the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.; *DSM-IV*; American Psychiatric Association, 1994) diagnostic criteria, clinical interviews with at least one guardian were conducted by a trained child psychiatrist. In the discovery stage, 411 children newly diagnosed with ADHD were recruited from Hunan Children's Hospital between July 2012 and June 2016, and 450 controls were enrolled among ADHD-free children from the same hospital by a screening program during the same period. In the validation stage, 458 cases and 517 controls were recruited from Wuhan Medical and Health Center for Women and Children between July 2016 and September 2018 according to the same inclusion method as the first stage. Participants who met the following criteria were included: (a) age between 6 and 16 years; (b) ethnic Han Chinese origin; (c) no history of neuropsychiatric diseases (such as intellectual disabilities, autism spectrum disorder [ASD], major depressive disorders, bipolar disorder, substance dependence, or any psychotic spectrum disorder); and (d) no history of psychostimulant or other psychiatric drug use.

The Ethics Committees of Tongji Medical College of Huazhong University of Science and Technology, Hunan Children's Hospital, and Wuhan Medical and Health Center for Women and Children approved this study protocol. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments. We informed the parents of each participant of the purpose of the study, and written informed consent was obtained from them.

Clinical Symptom Assessment

The Swanson, Nolan, and Pelham version IV (SNAP-IV) scale (Costa et al., 2019) is a 26-item questionnaire that is widely used for the assessment and classification of ADHD symptom severity. Of the 26 items, 18 are associated with ADHD symptoms (nine for inattention and nine for hyperactivity/impulsivity), and parents rated their children's inattentive and hyperactive/impulsive symptoms using a 4-point Likert-type scale ranging from 0 (*not at all*) to 3 (*very much*). We used the average score for each dimension to evaluate the severity of the symptoms.

The Integrated Visual and Auditory Continuous Performance Test (IVA-CPT) is a common instrument for assessing continuous attention. It is used not only as an important auxiliary diagnostic method for ADHD but also

to evaluate treatment efficacy (Moreno-Garcia et al., 2015). This study used the Full-Scale Attention Quotient (FAQ) and Full-Scale Response Control Quotient (FRCQ) to evaluate the attentional deficits and response control, respectively, of the participants.

Candidate Genes and Single Nucleotide Polymorphism Selection

First, GeneCards are used to screen ADHD genes with a cut-off score of ≥10, and we used GeneCards (https://www.genecards.org/) to query the genes associated with ADHD and found GRIN2B and GRIN2A. Second, we screened top 25 iGluRs genes ordered by significant in GWASs, with *p*-value less than 10^{-5}. GRIK4 ($p = 4.0 \times 10^{-5}$), GRIK1 ($p = 3.9 \times 10^{-6}$), and GRID2 ($p = 2.1 \times 10^{-5}$) were included in the study, based on the results of the GWASs (Hinney et al., 2011; Lasky-Su et al., 2008; Stergiakouli et al., 2012). Finally, we screened five genes: GRIN2A, GRIN2B, GRIK1, GRIK4, and GRID2.

We obtained single nucleotide polymorphisms (SNPs) of the target genes located in the promoter region (5′ near gene), non-coding region (5′UTR, 3′UTR), and exon region (missense, nonsynonymous) from the NCBI-dbSNP database (http://www.ncbi.nlm.nih.gov/snp/) and excluded SNPs with a minor allele frequency (MAF) < 0.1 in Han Chinese in Beijing China (CHB) based on the 1000 Genomes database (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). Second, combined with the SNPs filtered from dbSNP database, SNPs with disease susceptibility were labeled by consulting relevant literature, and functional SNPs were screened by accessing the SNPinfo web server (https://snpinfo.niehs.nih.gov/). The SNPs selected should meet at least any two of the criteria described above. Furthermore, SNPs that were reported to be positively associated with ADHD were also included in the study (Supplemental Table S1). Based on the above screening steps (Supplemental Figure S1), we finally selected 12 SNPs (Supplemental Table S2). Linkage disequilibrium (LD) analysis for the selected SNPs was performed using PLINK. The maximum r^2 value was 0.23, and the rest were less than 0.1.

DNA Extraction and Genotyping

According to the manufacturer's instructions, genomic DNA was extracted from peripheral blood samples (2 mL) using the Relax Gene Blood DNA System DP319-02 (Tiangen, Beijing, China). The concentration and purity of the DNA were determined with a spectrophotometer before genotyping was performed with the MassARRAY and iPlex systems of the Sequenom genotyping platform (Sequenom, San Diego, CA, USA). Genotyping was performed by experimenters blinded to the status of the participants. The process of genotyping is described in detail in a previous study (Gu et al., 2018).

In the discovery stage, we genotyped the 12 SNPs included in the study. In the validation stage, we genotyped the positive SNPs found in the discovery stage.

In Silico Analysis and Expression Quantitative Trait Locus Analysis

To evaluate whether the promising SNPs harbored regulatory elements, in silico analysis was performed with various website resources. First, the significant SNP functions were evaluated roughly though the SNPinfo database. Then, a particular database was used to perform specific functional predictions again. Specifically, for SNPs with a splicing function, we used the Human Splicing Finder V3.1 web server (http://www.umd.be/HSF3/index.html) and the ESEfinder database (http://krainer01.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi) to predict their specific splicing effects on pre-RNA.

Expression quantitative trait locus (e-QTL) is a locus that explains a fraction of the genetic variance of a gene expression phenotype, and e-QTL analysis is a feasible and effective strategy to explore the biological mechanism of SNPs in non-coding regions. BRAINEAC is a web-resource to access the U.K. Brain Expression Consortium dataset, which consists of genotypes and gene expression data of 10 brain areas (cerebella cortex, putamen, thalamus, occipital cortex, medulla, frontal cortex, temporal cortex, hippocampus, substantia nigra, intralobular white matter) of 134 neuropathologically normal donors. To evaluate the potential association between the positive SNPs and the expression of their nearby genes in the human brain, we used the data provided by the BRAINEAC database (https://caprica.genetics.kcl.ac.uk/BRAINEAC/) to conduct e-QTL analysis.

Statistical Analysis

Group differences in demographic characteristics (sex, age, IQ, BMI) were computed using Pearson's χ^2 test or *t*-test. The Hardy–Weinberg equilibrium (HWE) for genotypes in the control participants was assessed by a goodness-of-fit χ^2 test. Binary logistic regression analysis adjusting for age and sex was employed to examine the impact of iGluR gene polymorphisms on ADHD with codominant, dominant, recessive, and additive models. The associations of ADHD clinical feature scores with SNPs were explored using analysis of variance (ANOVA) with *post hoc* comparisons using Dunnett's *t* method. All statistical analyses were conducted using the IBM SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA). Multiple comparison results were corrected by Bonferroni adjustment based on the number of

Table 1. General Characteristics of the Participants in Two-Stage Case–Control Study.

Characteristics	Stage 1				Stage 2			
	Case (n = 411)	Control (n = 450)	t or χ^2	p	Case (n = 458)	Control (n = 517)	t or χ^2	p
Gender								
Boys	330	350	0.82	.403	349	371	2.480	.125
Girls	81	100			109	146		
Age (year), $M \pm SD$	8.76 ± 1.72	8.92 ± 1.96	1.32	.188	8.23 ± 1.63	8.41 ± 1.76	1.624	.105
BMI (kg/m^2), $M \pm SD$	17.12 ± 3.12	16.94 ± 5.39	–0.615	.539	16.55 ± 6.07	17.01 ± 6.78	1.121	.263
IQ, $M \pm SD$	97.91 ± 12.40	98.19 ± 12.41	0.33	.741	96.01 ± 12.41	95.64 ± 11.61	–0.487	.627
Inattention	1.77 ± 0.55	0.94 ± 0.39	–25.38	**<.001**	1.94 ± 0.44	1.01 ± 0.44	–32.730	**<.001**
Hyperactivity/impulsive	1.61 ± 0.60	0.84 ± 0.44	–21.45	**<.001**	1.78 ± 0.55	0.89 ± 0.44	–27.745	**<.001**
FAQ	81.53 ± 18.42	94.17 ± 17.49	10.33	**<.001**	78.45 ± 21.09	96.25 ± 16.84	14.012	**<.001**
FRCQ	84.78 ± 20.53	99.52 ± 18.12	11.13	**<.001**	83.12 ± 20.04	100.26 ± 17.91	14.440	**<.001**

Note. The significant results are in boldface. BMI = body mass index; IQ = intelligence quotient; FAQ = Full-Scale Attention Quotient; FRCQ = Full-Scale Response Control Quotient.

comparisons. The statistical power was calculated using PASS version 15.0 (NCSS, LLC, Kaysville, UT, USA) after the study was performed.

Results

Participants' Characteristics

The geographic characteristics for the discovery stage (450 controls and 411 ADHD) and the validation stage (517 controls and 458 ADHD) are summarized in Table 1. Briefly, no significant differences were detected for sex, age, IQ, and BMI (all $p > .05$) in the discovery stage or the validation stage. The proportions of inattention types, hyperactivity/impulsive types, and combined types of ADHD was 38.9%, 20.0%, and 41.1%, respectively, in the discovery stage and 45.9%, 23.1%, and 31.0%, respectively, in the validation stage. In addition, compared with the control group, the case group had lower FAQ scores and FRCQ scores and higher inattention scores and hyperactivity/impulsive scores ($p < .001$).

Association Analysis of Candidate SNPs With ADHD Risk

The genotyping call rates for the 12 selected SNPs were all above 95%. The observed genotypes for these SNPs were in agreement with HWE in the controls ($p > .05$). The genotype frequency distribution of GRID2 rs1385405 was significantly different between the case and the control groups after Bonferroni adjustment in the discovery stage and the validation stage ($p = .003$ and $p = .007$, respectively). The genotype distributions of the 12 candidate SNPs in the case and control groups are shown in Supplemental Table S3.

Associations of the candidate SNPs with ADHD under different models (codominant, dominant, recessive, and additive models) are shown in Table 2. In the discovery stage, we found that GRID2 rs1385405 T/T was associated with the increased risk of ADHD in the codominant model (OR = 2.208, 95% CI = [1.387, 3.515], $p = .0008$) after Bonferroni adjustment. GRIN2A rs1014531 was nominally associated with increased ADHD risk under the codominant and dominant models (GA:GG, OR = 1.403, 95% CI = [1.041, 1.890], $p = .026$; dominant model, OR = 1.358, 95% CI = [1.021, 1.804], $p = .035$), but the associations were not found after Bonferroni adjustment.

In view of this, rs1014531 and rs1385405 were further genotyped in the validation stage, and the relationship between rs1385405 and ADHD susceptibility was verified (TT:GG, OR = 1.874, 95% CI = [1.225, 2.869], $p = .0038$; recessive model, OR = 1.906, 95% CI = [1.265, 2.873], $p = .002$). We combined the two stages together for analysis and found that rs1385405 T/T was consistently associated with an increased risk for ADHD (TT:GG, OR = 2.019, 95% CI = [1.475, 2.762], $p < .001$; recessive model, OR = 1.994, 95% CI = [1.473, 2.700], $p < .001$; additive model, OR = 1.282, 95% CI = [1.118, 1.470], $p < .001$).

We calculated the power based on the positive correlation SNPs in this study. For significant SNP rs1385405 (MAF = 0.316 in CHB), the powers that detect an OR of 1.8 in the first stage and second stage were 0.98 and 0.99, respectively.

Association Analysis of the Significant SNP With Clinical Symptom Assessment

To explore the relationship between GRID2 rs1385405 and clinical symptoms of ADHD, the association of rs1385405 with two comprehensive quotients (FAQ and FRCQ) in the IVA-CPT and SNAP-IV scores was assessed.

Table 2. Association Between Candidate SNPs and ADHD Risk.

SNP	Major/minor allele	HT vs. HW		HV vs. HW		Dominant model		Recessive model		Additive model	
		p	OR [95% CI]	*p*	OR [95% CI]	*p*	OR [95% CI]	*p*	OR [95% CI]	*p*	OR [95% CI]
Stage 1											
rs1014531	G/A	.026	1.403 [1.041, 1.890]	.858	1.062 [0.548, 2.060]	.035	1.358 [1.021, 1.804]	.923	0.968 [0.504, 1.862]	.087	1.230 [0.971, 1.558]
rs3104709	A/T	.399	0.879 [0.652, 1.185]	.867	1.037 [0.677, 1.588]	.527	0.912 [0.686, 1.213]	.566	1.120 [0.761, 1.648]	.881	0.985 [0.805, 1.205]
rs4782258	G/A	.633	1.080 [0.788, 1.478]	.481	1.159 [0.769, 1.747]	.529	1.101 [0.816, 1.486]	.59	1.103 [0.773, 1.574]	.467	1.078 [0.881, 1.318]
rs890	A/C	.184	0.819 [0.610, 1.100]	.199	0.650 [0.337, 1.254]	.111	0.795 [0.599, 1.054]	.272	0.695 [0.363, 1.330]	.085	0.813 [0.642, 1.029]
rs7301328	G/C	.776	1.048 [0.758, 1.449]	.614	0.908 [0.622, 1.324]	.988	0.998 [0.737, 1.351]	.389	0.870 [0.634, 1.194]	.6	0.951 [0.788, 1.148]
rs1805502	A/G	.915	1.016 [0.757, 1.363]	.17	1.491 [0.843, 2.639]	.611	1.075 [0.813, 1.421]	.173	1.476 [0.843, 2.583]	.339	1.115 [0.892, 1.392]
rs2284411	C/T	.7	0.941 [0.692, 1.281]	.109	0.529 [0.243, 1.151]	.418	0.885 [0.659, 1.189]	.123	0.544 [0.251, 1.179]	.223	0.856 [0.666, 1.100]
rs363538	T/G	.56	0.918 [0.687, 1.226]	.955	1.013 [0.657, 1.560]	.646	0.938 [0.713, 1.234]	.785	1.058 [0.707, 1.583]	.827	0.978 [0.801, 1.194]
rs363512	G/A	.704	0.936 [0.667, 1.315]	.21	0.467 [0.142, 1.537]	.511	0.895 [0.643, 1.245]	.22	0.475 [0.145, 1.559]	.352	0.868 [0.644, 1.170]
rs2298725	G/A	.519	0.908 [0.676, 1.218]	.8	1.057 [0.687, 1.629]	.667	0.942 [0.717, 1.237]	.64	1.103 [0.731, 1.665]	.931	0.991 [0.815, 1.206]
rs1385405	G/T	.500	1.107 [0.824, 1.487]	**.0008**	**2.208 [1.387, 3.515]**	.063	1.295 [0.987, 1.701]	.0011	2.109 [1.344, 3.310]	.004	1.341 [1.097, 1.640]
rs6815704	G/A	.961	0.993 [0.744, 1.324]	.298	0.603 [0.233, 1.562]	.792	0.963 [0.726, 1.277]	.301	0.608 [0.236, 1.563]	.595	0.933 [0.722, 1.205]
Stage 2											
rs1014531	G/A	.433	1.119 [0.845, 1.480]	.66	1.152 [0.614, 2.160]	.419	1.117 [0.854, 1.460]	.715	1.123 [0.604, 2.088]	.421	1.096 [0.877, 1.370]
rs1385405	G/T	.888	0.980 [0.743, 1.293]	**.0038**	**1.874 [1.225, 2.869]**	.296	1.146 [0.887, 1.481]	**.002**	**1.906 [1.265, 2.873]**	.028	1.234 [1.024, 1.487]
Combined stage											
rs1385405	G/T	.724	1.037 [0.848, 1.269]	**<.001**	**2.019 [1.475, 2.762]**	.042	1.213 [1.007, 1.462]	**<.001**	**1.994 [1.473, 2.700]**	**<.001**	**1.282 [1.118, 1.470]**

Note. All the OR [95% CI] and *p* values were adjusted for age and gender. In Stage 1, the significant level was corrected with the formula of $\alpha' = 0.05/12/4 = 0.00104$ according to Bonferroni method. Similarly, $\alpha' = 0.05/2/4 = 0.00625$ in Stage 2. The significant results are in boldface. Codominant model: HT vs. HW and HV vs. HW. Dominant model: (HV+HT) vs. HW. Recessive model: HV vs. (HT+HW). Additive model: HV vs. HT vs. HW. SNP = single nucleotide polymorphism; HT = heterozygote; HW = wild-type homozygote; HV = variant homozygote; OR = odds ratio; CI = confidence interval

Table 3. Association Between rs1385405 and ADHD Clinical Characteristic Scores.

Clinical symptoms	Genotype	Stage 1			Stage 2			Combined stage		
		M ± *SD*	*F*	*p*	*M* ± *SD*	*F*	*p*	*M* ± *SD*	*F*	*p*
SNAP-IV/			3.055	.048		2.545	.079		5.218	**.006**
inattention	GG	1.308 ± 0.616		Ref	1.444 ± 0.644		Ref	1.380 ± 0.634		Ref
	GT	1.334 ± 0.637		.818	1.413 ± 0.634		.725	1.376 ± 0.636		.994
	TT	1.486 ± 0.685		.027	1.570 ± 0.606		.116	1.532 ± 0.643		**.004**
SNAP-IV/			4.485	**.0115**		4.001	.019		8.548	**<.001**
hyperactivity/	GG	1.172 ± 0.628		Ref	1.270 ± 0.644		Ref	1.224 ± 0.638		Ref
impulsivity	GT	1.219 ± 0.656		.541	1.312 ± 0.685		.579	1.269 ± 0.673		.308
	TT	1.340 ± 0.606		**.006**	1.466 ± 0.632		**.010**	1.431 ± 0.620		**<.001**
IVA-CPT/FAQ			4.154	.016		2.918	.055		6.818	**.001**
	GG	89.354 ± 18.686		Ref	88.748 ± 21.174		Ref	89.036 ± 20.022		Ref
	GT	87.623 ± 18.783		.386	87.923 ± 20.402		.813	87.783 ± 19.651		.385
	TT	83.213 ± 20.453		**.009**	83.409 ± 21.340		.031	83.320 ± 20.887		**<.001**
IVA-CPT/FRCQ			0.658	.518		1.822	.162		2.237	.107
	GG	93.112 ± 20.674		Ref	92.476 ± 21.204		Ref	92.778 ± 20.945		Ref
	GT	92.478 ± 20.796		.895	92.949 ± 20.189		.933	92.730 ± 20.458		.999
	TT	90.416 ± 20.159		.436	88.700 ± 20.131		.159	89.485 ± 20.112		.078

Note. The significant level was corrected with the formula of $\alpha' = 0.05/4 = 0.0125$ according to Bonferroni method. The significant results are in boldface. SNAP-IV = Swanson, Nolan, and Pelham version IV scale; IVA-CPT = Integrated Visual and Auditory Continuous Performance Test; FAQ = Full-Scale Attention Quotient; FRCQ = Full-Scale Response Control Quotient.

Table 4. Scores in the Sequence Containing the G Allele From ESEfinder Database.

Site[a]	SRSF1 (SF2/ASF) threshold: 1.956	SRSF1 (IgM-BRCA1) threshold: 1.867	SRSF2 (SC35) threshold: 2.383	SRSF5 (SRp40) threshold: 2.67	SRSF6 (SRp55) threshold: 2.676
GCTTGG**G**	–2.80546	–2.55004	–2.77735	–2.16640	–5.41021
CTTGG**G**T	–0.70938	0.74542	–1.30460	–2.05224	–1.12772
TTGG**G**TG	–4.08966	–2.42302	–8.45824	–2.89788	–2.60520
TGG**G**TGC	–4.69379	–2.74312	–5.18286	0.08137	1.31041
GG**G**TGCT	–1.66763	1.32382	**3.01556**	–7.23683	–2.85339
G**G**TGCTG	–3.50084	–2.96635	–0.86443	–3.53260	–3.39137
GTGCTGG	–0.60103	–0.96278	–0.94804	0.65349	–6.10680

[a]Target locus is in boldface. Scores above the threshold are in boldface.

In the discovery stage, rs1385405 was associated with hyperactivity/impulsive scores ($p = .012$) and FAQ ($p = .016$); TT variant homozygote showed a higher hyperactivity/impulsive scores ($p = .006$) and a lower FAQ ($p = .009$) than the GG wild-type homozygote. In the validation stage, the association of rs1385405 with hyperactivity/impulsive symptoms was confirmed; TT variant homozygote was more susceptible to hyperactivity/impulsivity than GG wild-type homozygote ($p = .010$). Besides, we found that rs1385405 was associated with attention-deficit and hyperactivity/impulsive symptoms ($p < .0125$) in combined stage, and detailed results are presented in Table 3.

In Silico Splicing Analysis for the Significant SNP

We predicted that rs1385405 had the function of splicing through the SNPinfo database (Supplemental Table S2). Then, using the Human Splicing Finder web server to query, it was found that the sequence containing the rs1385405 T allele could improve the splicing efficiency as an enhancer motif binding to SC35 or SRp40 (values 89.61 and 81.02, thresholds 75.05 and 78.08, respectively). In addition, the sequence containing the rs1385405 T allele also had the potential to reduce splicing efficiency as a silence motif, but with a value of 60.16, which is close to the threshold (60). Taken together, we speculated that the sequence containing the rs1385405 T allele was more prone to improve splicing efficiency as an exonic splicing enhancer (ESE).

We used the ESEfinder database to explore the effect of rs1385405 on pre-mRNA, and the results of the scores of the rs1385405 G allele and T allele sequences interacting with the SR proteins are shown in Tables 4 and 5, respectively. The sequence containing the G allele could bind to SC35, and the sequence containing the T allele could bind to SR35 or SRp40. We concluded that rs1385405 might

Table 5. Scores in the Sequence Containing the T Allele From ESEfinder Database.

Site[a]	SRSF1 (SF2/ASF) threshold: 1.956	SRSF1 (IgM-BRCA1) threshold: 1.867	SRSF2 (SC35) threshold: 2.383	SRSF5 (SRp40) threshold: 2.67	SRSF6 (SRp55) threshold: 2.676
GCTTGG**T**	–2.43191	–2.34237	–0.45735	–4.54814	–5.41021
CTTGG**T**T	–2.82941	–0.79789	–2.11830	–4.07479	–1.92549
TTGG**T**TG	–6.00927	–4.02688	–8.31980	–0.58905	–1.79133
TGG**T**TGC	–4.23609	–2.68393	–2.71556	–0.57893	–0.99356
GG**T**TGCT	–3.73142	–3.01141	**4.75421**	–5.67531	–1.34293
G**T**TGCTG	–4.17615	–3.69583	–1.51211	–3.02311	–5.69534
TTGCTGG	–1.97421	–1.16963	–2.99769	**3.15782**	–3.29753

[a]Target locus is in boldface. Scores above the threshold are in boldface.

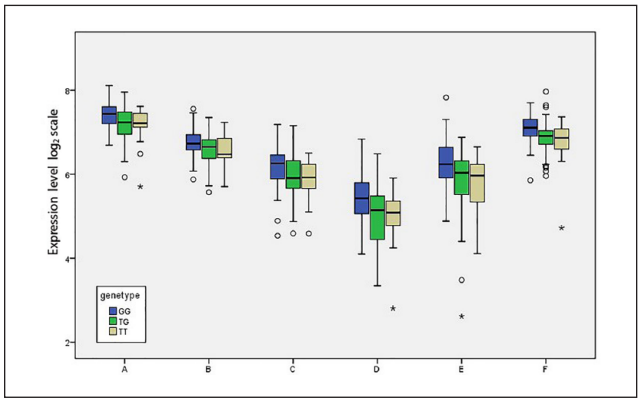

Figure 1. Association of rs1385405 with GRID2 expression in the frontal cortex in the BRAINEAC database.
Note. A:2736061 probe ($p = .0033$), B:2736146 probe ($p = .0083$), C:2736149 probe ($p = .0098$), D:2736176 probe ($p = .0028$), E:2736181 probe ($p = .0015$), F:2736209 probe ($p = .0011$).
*Represents extreme values.

change the binding of the SR protein to pre-mRNA, which might affect ESE activity.

e-QTL Analysis for rs1385405

We retrieved the available data through the BRAINEAC database for e-QTL analysis. The results showed that individuals carrying rs1385405-G had higher GRID2 mRNA expression than rs1385405-T carriers in the frontal cortex with 14 different probes ($p < .05$); among them, six p-values were less than .01 ($p = .0033$, .083, .0098, .0028, .0015, and .0011, respectively), as shown in Figure 1.

Discussion

In our study, we comprehensively investigated the association between ionotropic glutamate receptor gene polymorphisms and ADHD susceptibility in a two-stage case–control study in the Chinese population for the first time. We found that GRID2 rs1385405 T/T was associated with the increased risk of ADHD and was associated with hyperactivity/impulse and inattention symptoms. Bioinformatics analysis showed that rs1385405 interfered with ESE activity, and e-QTL analysis demonstrated that rs1385405 affected GRID2 gene expression in the frontal cortex.

There was no association between GRIN2A, GRIN2B, GRIK1, and GRIK4 polymorphisms and ADHD in our study. Similarly, Kim et al. (2017) did not find a relationship between GRIN2A and ADHD. However, Turic et al. (2004) and Kim et al. (2016) reported that GIRN2A might confer an increased risk for ADHD and be associated with attention problems in ADHD patients. In addition, some studies have suggested that GRIN2B is implicated in the risk of ADHD (Dorval et al., 2007; Kim et al., 2016, 2017). For GRIK1 and GRIK4, GWASs suggested that they might be associated with the risk of ADHD (Hinney et al., 2011; Lasky-Su et al., 2008). A potential explanation for the inconsistent correlation results may be partly due to differences in the allele frequencies among people of different races.

Some studies have investigated the relationship between GRID2 gene and ADHD in other populations. Stergiakouli et al. (2012) conducted a GWAS with 727 Caucasian children with ADHD and 5,081 comparison participants in 2012, which provided preliminary evidence that GRID2 rs6815704 might be associated with the risk of ADHD albeit below GWAS significance ($p = 2.09 \times 10^{-5}$); we failed to replicate the reported association. This might be due to the differences in the allele frequencies and LD patterns among populations. Moreover, the GRID2 rs1385405 was absent in significant GWAS results. Rs1385405 and rs6815704 are located on the exon 9/16 and intron 1/16 of the GRID2, respectively, non-LD. Using the Psychiatric Genomics Consortium (PGC) database (https://www.med.unc.edu/pgc/results-and-downloads/adhd/), we found that the smallest p-values of GRID2 were 1.29×10^{-5} that did not reach the GWAS significance as many other significant candidate genes did (Demontis et al., 2019). However, the GWAS was conducted in the European people while our research was based on Chinese Han population, so we will

verify our results in different genetic ancestry in future research. Although no SNP of GRID2 passed the rigorous significant level in GWASs, a GWAS meta-analysis of ADHD indicated that neurodevelopmental processes were likely to be associated with ADHD (Demontis et al., 2019), which suggested that GRID2 gene important for synapse formation and long-term depression (Kakegawa et al., 2008) might be relevant to ADHD.

GRID2 maps to chromosome 4q22.1-22.2 and codes for the glutamate receptor δ2 (GluRD2) protein, which is a multi-pass membrane protein and consists of two extracellular domains, an amino-terminal (ATL) and a ligand binding domain (LBD). GRID2 is expressed exclusively at postsynaptic site and is involved in a retrograde signaling pathway that modifies the presynaptic release probability (Kakegawa et al., 2008). It is mostly expressed in the brain regions throughout the lifespan of an individual, with detectable concentrations in the cerebellum, anterior cingulate cortex, caudate, frontal cortex, hippocampus, and putamen, all of which have been implicated in ADHD (Bauer et al., 2018; Dang et al., 2016; Hoogman et al., 2017; Stevens & Haney-Caron, 2012).

Using HaploReg as a tool for systematically mining these chromatin state data, along with conservation data and regulatory motif alterations (Ward & Kellis, 2012), we identified that rs1385405 was located in the region containing the enhancer histone marks of H9 Derived Neuronal Progenitor Cultured Cells and would change regulatory motifs for pax-6, and the role of Pax genes in the process of neurodevelopment (Thompson & Ziman, 2011) supported a functional role for rs1385405 in neuropsychiatric disorders. In addition, the e-QTL analysis showed that the expression level of GRID2 of carriers of the rs1385405 T allele was lower than that of carriers of the G allele in the frontal cortex, and studies have confirmed that the frontal cortex is involved in the pathogenesis of ADHD (Hoogman et al., 2019). The frontal cortex is a glutamate-rich brain region, and deficits in this area cause poor impulse control, distractibility, hyperactivity, and poor organization and planning (Stuss & Levine, 2002), which is consistent with our result that rs1385405 was associated with hyperactivity and distractibility symptoms. Hence, we speculated that decreased expression of GRID2 in the frontal cortex might be one of the reasons for the increased risk of ADHD, possibly through regulation of alternative splicing or instead containing another functional variant responsible for alterations in GRID2 expression or function.

In addition, we suspected that GRID2 might be involved in the pathogenesis of ADHD through its biological function in the cerebellum. GRID2 is selectively and highly expressed in cerebellar Purkinje cell (Jeromin et al., 1996). Indeed, the cerebellum was involved in the pathogenesis of ADHD, which was confirmed by numerous studies (Bruchhage et al., 2018; Goetz et al., 2017; Stoodley, 2016). The homozygous deletion of the GRID2 gene was associated with cerebellar atrophy, development delay (Ali et al., 2017; Taghdiri et al., 2019; Utine et al., 2013); patients with bilateral cerebellar atrophy showed similar clinical features as ADHD patients, such as hyperactivity and distraction, and ADHD symptoms significantly improved with methylphenidate treatment, which is a common drug for ADHD patients (C. Chang & Siao, 2016). In addition, a nonprogressive loss of volume was observed in the superior cerebellar vermis in ADHD patients (Mackie et al., 2007). Moreover, GRID2 plays essential roles in facilitating cerebellar synapse organization and transmission, motor coordination, and long-term depression of parallel fiber-Purkinje cell synaptic transmission (Kakegawa et al., 2008; Kashiwabuchi et al., 1995). In addition, Vloet et al. (2010) observed disrupted functional connectivity between the frontal cortex and the cerebellum during time discrimination in the ADHD group.

As members of the iGluRs family, NMDAR and AMPAR dysfunction might be one of the potential causes of ADHD pathogenesis (J. P. Chang et al., 2014; Cheng et al., 2017; Fan et al., 2018; Medin et al., 2019), and GRID2 is critical for correcting NMDAR function and maintaining proper transmission of AMPAR signals (Kumagai et al., 2014; Yamasaki et al., 2011). In addition, dysfunction in GluRD2 is associated with a host of psychiatric phenotypes, including ASD, which shares 50%–70% of its contributing genetic factors with ADHD (Kalkan et al., 2016; Pinto et al., 2014; Rommelse et al., 2010), obsessive–compulsive disorder (OCD) (Khramtsova et al., 2019), and schizophrenia endophenotypes (Greenwood et al., 2016). Furthermore, risperidone was combined with methylphenidate to treat ADHD with comorbidities, such as conduct disorder and oppositional defiant disorder (Gadow et al., 2014; Masi et al., 2017), and the GRID2 gene was significantly associated with the response of risperidone monotherapy to psychosis in a genome-wide association analysis (Stevenson et al., 2016).

Several limitations in the study should be noted. First, the sample size should be expanded to verify the results in Chinese population. Second, functional experiments have not been carried out to verify the possible biological mechanism of rs1385405 in the pathogenesis of ADHD.

In short, our research adds new evidence for GRID2 involved in the pathogenesis of ADHD. However, the exact molecular mechanisms through which GRID2 genetic polymorphisms underlie ADHD remain to be investigated.

Conclusion

The two-stage case–control study emphasized the role of GRID2 rs1385405 in ADHD susceptibility in the Chinese Han population, and further functional experiments are needed to verify the effect of rs1385405 on pre-RNA splicing. Future studies with larger sample sizes and different ethnicities are warranted to confirm these results.

Declaration of Conflicting Interests

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Supplemental material

Supplemental material for this article is available online.

References

Ali, Z., Zulfiqar, S., Klar, J., Wikstrom, J., Ullah, F., Khan, A., . . . Dahl, N. (2017). Homozygous GRID2 missense mutation predicts a shift in the D-serine binding domain of GluD2 in a case with generalized brain atrophy and unusual clinical features. *BMC Medical Genetics*, *18*(1), Article 144. https://doi.org/10.1186/s12881-017-0504-6

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.).

Asherson, P., Buitelaar, J., Faraone, S. V., & Rohde, L. A. (2016). Adult attention-deficit hyperactivity disorder: Key conceptual issues. *The Lancet Psychiatry*, *3*(6), 568–578. https://doi.org/10.1016/s2215-0366(16)30032-3

Bauer, J., Werner, A., Kohl, W., Kugel, H., Shushakova, A., Pedersen, A., & Ohrmann, P. (2018). Hyperactivity and impulsivity in adult attention-deficit/hyperactivity disorder is related to glutamatergic dysfunction in the anterior cingulate cortex. *World Journal of Biological Psychiatry*, *19*(7), 538–546. https://doi.org/10.1080/15622975.2016.1262060

Bruchhage, M. M. K., Bucci, M. P., & Becker, E. B. E. (2018). Cerebellar involvement in autism and ADHD. *Handbook of Clinical Neurology*, *155*, 61–72. https://doi.org/10.1016/b978-0-444-64189-2.00004-4

Chang, C., & Siao, S. W. (2016). Cerebellar cognitive affective syndrome: Attention deficit-hyperactivity disorder episode of adolescent with cerebellar atrophy in a psychiatric ward. *The Kaohsiung Journal of Medical Sciences*, *32*(1), 52–54. https://doi.org/10.1016/j.kjms.2015.10.006

Chang, J. P., Lane, H. Y., & Tsai, G. E. (2014). Attention deficit hyperactivity disorder and N-methyl-D-aspartate (NMDA) dysregulation. *Current Pharmaceutical Design*, *20*(32), 5180–5185. https://doi.org/10.2174/1381612819666140110115227

Cheng, J., Liu, A., Shi, M. Y., & Yan, Z. (2017). Disrupted glutamatergic transmission in prefrontal cortex contributes to behavioral abnormality in an animal model of ADHD. *Neuropsychopharmacology*, *42*(10), 2096–2104. https://doi.org/10.1038/npp.2017.30

Costa, D. S., de Paula, J. J., Malloy-Diniz, L. F., Romano-Silva, M. A., & Miranda, D. M. (2019). Parent SNAP-IV rating of attention-deficit/hyperactivity disorder: Accuracy in a clinical sample of ADHD, validity, and reliability in a Brazilian sample. *Jornal de Pediatria*, *95*, 736–743. https://doi.org/10.1016/j.jped.2018.06.014

Dang, L. C., Samanez-Larkin, G. R., Young, J. S., Cowan, R. L., Kessler, R. M., & Zald, D. H. (2016). Caudate asymmetry is related to attentional impulsivity and an objective measure of ADHD-like attentional problems in healthy adults. *Brain Structure & Function*, *221*(1), 277–286. https://doi.org/10.1007/s00429-014-0906-6

DasBanerjee, T., Middleton, F. A., Berger, D. F., Lombardo, J. P., Sagvolden, T., & Faraone, S. V. (2008). A comparison of molecular alterations in environmental and genetic rat models of ADHD: A pilot study. *American Journal of Medical Genetics: Part B, Neuropsychiatric Genetics, 147B*(8), 1554–1563. https://doi.org/10.1002/ajmg.b.30877

Dela Peña, I., Bang, M., Lee, J., de la Peña, J. B., Kim, B. N., Han, D. H., . . . Cheong, J. H. (2015). Common prefrontal cortical gene expression profiles between adolescent SHR/NCrl and WKY/NCrl rats which showed inattention behavior. *Behavioural Brain Research*, *291*, 268–276. https://doi.org/10.1016/j.bbr.2015.05.012

Demontis, D., Walters, R. K., Martin, J., Mattheisen, M., Als, T. D., Agerbo, E., . . . Neale, B. M. (2019). Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. *Nature Genetics*, *51*(1), 63–75. https://doi.org/10.1038/s41588-018-0269-7

Diana, M. C., Santoro, M. L., Xavier, G., Santos, C. M., Spindola, L. N., Moretti, P. N., . . .Belangero, S. I. (2015). Low expression of Gria1 and Grin1 glutamate receptors in the nucleus accumbens of Spontaneously Hypertensive Rats (SHR). *Psychiatry Research*, *229*(3), 690–694. https://doi.org/10.1016/j.psychres.2015.08.021

Dorval, K. M., Wigg, K. G., Crosbie, J., Tannock, R., Kennedy, J. L., Ickowicz, A., . . . Barr, C. L. (2007). Association of the glutamate receptor subunit gene GRIN2B with attention-deficit/hyperactivity disorder. *Genes, Brain and Behavior*, *6*(5), 444–452. https://doi.org/10.1111/j.1601-183X.2006.00273.x

Ende, G., Cackowski, S., Van Eijk, J., Sack, M., Demirakca, T., Kleindienst, N., . . . Schmahl, C. (2016). Impulsivity and aggression in female BPD and ADHD patients: Association with ACC glutamate and GABA concentrations. *Neuropsychopharmacology*, *41*(2), 410–418. https://doi.org/10.1038/npp.2015.153

Fan, Z., Qian, Y., Lu, Q., Wang, Y., Chang, S., & Yang, L. (2018). DLGAP1 and NMDA receptor-associated postsynaptic density protein genes influence executive function in attention deficit hyperactivity disorder. *Brain and Behavior*, *8*(2), e00914. https://doi.org/10.1002/brb3.914

Faraone, S. V., Perlis, R. H., Doyle, A. E., Smoller, J. W., Goralnick, J. J., Holmgren, M. A., & Sklar, P. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry*, *57*(11), 1313–1323. https://doi.org/10.1016/j.biopsych.2004.11.024

Gadow, K. D., Arnold, L. E., Molina, B. S., Findling, R. L., Bukstein, O. G., Brown, N. V., . . . Aman, M. G. (2014). Risperidone added to parent training and stimulant medication: Effects on

attention-deficit/hyperactivity disorder, oppositional defiant disorder, conduct disorder, and peer aggression. *Journal of the American Academy of Child & Adolescent Psychiatry*, *53*(9), 948–959.e1. https://doi.org/10.1016/j.jaac.2014.05.008

Gallo, E. F., & Posner, J. (2016). Moving towards causality in attention-deficit hyperactivity disorder: Overview of neural and genetic mechanisms. *The Lancet Psychiatry*, *3*(6), 555–567. https://doi.org/10.1016/s2215-0366(16)00096-1

Gizer, I. R., Ficks, C., & Waldman, I. D. (2009). Candidate gene studies of ADHD: A meta-analytic review. *Human Genetics*, *126*(1), 51–90. https://doi.org/10.1007/s00439-009-0694-x

Goetz, M., Schwabova, J., Hlavka, Z., Ptacek, R., Zumrova, A., Hort, V., & Doyle, R. (2017). Cerebellar symptoms are associated with omission errors and variability of response time in children With ADHD. *Journal of Attention Disorders*, *21*(3), 190–199. https://doi.org/10.1177/1087054713517745

Greenwood, T. A., Lazzeroni, L. C., Calkins, M. E., Freedman, R., Green, M. F., Gur, R. E., . . .Braff, D. L. (2016). Genetic assessment of additional endophenotypes from the Consortium on the Genetics of Schizophrenia Family Study. *Schizophrenia Research*, *170*(1), 30–40. https://doi.org/10.1016/j.schres.2015.11.008

Gu, X., Yuan, F. F., Huang, X., Hou, Y., Wang, M., Lin, J., & Wu, J. (2018). Association of PIK3CG gene polymorphisms with attention-deficit/hyperactivity disorder: A case-control study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *81*, 169–177. https://doi.org/10.1016/j.pnpbp.2017.10.020

Hinney, A., Scherag, A., Jarick, I., Albayrak, O., Putter, C., Pechlivanis, S., . . . Hebebrand, J. (2011). Genome-wide association study in German patients with attention deficit/hyperactivity disorder. *American Journal of Medical Genetics: Part B Neuropsychiatric Genetics*, *156B*(8), 888–897. https://doi.org/10.1002/ajmg.b.31246

Hoogman, M., Bralten, J., Hibar, D. P., Mennes, M., Zwiers, M. P., Schweren, L. S. J., . . . Franke, B. (2017). Subcortical brain volume differences in participants with attention deficit hyperactivity disorder in children and adults: A cross-sectional mega-analysis. *Lancet Psychiatry*, *4*(4), 310–319. https://doi.org/10.1016/s2215-0366(17)30049-4

Hoogman, M., Muetzel, R., Guimaraes, J. P., Shumskaya, E., Mennes, M., Zwiers, M. P., . . . Franke, B. (2019). Brain Imaging of the Cortex in ADHD: A Coordinated Analysis of Large-Scale Clinical and Population-Based Samples. *Am J Psychiatry*, *176*(7), 531–542. https://doi.org/10.1176/appi.ajp.2019.18091033

Huang, X., Wang, M., Zhang, Q., Chen, X., & Wu, J. (2019). The role of glutamate receptors in attention-deficit/hyperactivity disorder: From physiology to disease. *American Journal of Medical Genetics: Part B Neuropsychiatric Genetics*, *180*(4), 272–286. https://doi.org/10.1002/ajmg.b.32726

Jeromin, A., Huganir, R. L., & Linden, D. J. (1996). Suppression of the glutamate receptor delta 2 subunit produces a specific impairment in cerebellar long-term depression. *Journal of Neurophysiology*, *76*(5), 3578–3583. https://doi.org/10.1152/jn.1996.76.5.3578

Kakegawa, W., Miyazaki, T., Emi, K., Matsuda, K., Kohda, K., Motohashi, J., . . . Yuzaki, M. (2008). Differential regulation of synaptic plasticity and cerebellar motor learning by the C-terminal PDZ-binding motif of GluRdelta2. *Journal of Neuroscience*, *28*(6), 1460–1468. https://doi.org/10.1523/jneurosci.2553-07.2008

Kalkan, Z., Durasi, I. M., Sezerman, U., & Atasever-Arslan, B. (2016). Potential of GRID2 receptor gene for preventing TNF-induced neurodegeneration in autism. *Neuroscience Letters*, *620*, 62–69. https://doi.org/10.1016/j.neulet.2016.03.043

Kashiwabuchi, N., Ikeda, K., Araki, K., Hirano, T., Shibuki, K., Takayama, C., . . .Mishina, M. (1995). Impairment of motor coordination, Purkinje cell synapse formation, and cerebellar long-term depression in GluR delta 2 mutant mice. *Cell*, *81*(2), 245–252. https://doi.org/10.1016/0092-8674(95)90334-8

Khramtsova, E. A., Heldman, R., Derks, E. M., Yu, D., Davis, L. K., & Stranger, B. E. (2019). Sex differences in the genetic architecture of obsessive-compulsive disorder. *American Journal of Medical Genetics: Part B Neuropsychiatric Genetics*, *180*(6), 351–364. https://doi.org/10.1002/ajmg.b.32687

Kim, J. I., Kim, J. W., Park, J. E., Park, S., Hong, S. B., Han, D. H., . . . Kim, B. N. (2017). Association of the GRIN2B rs2284411 polymorphism with methylphenidate response in attention-deficit/hyperactivity disorder. *Journal of Psychopharmacology*, *31*(8), 1070–1077. https://doi.org/10.1177/0269881116667707

Kim, J. I., Kim, J. W., Park, S., Hong, S. B., Lee, D. S., Paek, S. H., . . . Kim, B. N. (2016). The GRIN2B and GRIN2A gene variants are associated with continuous performance test variables in ADHD. *Journal of Attention Disorders*. https://doi.org/10.1177/1087054716649665

Kumagai, A., Fujita, A., Yokoyama, T., Nonobe, Y., Hasaba, Y., Sasaki, T., . . . Takemori, H. (2014). Altered actions of memantine and NMDA-induced currents in a new Grid2-deleted mouse line. *Genes*, *5*(4), 1095–1114. https://doi.org/10.3390/genes5041095

Lasky-Su, J., Neale, B. M., Franke, B., Anney, R. J., Zhou, K., Maller, J. B., . . . Faraone, S. V. (2008). Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. *American Journal of Medical Genetics: Part B Neuropsychiatric Genetics*, *147B*(8), 1345–1354. https://doi.org/10.1002/ajmg.b.30867

Lehohla, M., Kellaway, L., & Russell, V. A. (2004). NMDA receptor function in the prefrontal cortex of a rat model for attention-deficit hyperactivity disorder. *Metabolic Brain Disease*, *19*(1–2), 35–42.

Mackie, S., Shaw, P., Lenroot, R., Pierson, R., Greenstein, D. K., Nugent, T. F., III, . . . Rapoport, J. L. (2007). Cerebellar development and clinical outcome in attention deficit hyperactivity disorder. *American Journal of Psychiatry*, *164*(4), 647–655. https://doi.org/10.1176/ajp.2007.164.4.647

Masi, G., Manfredi, A., Nieri, G., Muratori, P., Pfanner, C., & Milone, A. (2017). A naturalistic comparison of methylphenidate and risperidone monotherapy in drug-naive youth with attention-deficit/hyperactivity disorder comorbid with oppositional defiant disorder and aggression. *Journal of Clinical Psychopharmacology*, *37*(5), 590–594. https://doi.org/10.1097/jcp.0000000000000747

Medin, T., Jensen, V., Skare, O., Storm-Mathisen, J., Hvalby, O., & Bergersen, L. H. (2019). Altered alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor function

and expression in hippocampus in a rat model of attention-deficit/hyperactivity disorder (ADHD). *Behavioural Brain Research*, *360*, 209–215. https://doi.org/10.1016/j.bbr.2018.12.028

Michielsen, M., Semeijn, E., Comijs, H. C., van de Ven, P., Beekman, A. T., Deeg, D. J., & Kooij, J. J. (2012). Prevalence of attention-deficit hyperactivity disorder in older adults in the Netherlands. *British Journal of Psychiatry*, *201*(4), 298–305. https://doi.org/10.1192/bjp.bp.111.101196

Moreno-Garcia, I., Delgado-Pardo, G., & Roldan-Blasco, C. (2015). Attention and response control in ADHD. Evaluation through integrated visual and auditory continuous performance test. *Spanish Journal of Psychology*, *18*, E1. https://doi.org/10.1017/sjp.2015.2

Naaijen, J., Bralten, J., Poelmans, G., Glennon, J. C., Franke, B., & Buitelaar, J. K. (2017). Glutamatergic and GABAergic gene sets in attention-deficit/hyperactivity disorder: Association to overlapping traits in ADHD and autism. *Translational Psychiatry*, *7*(1), e999. https://doi.org/10.1038/tp.2016.273

Naumova, D., Grizenko, N., Sengupta, S. M., & Joober, R. (2019). DRD4 exon 3 genotype and ADHD: Randomised pharmacodynamic investigation of treatment response to methylphenidate. *World Journal of Biological Psychiatry*, *20*, 486–495. https://doi.org/10.1080/15622975.2017.1410221

Pinto, D., Delaby, E., Merico, D., Barbosa, M., Merikangas, A., Klei, L., . . . Scherer, S. W. (2014). Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. *American Journal of Human Genetics*, *94*(5), 677–694. https://doi.org/10.1016/j.ajhg.2014.03.018

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry*, *56*(3), 345–365. https://doi.org/10.1111/jcpp.12381

Rommelse, N. N., Franke, B., Geurts, H. M., Hartman, C. A., & Buitelaar, J. K. (2010). Shared heritability of attention-deficit/hyperactivity disorder and autism spectrum disorder. *European Child & Adolescent Psychiatry*, *19*(3), 281–295. https://doi.org/10.1007/s00787-010-0092-x

Stergiakouli, E., Hamshere, M., Holmans, P., Langley, K., Zaharieva, I., Hawi, Z., . . . Thapar, A. (2012). Investigating the contribution of common genetic variants to the risk and pathogenesis of ADHD. *American Journal of Psychiatry*, *169*(2), 186–194. https://doi.org/10.1176/appi.ajp.2011.11040551

Stevens, M. C., & Haney-Caron, E. (2012). Comparison of brain volume abnormalities between ADHD and conduct disorder in adolescence. *Journal of Psychiatry & Neuroscience*, *37*(6), 389–398. https://doi.org/10.1503/jpn.110148

Stevenson, J. M., Reilly, J. L., Harris, M. S., Patel, S. R., Weiden, P. J., Prasad, K. M., . . . Bishop, J. R. (2016). Antipsychotic pharmacogenomics in first episode psychosis: A role for glutamate genes. *Translational Psychiatry*, *6*, e739. https://doi.org/10.1038/tp.2016.10

Stoodley, C. J. (2016). The cerebellum and neurodevelopmental disorders. *Cerebellum*, *15*(1), 34–37. https://doi.org/10.1007/s12311-015-0715-3

Stuss, D. T., & Levine, B. (2002). Adult clinical neuropsychology: Lessons from studies of the frontal lobes. *Annual Review of Psychology*, *53*, 401–433. https://doi.org/10.1146/annurev.psych.53.100901.135220

Surman, C. B., Hammerness, P. G., Petty, C., Spencer, T., Doyle, R., Napolean, S., . . . Biederman, J. (2013). A pilot open label prospective study of memantine monotherapy in adults with ADHD. *World Journal of Biological Psychiatry*, *14*(4), 291–298. https://doi.org/10.3109/15622975.2011.623716

Taghdiri, M., Kashef, A., Abbassi, G., Moshtagh, A., Sadatian, N., Fardaei, M., . . . Kariminejad, R. (2019). Further delineation of the phenotype caused by a novel large homozygous deletion of GRID2 gene in an adult patient. *Clinical Case Reports*, *7*(6), 1149–1153. https://doi.org/10.1002/ccr3.2020

Thapar, A., & Cooper, M. (2016). Attention deficit hyperactivity disorder. *Lancet*, *387*(10024), 1240–1250. https://doi.org/10.1016/s0140-6736(15)00238-x

Thompson, J. A., & Ziman, M. (2011). Pax genes during neural development and their potential role in neuroregeneration. *Progress in Neurobiology*, *95*(3), 334–351. https://doi.org/10.1016/j.pneurobio.2011.08.012

Turic, D., Langley, K., Mills, S., Stephens, M., Lawson, D., Govan, C., . . . Thapar, A. (2004). Follow-up of genetic linkage findings on chromosome 16p13: Evidence of association of N-methyl-D aspartate glutamate receptor 2A gene polymorphism with ADHD. *Molecular Psychiatry*, *9*(2), 169–173. https://doi.org/10.1038/sj.mp.4001387

Tye, S. J., Miller, A. D., & Blaha, C. D. (2013). Ventral tegmental ionotropic glutamate receptor stimulation of nucleus accumbens tonic dopamine efflux blunts hindbrain-evoked phasic neurotransmission: Implications for dopamine dysregulation disorders. *Neuroscience*, *252*, 337–345. https://doi.org/10.1016/j.neuroscience.2013.08.010

Utine, G. E., Haliloglu, G., Salanci, B., Cetinkaya, A., Kiper, P. O., Alanay, Y., . . . Alikasifoglu, M. (2013). A homozygous deletion in GRID2 causes a human phenotype with cerebellar ataxia and atrophy. *Journal of child neurology*, *28*(7), 926–932. https://doi.org/10.1177/0883073813484967

Vloet, T. D., Gilsbach, S., Neufang, S., Fink, G. R., Herpertz-Dahlmann, B., & Konrad, K. (2010). Neural mechanisms of interference control and time discrimination in attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry*, *49*(4), 356–367.

Volkow, N. D., & Swanson, J. M. (2013). Clinical practice: Adult attention deficit-hyperactivity disorder. *New England Journal of Medicine*, *369*(20), 1935–1944. https://doi.org/10.1056/NEJMcp1212625

Wang, T., Liu, K., Li, Z., Xu, Y., Liu, Y., Shi, W., & Chen, L. (2017). Prevalence of attention deficit/hyperactivity disorder among children and adolescents in China: A systematic review and meta-analysis. *BMC Psychiatry*, *17*(1), Article 32. https://doi.org/10.1186/s12888-016-1187-9

Ward, L. D., & Kellis, M. (2012). HaploReg: A resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Research*, *40*, D930–D934. https://doi.org/10.1093/nar/gkr917

Yamasaki, M., Miyazaki, T., Azechi, H., Abe, M., Natsume, R., Hagiwara, T., . . . Watanabe, M. (2011). Glutamate receptor delta2 is essential for input pathway-dependent regulation

of synaptic AMPAR contents in cerebellar Purkinje cells. *Journal of Neuroscience*, *31*(9), 3362–3374. https://doi.org/10.1523/jneurosci.5601-10.2011

Yuen, E. Y., Zhong, P., Li, X., Wei, J., & Yan, Z. (2013). Restoration of glutamatergic transmission by dopamine D4 receptors in stressed animals. *Journal of Biological Chemistry*, *288*(36), 26112–26120. https://doi.org/10.1074/jbc.M112.396648

Yuen, E. Y., Zhong, P., & Yan, Z. (2010). Homeostatic regulation of glutamatergic transmission by dopamine D4 receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(51), 22308–22313. https://doi.org/10.1073/pnas.1010025108

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