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Association between the group III metabotropic glutamate receptor gene polymorphisms and attention-deficit/hyperactivity disorder and functional exploration of risk loci

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ABSTRACT

Existing evidence suggests that the group III metabotropic glutamate receptor (mGluR) gene variations are involved in attention-deficit/hyperactivity disorder (ADHD), but few studies have fully explored this association. We conducted a case-control study with 617 cases and 636 controls to investigate the association between functional single-nucleotide polymorphisms (SNPs) from the group III mGluR gene polymorphisms (GRM4, GRM7, GRM8) and ADHD in the Chinese Han population and initially explored the function of positive SNPs. The GRM4 rs1906953 T genotype showed a significant association with a decreased risk of ADHD (TT:CC, *OR* = 0.55, *95% CI* = 0.40–0.77; recessive model, *OR* = 0.58, *95% CI* = 0.43–0.78). GRM7 rs9826579 C showed a significant association with an increased risk of ADHD (TC:TT, OR = 1.81, *95% CI* = 1.39–2.36; dominant model, OR = 1.74, *95% CI* = 1.35–2.24; additive model, OR = 1.56, *95% CI* = 1.24–1.97). In addition, compared with subjects with the rs1906953 TT genotype, subjects with of the CC genotype showed more obvious attention deficit behaviours and hyperactivity/impulsive behaviours. Dual-luciferase reporter gene assays showed that a promoter reporter with the rs1906953 TT genotype significantly decreased luciferase activity compared with the CC genotype. According to electrophoretic mobility shift assays, the binding capacity of rs1906953 T probe with nucleoprotein was lower than that of the rs1906953 C probe. Our results revealed the association of GRM4 rs1906953 and GRM7 rs9826579 with ADHD. Moreover, we found that rs1906953 disturbs the transcriptional activity of GRM4.

1. Introduction

Attention deficit/hyperactivity disorder (ADHD) is a common childhood neurodevelopmental disorder with symptoms of inattentiveness, hyperactivity, and impulsiveness, leading to marked impairment in academic, family, social functioning, and driving activities and increased criminal offenses (O'Neill et al., 2017; Wolraich et al., 2019). Recently, meta-analysis results showed that the pooled prevalence of ADHD in China was 6.3%, with a worldwide prevalence of 7.2% (Thomas et al., 2015; T. Wang et al., 2017). It has been reported that the prevalence of ADHD in children and adolescents in the United States has risen by 41% in the past decade (Hinshaw, 2018).

It is generally believed that genetic components play an important role in the pathophysiology of ADHD, with a heritability ranging from 77 to 88% (Faraone and Larsson, 2019). Many studies have examined candidate genes in monoamine transmission pathways and found some related to the risk of ADHD (5HTT, DAT1, DRD4, DRD5, HTR1B, SLC6A3 and SNAP25) (Bonvicini et al., 2016; Faraone and Larsson, 2019), but without replication in genome-wide association studies (GWASs). To date, most GWASs have not identified genes that reach genome-wide significance ($P \leq 5 \times 10^{-8}$), and only a recent meta-analysis of ADHD GWASs detected 12 genomic loci with genome-wide significance (Demontis et al., 2019).

Glutamate serves as the major excitatory neurotransmitter in the

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mammalian central nervous system; it mainly mediates biological functions, including neuronal migration, synaptic transmission, long-term depression (LTD) and long-term potentiation (LTP) by binding to glutamate receptors (Pittenger et al., 2011). In a previous study, we found that gene variations in the ionotropic glutamate receptor were associated with the risk of ADHD (Zhang et al., 2020).

Metabotropic glutamate receptors (mGluRs) are seven transmembrane G-protein coupled receptors (GPCRs) that have been categorized into three subtypes based on their sequence homology, ligand selectivity, and G-protein coupling (Niswender and Conn, 2010). Group III mGluRs (mGluR4/mGluR6/mGluR7/mGluR8) are thought to be involved in long-term and short-term forms of synaptic plasticity in the brain and are associated with LTD, which plays an important role in working memory (Mukherjee and Manahan-Vaughan, 2013). Numerous studies have demonstrated that group III mGluR activation has neuroprotective effects (Mercier and Lodge, 2014) and exerts anxiolytic-like effects (Stachowicz et al., 2009).

Among Group III mGluRs, mGluR4, mGluR7, and mGluR8 are mainly expressed presynaptically in the central nervous system (CNS) and act as both auto- and heteroreceptors to modulate glutamate release and responses, i.e., by coupling to Gi proteins to subsequently inhibit adenyly1 cyclase and lowering the intracellular cyclic adenosine monophosphate (cAMP) concentration and protein kinase A (PKA) activation (Cartmell and Schoepp, 2000; Mercier and Lodge, 2014; Schoepp, 2001). Moreover, studies have shown that mGluR4, mGluR7, and mGluR8 have a role in neurodevelopmental disorders (Dadkhah et al., 2017; H. Li et al., 2008; Noroozi et al., 2016). Unlike mGluR4, mGluR7, and mGluR8, expression of mGluR6 is mostly restricted to the retina, and GRM6 gene variations are often associated with retinopathy, such as night blindness and high myopia (Sergouniotis et al., 2012; H. Wang et al., 2016).

A whole-genome copy number variation (CNV) study indicated that GRM7 and GRM8 deletions might be associated with the risk of ADHD (Elia et al., 2011). Candidate gene studies found that CNVs in the GRM8 gene are overrepresented in ADHD patients and that GRM7 polymorphisms are associated with the risk of ADHD (Akutagava-Martins et al., 2014; Noroozi et al., 2019). GRM4, GRM7 and GRM8 gene knockout mice exhibit impairments in spatial information processing, learning and memory functions (Davis et al., 2013; Gerlai et al., 2002; Gerlai et al., 1998; Holscher et al., 2005).

Although some studies suggest that group III mGluR gene variations may be related to the risk of ADHD, there is still a lack of related research, and this type of research has not been carried out in the Chinese population. Therefore, this study selected functional single-nucleotide polymorphisms (SNPs) in GRM4, GRM7, and GRM8, and focused on an analysis of the association between these SNPs and ADHD in Chinese individuals and possible associated biological mechanisms.

2. Materials and methods

2.1. Subjects and diagnostic assessment

In total, 617 patients newly diagnosed with ADHD and 636 healthy controls were recruited for the study from the Wuhan Medical and Health Center for Women and Children between December 2016 and January 2019. ADHD patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) diagnostic criteria. Clinical interviews with at least one guardian were conducted by a trained child psychiatrist. Furthermore, the Swanson, Nolan, and Pelham version IV (SNAP-IV) scale was used as an auxiliary diagnostic method for the evaluation and classification of ADHD symptoms. The guardians rated the children's inattentive and hyperactive/impulsive symptoms using the total score of each dimension, producing a raw score ranging from 0 to 27. The controls were selected among ADHD-free children who underwent the same screening programme.

Participants who met the following criteria were enrolled: (1) ethnic Han Chinese origin; (2) age between 6 and 18 years old; and (3) a full-scale IQ $\geq$ 70 according to the Chinese -Wechsler Intelligence Scale for Children. Children who had a history of psychostimulant or other psychiatric drug use or neuropsychiatric disease (such as autism spectrum disorder, bipolar disorder, or major depressive disorders) were excluded.

This study was approved by the Ethics Committees of Tongji Medical College of Huazhong University of Science and Technology and Wuhan Medical and Health Center for Women and Children and was carried out in accordance with the latest version of the Declaration of Helsinki. We informed the parents of each subject of the purpose of the study, and they provided written informed consent.

2.2. SNP selection and genotyping

First, we identified SNPs with a minor allele frequency (MAF) > 0.1 (Prakash et al., 2016) in the Han Chinese population in Beijing from the target genes located in the exon region, promoter region and noncoding regions (5′UTR and 3′UTR) in the NCBI-dbSNP database (http://www.ncbi.nlm.nih.gov/snp/). Second, functional SNPs were selected from the SNPinfo web server (https://snpinfo.niehs.nih.gov/) (Xu and Taylor, 2009) and the HaploReg v4.1 database (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) (Ward and Kellis, 2016). Third, SNPs with disease susceptibility were labelled by consulting relevant literature. The SNPs included in this study met any two of the screening criteria described above. Finally, we selected 9 SNPs, namely, rs2229901, rs1906953, rs2499730, rs3749380, rs9826579, rs9870680, rs1508724, rs10268335 and rs712723, and the detailed results are shown in Table S1. We used the linkage disequilibrium (LD) calculator provided by the Ensemble web server (http://asia.ensembl.org/Homo_sapiens/Tools/LD?Db=core) to perform LD analysis of the SNPs included in the study, and there was no strong LD among them (Table S2).

Genomic DNA was extracted from peripheral blood samples using the Relax Gene Blood DNA System DP319-02 (Tiangen, Beijing, China). Genotyping was performed with the MassARRAY and iPlex systems of the Sequenom genotyping platform (Sequenom, San Diego, CA, USA). The detailed process of genotyping has been described in a previous study (Yuan et al., 2017).

2.3. Dual-luciferase reporter gene assay

For SNPs associated with the risk of ADHD, we used dual-luciferase reporter gene assays to explore whether SNPs affect luciferase expression. rs9826579 is located in the 3′UTR region of GRM7. MicroRNAs (miRNAs) bind mostly to target sequences within the 3′UTR, thereby regulating gene expression (O'Brien et al., 2018). The sequence flanking GRM7 rs9826579 (±100 bp) was downloaded from the NCBI database and synthesized. Then, the synthesized sequence was inserted at the Xbal/Xbal site of the GV272 vector (GeneChem, Shanghai, China). Based on the SNPinfo database, we predicted that the sequence containing rs9826579 could bind to miRNA (Table S1). Using the databases that specifically predicted miRNAs the MirSNP database (http://bioinfo.bjmu.edu.cn/mirsnp/search/) and PolyRTS Database 3.0 (http://compbio.uthsc.edu/miRSNP/home.php) we speculate that rs9826579 T > C might regulate GRM7 expression by binding to hsa-miR-3180 (Table S3). The hsa-miR-3180 sequence was inserted at the *Bam*HI/-*Hin*dIII site in the GV272 vector (GeneChem, Shanghai, China). All vectors were validated by sequencing.

rs1906953 is located in the intron region of GRM4. We predicted that there would be a large difference in the binding capacity to transcription factors (TFs) between rs1906953 T and rs1906953 C according to the HaploReg v4.1 database (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) (Table S3). It was speculated that expression of GRM4 might be changed by interfering with the binding capacity of rs1906953 to TFs. When constructing an overexpression vector, a

sequence containing the GRM4 promoter (2000 bp, NM-000841) and rs1906953 (±300 bp) was inserted at Kpnl/Xhol in the GV238 vector (GeneChem, China). We used the assay to explore whether rs1906953 affected luciferase expression by interfering with promoter activity. The construction of the vectors used in the study is shown in Fig S1.

HEK 293T cells (GeneChem, China), which is a human kidney cell line, were used for the dual-luciferase reporter gene assay. The cells were cultured in Dulbecco's modified Eagle's medium with 15% foetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 and were seeded onto 24-well plates in the logarithmic growth phase. After the cells reached 60%–70% confluence, we used X-tremegene HP reagent to transfect the constructed vector (GV272 vector and GV251 vector, or GV238 vector) into the cells. After 48 h of transfection, the luciferase activities were measured. All transfection experiments were independently carried out in triplicate.

2.4. Electrophoretic mobility shift assay (EMSA)

An EMSA was performed to evaluate the binding of the TFs to DNA sequences, including rs1906953 C > T. Nuclear proteins were extracted from HEK 293T cells using a cytoplasmic and nuclear protein extraction kit (Beyotime, China). The probes for rs1906953 C and rs1906953 T were 5' -AGTCAGCCTGGGCGGGCTGAAAGGA- 3′ and 5' -AGTCAGCCTGGGTGGGCTGAAAGGA- 3', respectively. The probes were end-labelled with biotin (Invitrogen, USA). The competitive probe, which is also called the cold probe, had the same sequence as that of the labelled probes and was unlabelled. The probes used in this experiment included a biotin-labelled rs1906953 C probe, an unlabelled rs1906953 C probe, a biotin-labelled rs1906953 T probe and an unlabelled rs1906953 T probe.

EMSAs were performed according to the manufacturer's instructions provided in the Lightshift Chemiluminescent EMSA Kit (Thermo Scientific, USA). Briefly, 6 μg of nuclear protein was incubated at 20°C–25 °C for 15 min in the presence of 1.5 μl of binding buffer and 1 μl of Poly (DidC) in a reaction volume of 15 μl, and then 2 μl of bioprobe was added, mixed well, and allowed to sit at 20°C–25 °C for 30 min. For the competition experiments, an unlabelled wild-type probe or unlabelled mutant probe was added at a 10 × or a 100 × molar excess prior to the addition of the biotin-labelled probe. Samples were electrophoresed through 6% non-denaturing polyacrylamide gels in 0.5 × Tris/Borate/EDTA at 4 °C and 100 V until the bromophenol blue reached 2/3 of the gel length and were then electrotransferred and cross-linked by ultraviolet treatment. Chemiluminescence imaging showed bands of biotin-labelled DNA binding to TFs.

2.5. Statistical analysis

Categorical variables were analysed with Pearson $\chi 2$ tests. Continuous variables were analysed with *t* tests. The Hardy-Weinberg equilibrium (HWE) for genotype in control subjects was tested with a goodness-of-fit $\chi 2$ test. We used a binary logistic regression model to estimate the association between the SNPs and the risk of ADHD after adjustment for sex and age in the codominant, dominant, recessive and additive models. The association between ADHD clinical feature scores and SNPs was explored by one-way analysis of variance (ANOVA) with *post hoc* comparisons using the Dunnett *t* method. Luciferase expression between different groups was compared by Student's *t*-test. Multiple comparisons were corrected by Bonferroni adjustment to reduce type I errors. The statistical power was calculated using PASS v15.0 (NCSS, LLC, Kaysville, UT, USA) after the study was performed. All statistical analyses in this study were carried out with IBM SPSS v21.0 software (Inc., Chicago, IL, USA).

3. Results

3.1. Demographic and clinical characteristics of the participants

A total of 1253 subjects was recruited in the study, including 617 ADHD cases and 636 healthy controls. The ratio of boys to girls in cases and controls was 3.47:1 and 4.09:1, respectively. There were no differences in sex, age, BMI, and IQ distribution between the cases and the controls ($P > 0.05$). Compared with the control group, the case group had higher attention scores and hyperactivity scores ($P < 0.001$). The detailed results are summarized in Table 1.

3.2. Analysis of the association between candidate SNPs and ADHD risk

The genotype distributions of the SNPs included in the study in the case group and the control group are shown in Table S4. The call rates of the candidate SNPs were all more than 95%, and no genotypes deviated from HWE. The genotype distributions of GRM4 rs1906953 and GRM7 rs9826579 in the case group and control group were significantly different after Bonferroni adjustment ($P = 0.002$ and $P < 0.001$, respectively).

The associations between the candidate SNPs and the risk of ADHD under the different models (codominant, dominant, recessive and additive models) are shown in Table 2. The GRM8 rs712723 G genotype was nominally associated with an increased risk of ADHD under the codominant and dominant models (GG:AA, OR = 1.37, 95% *CI* = 1.07–1.76; dominant model, OR = 1.29, 95% *CI* = 1.02–1.63), but this association was not observed after Bonferroni adjustment. GRM4 rs1906953 T was associated with a decreased risk of ADHD under the codominant and recessive models (TT: CC, *OR* = 0.55, 95%*CI* = 0.40–0.77; recessive model, *OR* = 0.58, 95%*CI* = 0.43–0.78). GRM7 rs9826579 C was associated with an increased risk of ADHD under the codominant, dominant and recessive models (TC:TT, OR = 1.81, 95% *CI* = 1.39–2.36; dominant model, OR = 1.74, 95% *CI* = 1.35–2.24; additive model, OR = 1.56, 95% *CI* = 1.24–1.97).

Given that GRM4 rs1906953 and GRM7 rs9826579 were associated with the risk of ADHD in the recessive and dominant models, respectively, we further explored interaction between them. We did not observe a significant multiplicative interaction or additive interaction between rs1906953 in the recessive model and rs9826579 in the dominant model.

For rs1906953 (MAF = 0.403 in CHB), the power that detected an OR of 0.58 was 0.99. For rs9826579 (MAF = 0.141 in CHB), the power that detected an OR of 1.57 was 0.84.

3.3. Analysis of the association between the positive SNPs and the assessment of clinical symptoms

Attention scores and hyperactivity/impulsivity scores from the SNAP-IV scale were used to assess the clinical symptoms of ADHD in the

Table 1
Demographic characteristics and clinical features of the subjects.

Characteristics	Case (n = 617)	Control (n = 636)	*t* or χ^2	*p*
Age (year$\bar{\chi} \pm s$)	8.57 ± 1.61	8.71 ± 1.95	1.33	0.18
Sex				0.27
Boys	479	511	1.39	
Girls	138	125		
BMI (kg/m^2, $\bar{\chi} \pm s$)	16.46 ± 2.71	16.55 ± 3.38	0.54	0.59
IQ ($\bar{\chi} \pm s$)	94.03 ± 13.61	95.02 ± 11.67	1.37	0.17
Inattention	17.21 ± 4.93	5.86 ± 3.22	−47.64	< **0.001**
Hyperactivity/impulsive	14.53 ± 5.84	5.23 ± 2.98	−35.00	< **0.001**

Table 2
Associations of the candidate SNPs with the risk of ADHD.

SNP	Minor/major 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)*
rs2229901	C/T	0.87	1.02 (0.80, 1.30)	0.64	0.92 (0.64, 1.32)	0.98	1.00 (0.79, 1.26)	0.56	0.90 (0.64, 1.27)	0.76	0.97 (0.82, 1.15)
rs1906953	T/C	0.57	0.93 (0.72, 1.20)	**< 0.001**	0.55 (0.40, 0.77)	0.09	0.81 (0.64, 1.03)	**< 0.001**	0.58 (0.43, 0.78)	0.002	0.77 (0.66, 0.91)
rs2499730	T/G	0.07	0.80 (0.63, 1.02)	0.26	0.82 (0.57, 1.16)	0.06	0.80 (0.64, 1.01)	0.67	0.93 (0.68, 1.29)	0.12	0.88 (0.74, 1.04)
rs3749380	T/C	0.88	1.02 (0.80, 1.29)	0.21	1.28 (0.87, 1.89)	0.60	1.06 (0.85, 1.33)	0.21	1.27 (0.87, 1.84)	0.33	1.09 (0.92, 1.29)
rs9826579	C/T	**< 0.001**	1.81 (1.39, 2.36)	0.98	1.01 (0.43, 2.37)	**< 0.001**	1.74 (1.35, 2.24)	0.72	0.86 (0.37, 2.00)	**< 0.001**	1.56 (1.24, 1.97)
rs9870680	C/T	0.99	1.00 (0.79, 1.26)	0.20	0.76 (0.50, 1.16)	0.70	0.96 (0.77, 1.20)	0.20	0.76 (0.51, 1.15)	0.39	0.93 (0.78, 1.10)
rs1508724	A/G	0.29	1.15 (0.89, 1.48)	0.11	0.57 (0.29, 1.14)	0.58	1.07 (0.84, 1.37)	0.08	0.55 (0.28, 1.08)	0.93	0.99 (0.80, 1.22)
rs10268335	T/G	0.45	0.90 (0.69, 1.18)	0.28	0.69 (0.35, 1.36)	0.30	0.87 (0.68, 1.13)	0.30	0.70 (0.36, 1.38)	0.22	0.87 (0.70, 1.09)
rs712723	G/A	0.01	1.37 (1.07, 1.76)	0.61	1.09 (0.78, 1.53)	0.03	1.29 (1.02, 1.63)	0.52	0.91 (0.67, 1.23)	0.26	1.10 (0.94, 1.29)

HW, wild-type homozygote; HT, heterozygote; HV, variant homozygote. 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. All the OR (95% CI) and P were adjusted for age and gender. The significance level was corrected with the formula of α' = 0.05/9/4 = 0.0014 according to the Bonferroni method.

subjects. The relationships between the significant SNPs (rs1906953 and rs9826579) and the clinical symptoms are shown in Table 3 rs1906953 was associated with inattention and hyperactivity. Compared with subjects with the TT genotype, subjects with the CC genotype showed more obvious attention deficit behaviours and hyperactivity/impulsive behaviours. An association between rs9826579 and clinical symptoms was not found.

3.4. Dual-luciferase reporter gene assay

We explored whether rs9826579 affects reporter gene expression by binding to hsa-miR-3180, and the results are shown in Fig. 1. Although there was a significant difference in the relative luciferase activity between the "rs9826579-NC + miRNA" group and the "rs982679-T + miRNA" group, the difference was small (the difference < 0.2), and these results alone did not support that the difference between the two groups has actual biological significance. Moreover, we used the dual-luciferase reporter gene assay to test whether rs1906953 affects expression of the reporter gene by interfering with the promoter sequence. The results are shown in Fig. 2. Expression of luciferase in the rs1906953-T group was significantly reduced compared with that in the rs1906953-C group ($P < 0.001$).

Table 3
Association of the significant SNPs with the clinical characteristics.

SNP	Genotype	Inattention			Hyperactivity/impulsivity		
		$\bar{x} \pm s$	*F*	*P*	$\bar{x} \pm s$	*F*	*P*
rs1906953			6.24	**0.002**		5.55	**0.004**
	CC	11.64 ± 7.22		Ref	10.38 ± 6.39		Ref
	TC	11.78 ± 7.09		0.94	9.84 ± 6.77		0.34
	TT	9.91 ± 6.32		**0.006**	8.58 ± 6.07		**0.002**
rs9826579			3.05	0.124		1.87	0.582
	TT	11.20 ± 7.01		Ref	9.70 ± 6.51		Ref
	CT	12.14 ± 7.03		0.08	10.12 ± 6.61		0.54
	CC	11.52 ± 8.61		0.97	10.29 ± 7.82		0.90

The significance level was corrected with the formula of α' = 0.05/2/2 = 0.0125 according to the Bonferroni method.

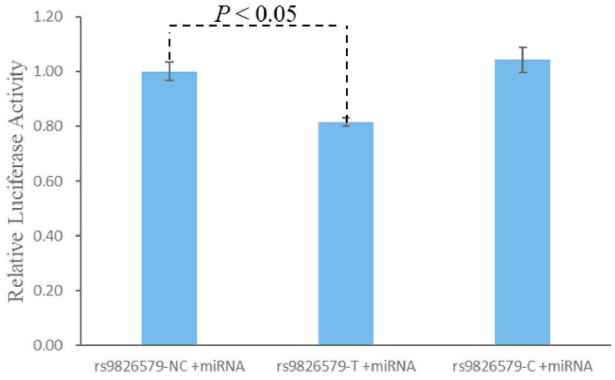

Fig. 1. Hsa-miR-3180 does not suppress reporter expression. "NC" represents the negative control. Although the relative luciferase activity in the ""rs9826579-T + miRNA" group is lower than that in the "rs9826579-NC + miRNA" group, the difference between the two groups is not significant (the difference < 0.2). Therefore, this result demonstrated that rs9826579 T might not bind to hsa-miR-3180 to affect expression of luciferase.

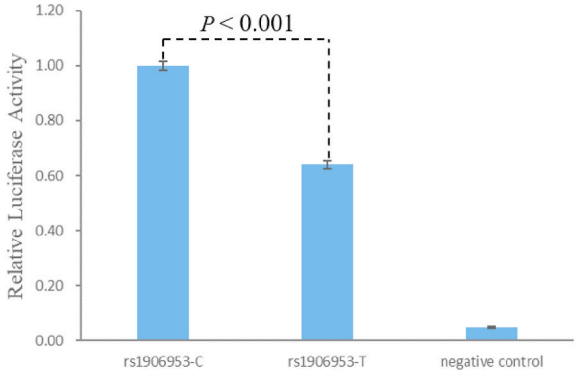

Fig. 2. rs1906953 might suppress the reporter gene expression by reducing promoter activity.

3.5. EMSA

We used an EMSA to explore whether rs1906953 C > T interferes with the binding of sequences containing rs1906953 to TFs. The results showed that the rs1906953 C probe binds strongly to nucleoprotein but that the rs1906953 T probe binds weakly, indicating that the mutant allele interferes with the binding of TFs to DNA sequences, including rs1906953 C > T (Fig. 3).

4. Discussion

We investigated the relationship of functional SNPs of GRM4, GRM7, and GRM8 with the risk of ADHD in the Chinese Han population for the first time and initially explored the function of the significant SNPs in the study. We found that GRM4 rs1906953 T was associated with a reduced risk of ADHD but that GRM7 rs9826579 C was associated with an increased risk of ADHD. The dual-luciferase reporter gene assay showed that rs1906953 T reduced luciferase expression. The electrophoretic mobility shift assay showed that rs1906953 T interfered with the binding of the TFs to DNA sequences, including rs1906953 C > T. We speculate that rs1906953 C > T might affect expression of the GRM4 gene by interfering with the binding of DNA sequences to TFs. However, we did not have sufficient evidence to show that hsa-miR-3180 could affect expression of the GRM7 gene.

Although it is not yet possible to conclude that hsa-miR-3180 affected GRM7 gene expression by binding to DNA fragments containing the rs9826579 allele, the Braineac database (http://www.braineac.org/) shows that rs9826579 can affect expression of the GRM7 gene in the brain. We speculate that there may be other potential factors that affect gene expression through interaction with rs9826579. GRM7 is the most widely expressed group III mGluR in the CNS, particularly in the frontal cortex, hippocampus and hypothalamus. Studies have found that the frontal cortex and hippocampus are involved in the pathophysiology of ADHD (Medin et al., 2019; Miller et al., 2019; Salavert et al., 2018). Presynaptic mGluR7 modulates the release of neurotransmitters such as GABA, glutamate, and, possibly, monoamines (Schoepp, 2001), which are associated with the risk of ADHD (Ende et al., 2016; Naaijen et al., 2017). Postsynaptic mGluR7 could regulate neuronal activity, for example, via modulation of N-methyl-D-aspartic acid (NMDA) receptor activity (Gu et al., 2012), while NMDA receptors have been implicated in ADHD (Kim et al., 2016).

To date, some studies have found that GRM7 gene variations are associated with the risk of ADHD in other populations. A genome-wide copy number variation study was performed in children of European ancestry and indicated that CNVs in GRM7 were associated with ADHD (Elia et al., 2011). Several studies in different populations found that GRM7 rs3792452 played a role in the treatment response to methylphenidate (Mick et al., 2008; S. Park et al., 2014) and showed biased transmission of the G allele (S. Park et al., 2013) in ADHD patients. In addition, a recent study reported that GRM7 rs6782011 is associated with the risk of ADHD in patients recruited from an Iranian hospital (Noroozi et al., 2019). rs3792452, rs6782011, and rs9826579 are located on intron 8/10, intron 4/10, and exon 10/10 of GRM7, respectively, and do not show LD. Furthermore, we found that the smallest *P* value of GRM7 SNPs associated with ADHD was 2.8×10^{-4} by searching the Psychiatric Genomics Consortium (PGC) database (https://www.med.unc.edu/pgc/results-and-downloads/adhd/) (Demontis et al., 2019), which did not reach GWAS significance ($P \leq 5 \times 10^{-8}$), like many other candidate genes associated with ADHD (Faraone and Larsson, 2019).

GRM7 knockdown increases neural progenitor cell (NPC) proliferation, decreases neuronal differentiation, and leads to abnormal neuronal morphology, which indicates that GRM7 signalling is necessary for neurogenesis in the brain (Xia et al., 2015). mGluR7-deficient mice also exhibit prominent anxiolytic behaviour and impaired working memory (Callaerts-Vegh et al., 2006; Cryan et al., 2003). Approximately 25% of patients in each population have comorbid ADHD and anxiety (Reimherr et al., 2017), and working memory dysfunctions have been identified as potential core factors in the development of ADHD, especially in regulating attention (Kofler et al., 2018).

The rs1906953 of GRM4, which is another mGluR gene in the glutamatergic pathway, is located in the activated enhancer region or transcriptional region in brain cells, embryonic stem cells, and induced pluripotent stem cells (the HaploReg v4.1 database). In addition, rs1906953 C was associated with increased GRM4 gene expression in brain tissue observed in the Braineac database, which was consistent with the results of this study. We speculate that rs1906953 C might increase the risk of ADHD by affecting expression of the GRM4 gene. GRM4 plays an important role in the central nervous system and is highly expressed in the cerebellum, and it is well known that the cerebellum is involved in the pathogenesis of ADHD (Bruchhage et al., 2018; Stoodley, 2016). Recent work suggests that activation of mGluR4 may inhibit neuroinflammatory processes. Interestingly, some studies have proposed that ADHD may be a highly inflammatory and immune-associated disease (Zhou et al., 2017) and indicated neuroinflammation as a risk factor for ADHD (Dunn et al., 2019). It is worth noting that mGluR4 and mGluR7 can negatively regulate the cAMP/PKA pathway, while cAMP signalling contributes to nicotine- and ethanol-induced hyperactivity and memory/learning deficits (Abreu-Villaca et al., 2018). Furthermore, the neuronal membrane trafficking

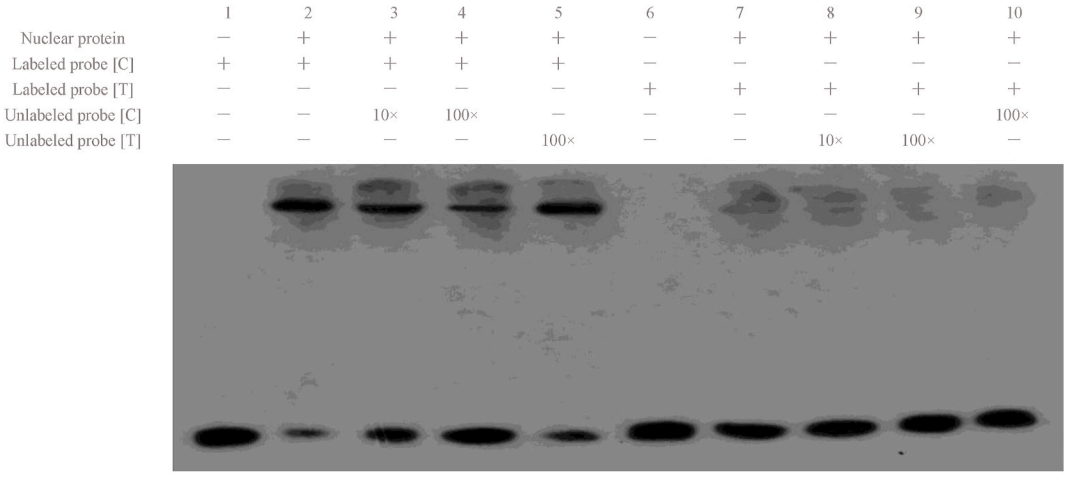

Fig. 3. The result of the electrophoretic mobility shift assay. Lanes 1–5 and 6–10 were used to explore the binding capacities of rs1906953 C and rs1906953 T to nuclear proteins, respectively. We set 10 × and 100 × concentration gradients for the competition experiments.

regulated by several protein kinases including cAMP-dependent PKA, is involved in ADHD. Diets may contribute to the improved membrane trafficking in ADHD via the modulation of PKA signalling (Kitagishi et al., 2015).

Despite little evidence regarding the association between GRM4 and ADHD, many studies have found that GRM4 variations are associated with neurodevelopmental disorders and suggested that mGluR4 activators play a role in the treatment of CNS disorders (Célanire and Campo, 2012). Expression of GRM4 is increased in the brains of patients with depression (Dadkhah et al., 2017). Similarly, this study speculated that the increase in GMR4 gene expression may be associated with the risk of ADHD. GRM4 gene variations are associated with major depressive disorder (MDD), BD and schizophrenia (Kato, 2007; J. Li, Meng, Cao and Qiu, 2015; Shibata et al., 2009). Patients with ADHD are often also diagnosed with depression or BD (Bron et al., 2016; Pinna et al., 2019). Schizophrenia and ADHD likely have shared mechanisms; for example, similar deficits in social cognition, cognitive function and language abilities are observed (M. T. M. Park et al., 2018). Moreover, a gene-set analysis identified 19 gene sets that were significantly associated with ADHD, schizophrenia, MDD, bipolar disorder and ASD (Hammerschlag et al., 2019). In addition, we found that the smallest *P* value of GRM4 SNPs associated with ADHD was 2.9×10^{-4} by searching the PGC database (Neale et al., 2010).

Furthermore, animal studies have suggested that the absence of mGluR4 affects spatial learning and memory (Gerlai et al., 1998), and mGluR4 receptor activation could lead to the deterioration of hippocampus-dependent tasks (Pershina and Arkhipov, 2016). Furthermore, mGluR4$^{-/-}$ male mice showed increased anxiety and impaired sensorimotor function, and mGluR4$^{-/-}$ female mice showed reduced anxiety and enhanced rotarod performance, which indicated the effects of mGluR4 on sensorimotor function and anxiety (Davis et al., 2012).

This study has the following limitations. First, the association of functional SNPs in GRM7 and GRM4 with ADHD needs to be verified in a larger population with different genetic ancestries in further research. Second, our functional exploration of positive SNPs was limited to *in vitro* experiments and should be verified with *in vivo* experiments or in brain tissue in future research.

5. Conclusion

This case-control study found associations between rs9826579 and rs1906953 and the risk of ADHD in a Chinese Han population. Furthermore, we speculate that GRM4 rs1906953 C > T might weaken the binding to TFs to affect expression of GRM4, as evidenced by EMSA and dual-luciferase reporter gene assay results.

CRediT authorship contribution statement

Qi Zhang: Project administration, Investigation, Data curation, Methodology, Formal analysis, Writing - original draft. **Xinzhen Chen:** Investigation, Validation, Writing - review & editing. **Shanyawen Li:** Investigation, Data curation. **Ting Yao:** Investigation. **Jing Wu:** Project administration, Conceptualization, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2020.09.035.

References

Abreu-Villaca, Y., Carvalho-Graca, A.C., Skinner, G., Lotufo, B.M., Duarte-Pinheiro, V.H. S., Ribeiro-Carvalho, A., Filgueiras, C.C., 2018. Hyperactivity and memory/learning deficits evoked by developmental exposure to nicotine and/or ethanol are mitigated by cAMP and cGMP signaling cascades activation. Neurotoxicology 66, 150–159. https://doi.org/10.1016/j.neuro.2018.04.003.

Akutagava-Martins, G.C., Salatino-Oliveira, A., Genro, J.P., Contini, V., Polanczyk, G., Zeni, C., Hutz, M.H., 2014. Glutamatergic copy number variants and their role in attention-deficit/hyperactivity disorder. Am. J. Med. Genet. Neuropsychiatr. Genet. 165b (6), 502–509. https://doi.org/10.1002/ajmg.b.32253.

Bonvicini, C., Faraone, S.V., Scassellati, C., 2016. Attention-deficit hyperactivity disorder in adults: a systematic review and meta-analysis of genetic, pharmacogenetic and biochemical studies. Mol. Psychiatr. 21 (7), 872–884. https://doi.org/10.1038/mp.2016.74.

Bron, T.I., Bijlenga, D., Verduijn, J., Penninx, B.W., Beekman, A.T., Kooij, J.J., 2016. Prevalence of ADHD symptoms across clinical stages of major depressive disorder. J. Affect. Disord. 197, 29–35. https://doi.org/10.1016/j.jad.2016.02.053.

Bruchhage, M.M.K., Bucci, M.P., Becker, E.B.E., 2018. Cerebellar involvement in autism and ADHD. Handb. Clin. Neurol. 155, 61–72. https://doi.org/10.1016/b978-0-444-64189-2.00004-4.

Callaerts-Vegh, Z., Beckers, T., Ball, S.M., Baeyens, F., Callaerts, P.F., Cryan, J.F., D'Hooge, R., 2006. Concomitant deficits in working memory and fear extinction are functionally dissociated from reduced anxiety in metabotropic glutamate receptor 7-deficient mice. J. Neurosci. 26 (24), 6573–6582. https://doi.org/10.1523/jneurosci.1497-06.2006.

Cartmell, J., Schoepp, D.D., 2000. Regulation of neurotransmitter release by metabotropic glutamate receptors. J. Neurochem. 75 (3), 889–907. https://doi.org/10.1046/j.1471-4159.2000.0750889.x.

Célanire, S., Campo, B., 2012. Recent advances in the drug discovery of metabotropic glutamate receptor 4 (mGluR4) activators for the treatment of CNS and non-CNS disorders. Expet Opin. Drug Discov. 7 (3), 261–280. https://doi.org/10.1517/17460441.2012.660914.

Cryan, J.F., Kelly, P.H., Neijt, H.C., Sansig, G., Flor, P.J., van Der Putten, H., 2003. Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. Eur. J. Neurosci. 17 (11), 2409–2417. https://doi.org/10.1046/j.1460-9568.2003.02667.x.

Dadkhah, T., Rahimi-Aliabadi, S., Jamshidi, J., Ghaedi, H., Taghavi, S., Shokraeian, P., Darvish, H., 2017. A genetic variant in miRNA binding site of glutamate receptor 4, metabotropic (GRM4) is associated with increased risk of major depressive disorder. J. Affect. Disord. 208, 218–222. https://doi.org/10.1016/j.jad.2016.10.008.

Davis, M.J., Haley, T., Duvoisin, R.M., Raber, J., 2012. Measures of anxiety, sensorimotor function, and memory in male and female mGluR4$^{-/-}$ mice. Behav. Brain Res. 229 (1), 21–28. https://doi.org/10.1016/j.bbr.2011.12.037.

Davis, M.J., Iancu, O.D., Acher, F.C., Stewart, B.M., Eiwaz, M.A., Duvoisin, R.M., Raber, J., 2013. Role of mGluR4 in acquisition of fear learning and memory. Neuropharmacology 66, 365–372. https://doi.org/10.1016/j.neuropharm.2012.07.038.

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. Nat. Genet. 51 (1), 63–75. https://doi.org/10.1038/s41588-018-0269-7.

Dunn, G.A., Nigg, J.T., Sullivan, E.L., 2019. Neuroinflammation as a risk factor for attention deficit hyperactivity disorder. Pharmacol. Biochem. Behav. 182, 22–34. https://doi.org/10.1016/j.pbb.2019.05.005.

Elia, J., Glessner, J.T., Wang, K., Takahashi, N., Shtir, C.J., Hadley, D., Hakonarson, H., 2011. Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder. Nat. Genet. 44 (1), 78–84. https://doi.org/10.1038/ng.1013.

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.

Faraone, S.V., Larsson, H., 2019. Genetics of attention deficit hyperactivity disorder. Mol. Psychiatr. 24 (4), 562–575. https://doi.org/10.1038/s41380-018-0070-0.

Gerlai, R., Adams, B., Fitch, T., Chaney, S., Baez, M., 2002. Performance deficits of mGluR8 knockout mice in learning tasks: the effects of null mutation and the background genotype. Neuropharmacology 43 (2), 235–249. https://doi.org/10.1016/s0028-3908(02)00078-3.

Gerlai, R., Roder, J.C., Hampson, D.R., 1998. Altered spatial learning and memory in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. Behav. Neurosci. 112 (3), 525–532. https://doi.org/10.1037//0735-7044.112.3.525.

Gu, Z., Liu, W., Wei, J., Yan, Z., 2012. Regulation of N-methyl-D-aspartic acid (NMDA) receptors by metabotropic glutamate receptor 7. J. Biol. Chem. 287 (13), 10265–10275. https://doi.org/10.1074/jbc.M111.325175.

Hammerschlag, A.R., de Leeuw, C.A., Middeldorp, C.M., Polderman, T.J.C., 2019. Synaptic and brain-expressed gene sets relate to the shared genetic risk across five

psychiatric disorders. Psychol. Med. 1–11. https://doi.org/10.1017/s0033291719001776.
Hinshaw, S.P., 2018. Attention deficit hyperactivity disorder (ADHD): controversy, developmental mechanisms, and multiple levels of analysis. Annu. Rev. Clin. Psychol. 14, 291–316. https://doi.org/10.1146/annurev-clinpsy-050817-084917.
Holscher, C., Schmid, S., Pilz, P.K., Sansig, G., van der Putten, H., Plappert, C.F., 2005. Lack of the metabotropic glutamate receptor subtype 7 selectively modulates Theta rhythm and working memory. Learn. Mem. 12 (5), 450–455. https://doi.org/10.1101/lm.98305.
Kato, T., 2007. Molecular genetics of bipolar disorder and depression. Psychiatr. Clin. Neurosci. 61 (1), 3–19. https://doi.org/10.1111/j.1440-1819.2007.01604.x.
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. J. Atten. Disord. https://doi.org/10.1177/1087054716649665.
Kitagishi, Y., Minami, A., Nakanishi, A., Ogura, Y., Matsuda, S., 2015. Neuron membrane trafficking and protein kinases involved in autism and ADHD. Int. J. Mol. Sci. 16 (2), 3095–3115. https://doi.org/10.3390/ijms16023095.
Kofler, M.J., Sarver, D.E., Harmon, S.L., Moltisanti, A., Aduen, P.A., Soto, E.F., Ferretti, N., 2018. Working memory and organizational skills problems in ADHD. JCPP (J. Child Psychol. Psychiatry) 59 (1), 57–67. https://doi.org/10.1111/jcpp.12773.
Li, H., Li, Y., Shao, J., Li, R., Qin, Y., Xie, C., Zhao, Z., 2008. The association analysis of RELN and GRM8 genes with autistic spectrum disorder in Chinese Han population. Am. J. Med. Genet. Neuropsychiatr. Genet. 147b (2), 194–200. https://doi.org/10.1002/ajmg.b.30584.
Li, J., Meng, H., Cao, W., Qiu, T., 2015. MiR-335 is involved in major depression disorder and antidepressant treatment through targeting GRM4. Neurosci. Lett. 606, 167–172. https://doi.org/10.1016/j.neulet.2015.08.038.
Medin, T., Jensen, V., Skare, Ø., Storm-Mathisen, J., Hvalby, Ø., Bergersen, L.H., 2019. Altered α-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). Behav. Brain Res. 360, 209–215. https://doi.org/10.1016/j.bbr.2018.12.028.
Mercier, M.S., Lodge, D., 2014. Group III metabotropic glutamate receptors: pharmacology, physiology and therapeutic potential. Neurochem. Res. 39 (10), 1876–1894. https://doi.org/10.1007/s11064-014-1415-y.
Mick, E., Neale, B., Middleton, F.A., McGough, J.J., Faraone, S.V., 2008. Genome-wide association study of response to methylphenidate in 187 children with attention-deficit/hyperactivity disorder. Am. J. Med. Genet. Neuropsychiatr. Genet. 147b (8), 1412–1418. https://doi.org/10.1002/ajmg.b.30865.
Miller, E.M., Quintero, J.E., Pomerleau, F., Huettl, P., Gerhardt, G.A., Glaser, P.E.A., 2019. Chronic methylphenidate alters tonic and phasic glutamate signaling in the frontal cortex of a freely-moving rat model of ADHD. Neurochem. Res. 44 (1), 89–101. https://doi.org/10.1007/s11064-018-2483-1.
Mukherjee, S., Manahan-Vaughan, D., 2013. Role of metabotropic glutamate receptors in persistent forms of hippocampal plasticity and learning. Neuropharmacology 66, 65–81. https://doi.org/10.1016/j.neuropharm.2012.06.005.
Naaijen, J., Forde, N.J., Lythgoe, D.J., Akkermans, S.E., Openneer, T.J., Dietrich, A., Buitelaar, J.K., 2017. Fronto-striatal glutamate in children with Tourette's disorder and attention-deficit/hyperactivity disorder. Neuroimage Clin. 13, 16–23. https://doi.org/10.1016/j.nicl.2016.11.013.
Neale, B.M., Medland, S.E., Ripke, S., Asherson, P., Franke, B., Lesch, K.P., Nelson, S., 2010. Meta-analysis of genome-wide association studies of attention-deficit/hyperactivity disorder. J. Am. Acad. Child Adolesc. Psychiatry 49 (9), 884–897. https://doi.org/10.1016/j.jaac.2010.06.008.
Niswender, C.M., Conn, P.J., 2010. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annu. Rev. Pharmacol. Toxicol. 50, 295–322. https://doi.org/10.1146/annurev.pharmtox.011008.145533.
Noroozi, R., Taheri, M., Movafagh, A., Mirfakhraie, R., Solgi, G., Sayad, A., Darvish, H., 2016. Glutamate receptor, metabotropic 7 (GRM7) gene variations and susceptibility to autism: a case-control study. Autism Res. 9 (11), 1161–1168. https://doi.org/10.1002/aur.1640.
Noroozi, R., Taheri, M., Omrani, M.D., Ghafouri-Fard, S., 2019. Glutamate receptor metabotropic 7 (GRM7) gene polymorphisms in mood disorders and attention deficit hyperactive disorder. Neurochem. Int. 129, 104483. https://doi.org/10.1016/j.neuint.2019.104483.
O'Brien, J., Hayder, H., Zayed, Y., Peng, C., 2018. Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. Front. Endocrinol. 9, 402. https://doi.org/10.3389/fendo.2018.00402.
O'Neill, S., Rajendran, K., Mahbubani, S.M., Halperin, J.M., 2017. Preschool predictors of ADHD symptoms and impairment during childhood and adolescence. Curr. Psychiatr. Rep. 19 (12), 95. https://doi.org/10.1007/s11920-017-0853-z.
Park, M.T.M., Raznahan, A., Shaw, P., Gogtay, N., Lerch, J.P., Chakravarty, M.M., 2018. Neuroanatomical phenotypes in mental illness: identifying convergent and divergent cortical phenotypes across autism, ADHD and schizophrenia. J. Psychiatry Neurosci. 43 (3), 201–212.
Park, S., Jung, S.W., Kim, B.N., Cho, S.C., Shin, M.S., Kim, J.W., Kim, H.W., 2013. Association between the GRM7 rs3792452 polymorphism and attention deficit hyperacitiveity disorder in a Korean sample. Behav. Brain Funct. 9, 1. https://doi.org/10.1186/1744-9081-9-1.
Park, S., Kim, B.N., Cho, S.C., Kim, J.W., Kim, J.I., Shin, M.S., Cheong, J.H., 2014. The metabotropic glutamate receptor subtype 7 rs3792452 polymorphism is associated with the response to methylphenidate in children with attention-deficit/hyperactivity disorder. J. Child Adolesc. Psychopharmacol. 24 (4), 223–227. https://doi.org/10.1089/cap.2013.0079.
Pershina, E.V., Arkhipov, V.I., 2016. Subacute activation of mGlu 4 receptors causes the feedback inhibition of its gene expression in rat brain. Life Sci. 153, 50–54. https://doi.org/10.1016/j.lfs.2016.03.033.
Pinna, M., Visioli, C., Rago, C.M., Manchia, M., Tondo, L., Baldessarini, R.J., 2019. Attention deficit-hyperactivity disorder in adult bipolar disorder patients. J. Affect. Disord. 243, 391–396. https://doi.org/10.1016/j.jad.2018.09.038.
Pittenger, C., Bloch, M.H., Williams, K., 2011. Glutamate abnormalities in obsessive compulsive disorder: neurobiology, pathophysiology, and treatment. Pharmacol. Ther. 132 (3), 314–332. https://doi.org/10.1016/j.pharmthera.2011.09.006.
Prakash, J., Williams, F.M., Trofimov, S., Surdulescu, G., Spector, T., Livshits, G., 2016. Quantitative genetics of circulating Dickkopf-related protein 1 (DKK1) in community-based sample of UK twins. Osteoporos. Int. 27 (6), 2065–2075. https://doi.org/10.1007/s00198-016-3486-x.
Reimherr, F.W., Marchant, B.K., Gift, T.E., Steans, T.A., 2017. ADHD and anxiety: clinical significance and treatment implications. Curr. Psychiatr. Rep. 19 (12), 109. https://doi.org/10.1007/s11920-017-0859-6.
Salavert, J., Ramos-Quiroga, J.A., Moreno-Alcázar, A., Caseras, X., Palomar, G., Radua, J., Pomarol-Clotet, E., 2018. Functional imaging changes in the medial prefrontal cortex in adult ADHD. J. Atten. Disord. 22 (7), 679–693. https://doi.org/10.1177/1087054715611492.
Schoepp, D.D., 2001. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J. Pharmacol. Exp. Therapeut. 299 (1), 12–20.
Sergouniotis, P.I., Robson, A.G., Li, Z., Devery, S., Holder, G.E., Moore, A.T., Webster, A. R., 2012. A phenotypic study of congenital stationary night blindness (CSNB) associated with mutations in the GRM6 gene. Acta Ophthalmol. 90 (3), e192–197. https://doi.org/10.1111/j.1755-3768.2011.02267.x.
Shibata, H., Tani, A., Chikuhara, T., Kikuta, R., Sakai, M., Ninomiya, H., Fukumaki, Y., 2009. Association study of polymorphisms in the group III metabotropic glutamate receptor genes, GRM4 and GRM7, with schizophrenia. Psychiatr. Res. 167 (1–2), 88–96. https://doi.org/10.1016/j.psychres.2007.12.002.
Stachowicz, K., Klodzinska, A., Palucha-Poniewiera, A., Schann, S., Neuville, P., Pilc, A., 2009. The group III mGlu receptor agonist ACPT-I exerts anxiolytic-like but not antidepressant-like effects, mediated by the serotonergic and GABA-ergic systems. Neuropharmacology 57 (3), 227–234. https://doi.org/10.1016/j.neuropharm.2009.06.005.
Stoodley, C.J., 2016. The cerebellum and neurodevelopmental disorders. Cerebellum 15 (1), 34–37. https://doi.org/10.1007/s12311-015-0715-3.
Thomas, R., Sanders, S., Doust, J., Beller, E., Glasziou, P., 2015. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Pediatrics 135 (4), e994–1001. https://doi.org/10.1542/peds.2014-3482.
Wang, H., Su, S., Yang, M., Hu, N., Yao, Y., Zhu, R., Guan, H., 2016. Association of ZNF644, GRM6, and CTNND2 genes with high myopia in the han Chinese population: jiangsu eye study. Eye 30 (7), 1017–1022. https://doi.org/10.1038/eye.2016.8.
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 Psychiatr. 17 (1), 32. https://doi.org/10.1186/s12888-016-1187-9.
Ward, L.D., Kellis, M., 2016. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. Nucleic Acids Res. 44 (D1), D877–D881. https://doi.org/10.1093/nar/gkv1340.
Wolraich, M.L., Hagan Jr., J.F., Allan, C., Chan, E., Davison, D., Earls, M., Zurhellen, W., 2019. Clinical practice guideline for the diagnosis, evaluation, and treatment of attention-deficit/hyperactivity disorder in children and adolescents. Pediatrics 144 (4). https://doi.org/10.1542/peds.2019-2528.
Xia, W., Liu, Y., Jiao, J., 2015. GRM7 regulates embryonic neurogenesis via CREB and YAP. Stem Cell Rep. 4 (5), 795–810. https://doi.org/10.1016/j.stemcr.2015.03.004.
Xu, Z., Taylor, J.A., 2009. SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res. 37 (Web Server issue), W600–W605. https://doi.org/10.1093/nar/gkp290.
Yuan, F.F., Gu, X., Huang, X., Zhong, Y., Wu, J., 2017. SLC6A1 gene involvement in susceptibility to attention-deficit/hyperactivity disorder: a case-control study and gene-environment interaction. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 77, 202–208. https://doi.org/10.1016/j.pnpbp.2017.04.015.
Zhang, Q., Huang, X., Chen, X.Z., Li, S.Y., Yao, T., Wu, J., 2020. Association of gene variations in ionotropic glutamate receptor and attention-deficit/hyperactivity disorder in the Chinese population: a two-stage case-control study. J. Atten. Disord. 1087054720905089 https://doi.org/10.1177/1087054720905089.
Zhou, R.Y., Wang, J.J., Sun, J.C., You, Y., Ying, J.N., Han, X.M., 2017. Attention deficit hyperactivity disorder may be a highly inflammation and immune-associated disease (Review). Mol. Med. Rep. 16 (4), 5071–5077. https://doi.org/10.3892/mmr.2017.7228.