Neuroscience Letters 760 (2021) 136086

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Polygenic risk of genes involved in the catecholamine and serotonin pathways for ADHD in children

Yanni Wang [a,*,1], Tingwei Wang [a,1], Yukai Du [b], Dan Hu [b], Yu Zhang [c], Honghui Li [c], Wenyan Pei [d]

[a] Department of Maternal, Child and Adolescent Health, School of Public Health, Lanzhou University, Lanzhou, Gansu, PR China
[b] Department of Maternal and Child Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China
[c] Department of Psychological and Behavioral Development, Liuzhou Women and Children Healthcare Hospital, Liuzhou, Guangxi, PR China
[d] Lanzhou Military Region Out-patient Department, Lanzhou, Gansu, PR China

ARTICLE INFO

Keywords:
Attention deficit/ hyperactivity disorder
Gene-gene interaction
Polygenic risk
Child
Dopamine
Norepinephrine
Serotonin

ABSTRACT

It is general acknowledged that genes play a vital role in the etiology of attention deficit/hyperactivity disorder (ADHD). The relationship between the genes involved in catecholamine (dopamine, noradrenaline)/serotonin transmissions and ADHD has been widely described in medical literature. A pathway-based study was conducted in this study to test the association of gene-gene interaction and the cumulative effect of genetic polymorphisms within the dopamine, norepinephrine, and serotonin neurotransmitter pathways with ADHD susceptibility. A case-control study was conducted among Chinese children, and 168 ADHD patients and 233 controls were recruited using a combination diagnosis according to the DSM-IV ADHD rating scale. Classification and regression tree (CART) analysis was conducted to explore the gene-gene interaction, and logistic regression modal was applied to estimate the polygenic risk of the potential multiple genetic variants. The results of CART analyses indicated that the children carrying the combination of *ADRA2A* rs553668GG/GA and *SLC6A4* rs6354 GG/GT genotypes displayed a 6.15-fold increased risk of ADHD, compared to those with the combination of *ADRA2A* rs553668 AA and *ANKK1* rs1800497 AA genotypes. The unfavorable alleles of *ADRA2A* rs553668 G, *DRD2* rs1124491 G and *SLC6A4* rs6354 G showed cumulative effects on ADHD, and the OR for ADHD may increase by 1.42 times when the number of unfavorable allele number increased by one. Those findings reveal the importance of the gene-gene interactions and polygenic effects of many common variants to ADHD susceptibility, even the effect of each variant is very small.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is typically characterized by developmentally age-inappropriate levels of inattentiveness and/or hyperactivity/impulsivity. It is one of the most common neurodevelopmental psychiatric disorders, with a prevalence of 7.2% worldwide [1]. Children with ADHD may have trouble focusing on a task, paying attention to homework, completing tasks, being organized, and remembering things [2]. Children with ADHD are at risk for a wide range of social functional impairments, such as school failure, peer rejection, injuries due to accidents, suicide personality disorder, substance abuse, and criminality [3]. Although the disorder is commonly diagnosed during childhood, two-thirds of ADHD children will continue to have impairing symptoms of ADHD in adulthood [4].

Although the exact etiology of ADHD is still unknown, plenty of twin, adoption, and family studies indicated that ADHD is a highly heritable disease with a heritability of 70–80% [5,6], and the genetic risk factors affect ADHD over the course of the development from childhood to early adulthood [7].

A series of drug studies on neurotransmitters found that drugs acting on the central neurotransmitter system could significantly improve the symptoms of ADHD [8]. Subsequent neurotransmitter, neurobiochemistry and psychopharmacological studies suggested that dysregulation in neurochemical signal transduction, particularly in monoamine neurotransmitters in the brain, might be the putative mechanism behind the symptoms of ADHD [9]. Thus, the dysfunction of

* Corresponding author at: School of Public Health, Lanzhou University, 222th South Tianshui Rd, Lanzhou 730000, Gansu, PR China.
E-mail address: wangyann@lzu.edu.cn (Y. Wang).
[1] Contributed equally.

https://doi.org/10.1016/j.neulet.2021.136086
Received 10 October 2020; Received in revised form 17 June 2021; Accepted 21 June 2021
Available online 24 June 2021

monoamine neurotransmitters, such as norepinephrine, serotonin and dopamine, is thought to be important to the etiology of ADHD. The dysfunction of these monoamines may be due to the disturbance of their synthesis or storage or the change of receptor function, and the genes involved in catecholamine (dopamine, noradrenaline) and serotonin transmissions have become the most common candidate genes for ADHD studies [10]. A genealogical association study including 674 children with ADHD in Europe and Israel showed that SNPs involved in norepinephrine, serotonin and dopamine neurotransmitter pathways are associated with susceptibility to ADHD [5,11]. Some meta-analysis studies also noted the significant associations between ADHD and candidate DNA variants in these pathways, such as the serotonin transporter gene(*5HTT*), the dopamine transporter gene(*DAT1*), the D4 dopamine receptor gene(*DRD4*), the D5 dopamine receptor gene(*DRD5*), the serotonin 1B receptor gene(*HTR1B*), the serotonin 2C receptor gene (*HTR2C*) and synaptosome associated protein 25(*SNAP25*) [12,13].

There is increasing evidence that multiple genetic variants contribute to the risk of psychiatric conditions [14]. Therefore, polygenic analyses have been used in the genetic research of psychiatry in recent years [15], such as in schizophrenia and Parkinson's disease [15,16]. However, polygenic risk research for ADHD is still relatively limited. A previous GWAS study found that the heritability of ADHD mostly depended on the effects of polygenes of many common variants, although each effect on ADHD was very small [17]. Ximena Carrasco et al. [18] indicated that the gene-gene interaction of *DRD4* 7- repeat allele and *DAT1* 10-repeat allele had an essential role in the onset of ADHD.

Polygenic research based on functionally related genes involved in molecular pathways or biological processes may be an effective way to identify the overall effect of polygenic risk [19] (Winham and Biernacka, 2013). In the present study, we performed the polygene cumulative study and gene-gene interaction study to explore the associations between genes involved in catecholamine and serotonin transmissions pathways and ADHD susceptibility in a Chinese children population.

2. Methods

2.1. Participants

This was a case-control study. ADHD patients were recruited from the Liuzhou Women and Children Healthcare Hospital, and healthy controls were recruited from 2 elementary schools in Liuzhou city. Inclusion criteria were as follows: 1) age 6 to 12 years and 2) Han Chinese. The exclusion criteria were: 1) any other mental disorder, such as autism spectrum disorder, bipolar disorder; 2) brain disorders, seizure disorders or other neurological disorders; 3) a history of mental retardation or an intelligence quotient (IQ) < 70; 4) the presence of any chronic physical diseases and 5) a blood lead concentration ≥ 10 (μg/dL). As a result, a total of 168 cases and 233 controls (6–12 years of age) were recruited.

Ethics approval was obtained from the Ethics Committee of School of Public Health of Lanzhou University.

2.2. Assessments of the children's ADHD symptoms

A face-to-face questionnaire survey was administered to the parents by a trained interviewer to collect socio-demographic variables. The statuses of both the case and control subjects were confirmed with a semi-structured clinical interview conducted according to the DSM-IV ADHD rating scale [20] by a pediatric psychiatrist. The Wechsler Intelligence Scale for Children 4th edition (WISC-IV) [21] was used to evaluate the full-scale IQ of all participants.

2.3. Candidate SNPs selection and genotyping

Genomic DNA was extracted from peripheral blood using the TIANamp® Blood DNA Kit DP318 (TIANGEN BIOTECH CO., LTD., Beijing, China).

The candidate genes within the dopamine, norepinephrine and serotonin neurotransmitter pathways were selected based on recent findings [22], including seven genes (i.e., *SLC6A3, SLC6A4, DRD2, ANKK1, DRD4, ADRA2A* and *SNAP25*). The screening process for the candidate SNPs was performed as follow: Firstly, we searched the F-SNP database [23] to collect information about the predictive functions of the SNPs within these genes and those of potential effects on splicing, transcription, translation, and post-translation processes were elected. Secondly, we selected SNPs with MAF (minor allele frequency) $> 5\%$ according to the SNPs by the MAF of CHB (minor allele frequency for Han Chinese in Beijing) in the 1000 genomes database (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). Thirdly, we examined the linkage disequilibria (LDs) for the Han Chinese population among selected SNPs by SNAP Pairwise LD (http://www.broadinstitute.org/mpg/snap/ldsearchpw. php), and only one was retained if several SNPs were in strong LD with each other ($r^2 \geq 0.80$).

High-quality genotyping was performed on the Sequenom MassARRAY platform (San Diego, USA) by BIO MIAO BIOLOGICAL TECHNOLOGY (Beijing, China) following the standard experimental procedures of the manufacturer. The SNPs with a call rate $< 80\%$ or Hardy-Weinberg equilibrium (HWE) < 0.05 in the control group were removed.

2.4. Statistical analysis

The statistical power to detect the effects of the SNPs was calculated by PS software (Power and Sample Size Program v3.1.2). For SNPs with MAF (minor allele frequency) of 0.11(rs6354) and 0.44(rs553668), for example, we calculated that the powers for our sample size to detect an OR of 1.50 were 43.6% and 74.8%, respectively.

2.4.1. Gene-gene interaction for ADHD risk

To estimate the high-order interactions of the SNPs associated with ADHD, classification and regression tree analysis(CART) [2425] was performed. CART analysis was performed by SPSS software to build a decision tree, which depicted how well each genotype predicted class. The CART tree was grown by splitting the root node into two offspring nodes and repeating the process via recursive partitioning. A series of 10-fold cross-validations were performed, and the Gini index was used as the splitting rule for the classification trees. The root node contains the entire sample. The best candidate SNP was the one located at the root node of the tree [25]. This process continued until the classification reached the lowest misclassification error in the terminal node. Post-process pruning was performed to avoid overfitting after the tree was grown. The different terminal nodes indicated the distinct interaction patterns of the genetic polymorphisms. Additionally, odds ratio (OR) and 95% confidence interval (CI) of these subgroups in different terminal nodes were computed using logistic regression analysis(LR), treating the lowest percentage of cases of the terminal node as the reference.

2.4.2. Cumulative effect analysis

Cumulative effect of the genetic variants of rs553668, rs1124491 and rs6354 was estimated using unconditional LR, by computing the ORs and 95%CIs [24]. We tailed the total number of unfavorable alleles for each individual and set individuals with ≦1 unfavorable allele as the reference group. Cochran-Armitage trend test [26] was applied to evaluate the cumulative effect. A receiver-operating characteristic curve (ROC) analysis was carried out to quantify the gain obtained of the three SNP. The value of area under the ROC curve (AUC) was calculated, and the chi2 test was used to compare the AUC of the cumulative effect of the three SNP.

The CART and LR analyses were performed using SPSS 22.0 software (SPSS Inc., Chicago, Illinois, USA). Cochran–Armitage test was performed by PLINK software. All tests were two-tailed, and statistical

significance was defined at $P < 0.05$.

3. Results

3.1. Characteristics of the participants

A total of 168 ADHD patients and 233 controls were included in the study. The average age of the patients was 8.55 ± 1.62 years, and that of the control group was 8.60 ± 1.76. Three SNPs of *ADRA2A* rs553668, *DRD2* rs1124491 and SLC6A4 rs6354 were significantly linked to ADHD at the uncorrected 0.05 significance level adjusted for age and sex. The details can be found in the previous study from our research group [27].

3.2. Gene-gene interaction for ADHD risk

As illustrated in Fig. 1, the SNP located at Node 0 of the tree was *ADRA2A* rs553668, which indicated that *ADRA2A* rs553668 exhibited the strongest association with ADHD among the 12 examined SNPs [25]. Five different interaction patterns for individuals carrying different *ADRA2A* rs553668 genotypes were identified in the CART tree (Node3, Node4, Node6, Node9 and Node10). Children carrying the combination of *ADRA2A* rs553668 AA and ANKK1 rs1800497 AA genotypes exhibited the lowest distribution of ADHD cases of 20.0% (Node 3). In contrast, children carrying the combination of *ADRA2A* rs553668 GG/GA and *SLC6A4* rs6354 GG/GT genotypes (Node 6)displayed the highest distribution of ADHD cases of 60.6%.

The risks of the four subgroups(Node4, Node6, Node9 and Node10) were estimated, treating carriers with the combination of *ADRA2A* rs553668 AA and *ANKK1* rs1800497 AA genotype (Node 3) as the reference who had the lowest distribution of ADHD cases. As a result, children carrying the combination of *ADRA2A* rs553668GG/GA, *SLC6A4* rs6354 GG/GT genotypes displayed the strongest risk association with ADHD, compared with other groups (adjusted OR = 6.15, 95% CI = 1.58–23.93, $P = 0.009$, Table 1).

3.3. Cumulative effect of genetic variants

Cumulative effect analysis was used to estimate the additive effect of multiple genetic variants based on the unfavorable genotypes identified in the single-loci association analysis. The cumulative effect of the three SNPs was evaluated treating *ADRA2A* rs553668 G allele, *DRD2* rs1124491 G allele and *SLC6A4* rs6354 G alleles as unfavorable alleles. We calculated the total number of unfavorable alleles in each subject and set subjects with 0–1 unfavorable alleles as the reference group. The

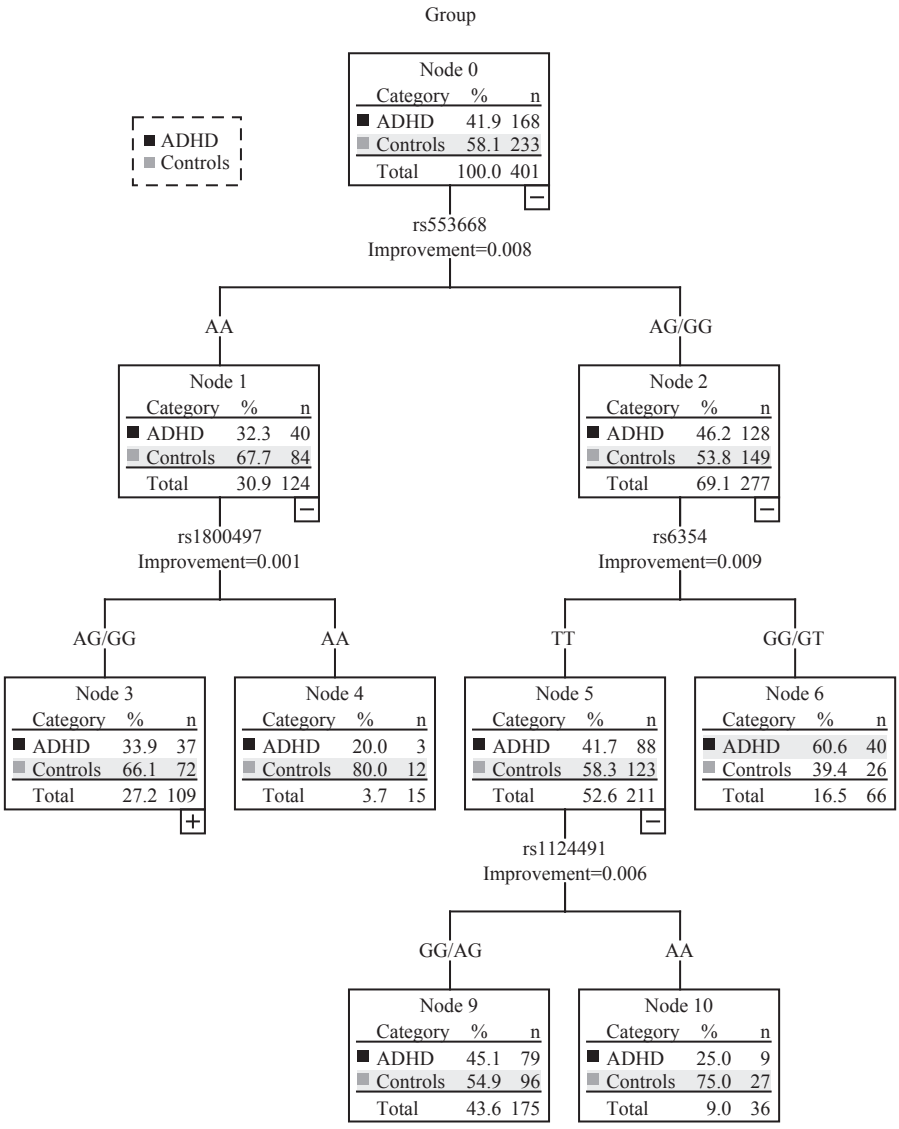

Fig.1. CART analysis of genetic polymorphisms of catecholamine and serotonin transmissions genes for ADHD risk. Frequency and percentage of cases and controls of subgroup were showed in each node.

Table 1
Risk estimates of the CART terminal nodes.

Node	Genotype	ADHD, *N*(%)	Controls, *N*(%)	Percentage of ADHD (%)	OR(95%CI)[a]	*P*
3	rs553668(AA)-rs1800497(AA)	3(1.7)	12(5.2)	20.0	Ref.	
9	rs553668(GG/GA)-rs6354(TT)-rs1124491(AA)	9(5.4)	27(11.6)	25.0	1.33(0.31–5.81)	0.702
4	rs553668(AA)-rs1800497 (GG/AG)	37(22.0)	72(30.9)	33.9	2.06(0.55–7.74)	0.287
10	rs553668(GG/GA)-rs6354(TT)-rs1124491(GG/AG)	79(47.1)	96(41.2)	45.1	3.29(0.90–12.08)	0.072
6	rs553668(GG/GA)-rs6354(GG/GT)	40(23.8)	26(11.1)	60.6	**6.15(1.58–23.93)**	**0.009**

Percentage of ADHD: the percentage of ADHD among all individuals in each node
a: The ORs and 95%CIs of the terminal nodes were calculated by logistic regression analysis adjusted for sex and age.

results showed that the risk of ADHD increased as the number of unfavorable alleles increased after controlling for age and sex. Compared with individuals carrying 0–1 unfavorable alleles, individuals carrying 4–6 unfavorable alleles had a 3.60-fold(95%CI:1.72–7.52, $P = 0.001$) increased risk of ADHD. As Fig. 2 shown, the AUC area of rs1124491 was 0.54(95%CI: 0.48–0.60). When rs6354 was added, the AUC increased moderately to 0.57(95%CI: 0.51–0.62, Chi2 = 2.80, $P = 0.094$). When rs6354 and rs553668 were added, the AUC increased to 0.59(95%CI: 0.53–0.65, Chi2 = 5.69, $P = 0.017$). The Cochran–Armitage trend test showed a significant dose–effect between cases distribution and unfavorable alleles, with a *P*-value of 0.006. To elaborate the effect of each additional unfavorable allele, the OR for trend was further calculated and the estimated OR was 1.42. It meant that the OR of ADHD might increase by 1.42 times when the number of unfavorable allele numbers increased by one. For example, the OR for ADHD would be 2.86 (1.42^3) for individuals carrying 3 unfavorable alleles (Table 2).

Table 2
Cumulative effects of the 3 SNPs (rs553668, rs1124491 and rs6354) between ADHD children and controls.

Number of unfavourable alleles	ADHD, *N* (%)	Controls, *N* (%)	Adjusted OR (95% CI)[a]	*P*-value[a]
0 ~ 1	12(7.1)	25(10.7)	Ref.	
2 ~ 3	112(66.6)	16872.1)	1.62(0.90–2.83)	0.079
4 ~ 6	44(26.3)	40(17.2)	**3.60(1.72–7.52)**	**0.001**
OR of per 1 unfavourable allele increase			**1.42(1.15–1.77)**	**0.001**
Cochran–Armitage trend test $\boldsymbol{P = 0.006}$				

a: The ORs and 95%CIs were calculated by logistic regression analysis adjusted for sex and age.

4. Discussion

In the current study, we applied the strategy of multi-loci analyses to systematically estimate the effects of the potentially functional polymorphisms of genes involved in catecholamine and serotonin transmissions pathways on susceptibility to ADHD. Both CART and LR analyses showed that gene-gene interactions in dopamine, norepinephrine, and serotonin transmission pathways, especially between *ADRA2A* rs553668G and *SLC6A4* rs6354G, were associated with the increased risk of ADHD. The combined effect of the gene-gene interaction enhanced the power for ADHD susceptibility. Compared to children with the combination of *ADRA2A* rs553668 AA and *ANKK1* rs1800497 AA

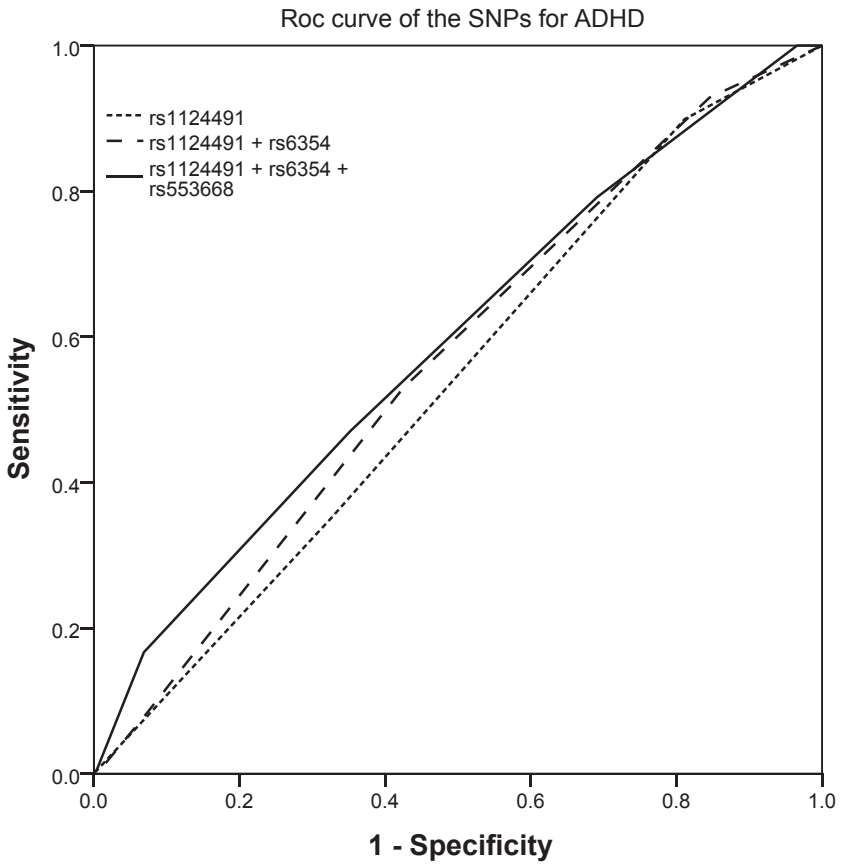

Fig. 2. ROC curves of the three SNPs for ADHD. The AUC of rs1124491 was 0.54((95% CI: 0.48–0.60); the AUC of rs1124491 + rs6354 was 0.566((95% CI: 0.51–0.62); and the AUC of rs1124491 + rs6354 + rs553668 was 0.59 (95%CI: 0.53–0.65). There was no significant difference between the AUC of rs1124491 and the AUC of rs1124491 + rs6354 (Chi2 = 2.80, $P = 0.094$). Compared with the AUC of rs1124491, the AUC of rs1124491 + rs6354 + rs553668 was significantly increased (Chi2 = 5.69, $P = 0.017$).

genotype, those carrying the combination of *ADRA2A* rs553668GG/GA and *SLC6A4* rs6354 GG/GT genotypes displayed a 6.15-fold increased risk of ADHD. In addition, *ADRA2A*rs553668 G allele, *DRD2* rs1124491 G allele and *SLC6A4* rs6354 G alleles had an additive effect on the risk of ADHD, and an incremental trend for ADHD risk was found as the number of unfavorable alleles increased.

There is growing evidence that ADHD is influenced by many common genetic variants, and about one-third of the heritability of ADHD is due to polygenic components containing many common variants [28]. A recent study found that the high polygenic risk score for ADHD was strongly related to the development of ADHD, but the presence of ADHD in parents is not a requirement for the transmission of genetic risk for ADHD [29]. Takahashi et al. [30] adopted a gene-set enrichment analysis, and observed that the cumulative polygenic risk of the dopaminergic signaling pathway was related to ADHD. Furthermore, the high polygenic risk score for ADHD was found to be associated with lower working memory performance [31], lower cognitive [32], and worse school performance [33]. These studies might explain the social functional impairments of ADHD patients. Additionally, the high polygenic risk score for ADHD was also reported to be in association with depressive symptoms [34], substance use disorder [35], and narcolepsy [30]. In line with the previous studies, our findings found an incremental trend for ADHD risk as the number of adverse alleles increased. However, it is noteworthy that the result of AUC and Chi2 analyses showed that cumulative effect of genetic variants having a moderate prediction for ADHD. These results may confirm that each SNP contributes a modest amount of risk, but the risks of SNPs are additive [36]. Therefore, cumulative polygenic risk analysis may offer a potential way to predict the development of a psychiatric disorder by acting as a biomarker for the disorder [14].

Our results suggested that the gene-gene interaction between *ADRA2A* and *SLC6A4* was associated with a high risk of ADHD. The combination of *ADRA2A* rs553668GG/GA and *SLC6A4* rs6354 GG/GT genotypes was associated with a 6.15-fold increased risk of ADHD, compared to the combination of *ADRA2A* rs553668 AA and *ANKK1* rs1800497 AA genotype. In contrast, cumulative effect analysis showed that the additive risk of ADHD in children carrying *ADRA2A* rs553668GG/GA and *SLC6A4* rs6354 GG/GT genotypes was 2.02 (1.42^2)-4.06 (1.42^4) times higher than the risk in children carrying *ADRA2A* rs553668 AA and *ANKK1* rs1800497 AA genotypes. The difference of the ORs between the gene-gene interaction and cumulative effect indicated that, besides the cumulative polygenic risk for ADHD, gene-gene interactions might be an vital component of the genetic architecture of ADHD [37]. Given this, the functional role of gene-gene interactions within the biological pathways in ADHD susceptibility is worthy of exploring.

Interestingly, there was not any gene-gene interaction between these polymorphisms and the polymorphisms within clock genes(data showed in [38]). It can be inferred that the association between genes involved in the dopamine, norepinephrine, and serotonin neurotransmitter pathways and ADHD susceptibility may not be accidental, and there should be a biological link between these genes and ADHD. Both *ADRA2A* and *SLC6A4* genes play important roles in regulating neurotransmitters. *ADRA2A* gene, encoding alpha-2A adrenergic receptor, is a member of the G protein-coupled receptor superfamily, which serves as the primary autoreceptor in sympathetic neurons [39] and plays a critical role in regulating the release of neurotransmitter from adrenergic neurons in the central nervous system [40,41]. Evidence proved that most of the alpha-2A adrenergic receptors might been located in the postsynaptic membrane in the brain, which was involved in regulating the brain's cognitive and attention function [42]. Previous studies noted that alpha-2A-adrenergic receptors in the prefrontal cortex could influence executive functions, such as attention and inhibitory control [43]. The most commonly used drugs for ADHD children—guanfacine and clonidine—exert therapeutic effects by stimulating the postsynaptic alpha-2A adrenergic receptor [44]. Evidence in rat models suggested that repeated administration of alpha-2A adrenergic receptor agonist significantly reduced impulsive choice behavior, which was related to direct stimulation of alpha-2A adrenergic receptors [45]. *SLC6A4* is one of the most important modulators of serotonergic neurotransmission [46]. The serotonin transporter encoded by *SLC6A4*, can reuptake serotonin from synapse or extracellular space, thereby regulating serotonergic activity in the brain [47]. A recent study revealed that alterations in the serotoninergic system, especially in *SLC6A4* gene, might be associated with psychiatric disorders [46]. The animal experiments showed that when *SLC6A4* heterozygous knockout mothers were exposed to stress, their offspring showed more signs of anxiety and increased risks of autism-like traits, including reduced social interaction and social interest [48].

Recently, researchers used neuroimaging to examine the gene-brain-ADHD relationships in order to explore the putative etiopathogenetic mechanisms. Kautzky et al. [49] identified the relevance of serotonin transporter and *HTR1B* rs130058 as well as *HTR2A* rs1328684 and rs6311 using a genetic and PET imaging classification model and implied a specific effect of serotonin transporter on ADHD. The study also suggested that the binding potential of serotonin transporter might be reduced in the striatum, insula, and anterior cingulate cortex in ADHD patients [49].

However, several limitations should be noted in the current study. First, the small sample size limited the statistical power of the effects of the SNPs. A large sample genetic study is needed to confirm the gene-gene interaction in these pathways. Second, the study did not include all the genes of the dopamine, norepinephrine, and serotonin neurotransmitter pathways, which might make the findings unable to fully reflect the genetic effects of these pathways on ADHD susceptibility. Third, although we observed the synergy of genes related to ADHD, the synergy effects were modest since the additive effect concerns only one polymorphism. The limitation might due to the limited number of SNPs examined. Further studies with more genes are warranted to confirm our findings.

In conclusion, this study highlighted the importance of the gene-gene interaction of the variants in the dopamine, norepinephrine, and serotonin neurotransmitter pathways. Our findings suggested that children carrying the combination of ADRA2A rs553668GG/GA and SLC6A4 rs6354 GG/GT genotypes were associated with a 6.15-fold increased risk of ADHD, compared to children with the combination of *ADRA2A* rs553668 AA and *ANKK1* rs1800497 AA genotype. The effect of the gene-gene interaction enhanced susceptibility to ADHD. The results collectively indicated that the gene-gene interaction might help to explore the etiology of ADHD.

CRediT authorship contribution statement

Yanni Wang: Project administration, Methodology, Writing - review & editing, Formal analysis. **Tingwei Wang:** Writing - original draft, Formal analysis. **Yukai Du:** Project administration, Supervision, Methodology. **Dan Hu:** Investigation, Methodology. **Yu Zhang:** Investigation. **Honghui Li:** Investigation. **Wenyan Pei:** Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the study participants and their parents. We also acknowledge the collaboration of the staff in the Liuzhou maternal and child care hospital.

Funding

This work was supported by the Fundamental Research Funds for the Central Universities (Grand number: lzujbky-2017-87, lzujbky-2019-5). The funder had no role in the study design, data collection, analysis and interpretation, writing of the report, and the decision to submit the article for publication.

Informed consent

Written informed consent was obtained from participants and their legal guardians.

References

[1] R. Thomas, S. Sanders, J. Doust, et al., Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis, Pediatrics 135 (4) (2015) e994–e1001, https://doi.org/10.1542/peds.2014-3482.
[2] W. Ten, C.-C. Tseng, Y.-S. Chiang, et al., Creativity in children with ADHD: Effects of medication and comparisons with normal peers, Psych Res 284 (2020), 112680, https://doi.org/10.1016/j.psychres.2019.112680.
[3] S. Kim, M. Arora, C. Fernandez, et al., Lead, mercury, and cadmium exposure and attention deficit hyperactivity disorder in children, Environ. Res. 126 (2013) 105–110, https://doi.org/10.1016/j.envres.2013.08.008.
[4] B.D. Clemow, Misuse of Methylphenidate, Switzerland (2017).
[5] S.V. Faraone, E. Mick, Molecular genetics of attention deficit hyperactivity disorder, The Psychiatric clinics of North America 33 (1) (2010) 159–180, https://doi.org/10.1016/j.psc.2009.12.004.
[6] A. Fernandez-Jaen, J. Albert, D.M. Fernandez-Mayoralas, et al., Cingulate cortical thickness and dopamine transporter (DAT1) genotype in children and adolescents with ADHD, J. Attent. Disord. 22 (7) (2018) 651–660, https://doi.org/10.1177/1087054716647483.
[7] Z. Chang, P. Lichtenstein, P.J. Asherson, et al., Developmental twin study of attention problems: high heritabilities throughout development, JAMA Psych. 70 (3) (2013) 311–318, https://doi.org/10.1001/jamapsychiatry.2013.287.
[8] A.F.T. Arnsten, K. Rubia, Neurobiological circuits regulating attention, cognitive control, motivation, and emotion: disruptions in neurodevelopmental psychiatric disorders, J. Am. Acad. Child Adolesc. Psychiatry 51 (4) (2012) 356–367, https://doi.org/10.1016/j.jaac.2012.01.008.
[9] A. Thapar, M. Cooper, Attention deficit hyperactivity disorder, Lancet 387 (10024) (2016) 1240–1250, https://doi.org/10.1016/s0140-6736(15)00238-x.
[10] M. Klein, M. Onnink, M. van Donkelaar, et al., Brain imaging genetics in ADHD and beyond - Mapping pathways from gene to disorder at different levels of complexity, Neurosci. Biobehav. Rev. 80 (2017) 115–155, https://doi.org/10.1016/j.neubiorev.2017.01.013.
[11] J. Lasky-Su, B.M. Neale, B. Franke, et al., Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations, Am. J. Med. Genet. Part B, Neuropsych. Genet. 147B (8) (2008) 1345–1354, https://doi.org/10.1002/ajmg.b.30867.
[12] I.R. Gizer, C. Ficks, I.D. Waldman, Candidate gene studies of ADHD: a meta-analytic review, Hum. Genet. 126 (1) (2009) 51–90, https://doi.org/10.1007/s00439-009-0694-x.
[13] Y.W. Hou, P. Xiong, X. Gu, et al., Association of serotonin receptors with attention deficit hyperactivity disorder: A systematic review and meta-analysis, Curr. Med. Sci. 38 (3) (2018) 538–551, https://doi.org/10.1007/s11596-018-1912-3.
[14] A.R. Martin, M.J. Daly, E.B. Robinson, et al., Predicting polygenic risk of psychiatric disorders, Biol. Psych. 86 (2) (2019) 97–109, https://doi.org/10.1016/j.biopsych.2018.12.015.
[15] C.M. Middeldorp, N.R. Wray, The value of polygenic analyses in psychiatry, World Psych. 17 (1) (2018) 26–28, https://doi.org/10.1002/wps.20480.
[16] A. Gialluisi, M.G. Reccia, A. Tirozzi, et al., Whole exome sequencing study of parkinson disease and related endophenotypes in the Italian population, Front. Neurol. 10 (2020), https://doi.org/10.3389/fneur.2019.01362.
[17] S.V. Faraone, R.H. Perlis, A.E. Doyle, et al., Molecular genetics of attention-deficit/hyperactivity disorder, Biol. Psych. 57 (11) (2005) 1313–1323, https://doi.org/10.1016/j.biopsych.2004.11.024.
[18] X. Carrasco, P. Rothhammer, M. Moraga, H. Henríquez, et al., Genotypic interaction between DRD4 and DAT1 loci is a high risk factor for attention-deficit/hyperactivity disorder in Chilean families, Am. J. Med. Genet. B Neuropsychiatr. Genet. 141B (1) (2006) 51–54.
[19] J.M. Biernacka, J. Geske, G.D. Jenkins, et al., Genome-wide gene-set analysis for identification of pathways associated with alcohol dependence, Int. J. Neuropsychopharmacol. 16 (2) (2013) 271–278, https://doi.org/10.1017/S1461145712000375.
[20] American Psychiatric Association, Diagnostic and statistical manual of mental disorders, fourth ed., DC American Psychiatric Publishing, Washington, 2000.
[21] Y.F. Feis, Wechsler intelligence scale for children-IV (WISC-IV), in: C.S. Clauss-Ehlers (Ed.), Encyclopedia of Cross-Cultural School Psychology, Springer, US, Boston, MA, 2010, pp. 1030–1032.
[22] J.M. Kamradt, J.T. Nigg, K.H. Friderici, et al., Neuropsychological performance measures as intermediate phenotypes for attention-deficit/hyperactivity disorder: A multiple mediation analysis, Dev. Psychopathol. (2016) 1–14, https://doi.org/10.1017/S0954579416000195.
[23] Lee PH, Shatkay H. F-SNP: computationally predicted functional SNPs for disease association studies. Nucleic acids research 2008;36(Database issue):D820-4 doi: 10.1093/nar/gkm904.
[24] L. Liu, J. Wu, R. Zhong, et al., Multi-loci analysis reveals the importance of genetic variations in sensitivity of platinum-based chemotherapy in non-small-cell lung cancer, Mol. Carcinog. 52 (12) (2013) 923–931, https://doi.org/10.1002/mc.21942.
[25] M. Garcia-Magarinos, I. Lopez-de-Ullibarri, R. Cao, et al., Evaluating the ability of tree-based methods and logistic regression for the detection of SNP-SNP interaction, Ann. Hum. Genet. 73 (Pt 3) (2009) 360–369, https://doi.org/10.1111/j.1469-1809.2009.00511.x.
[26] Armitage P. Armitage, P. Tests for Linear Trends in Proportions and Frequencies. Biometrics 11, 375-386. Biometrics 1955;11:375–86.
[27] Y. Wang, D. Hu, W. Chen, et al., Prenatal tobacco exposure modulated the association of genetic variants with diagnosed ADHD and its symptom domain in children: A Community Based Case-Control Study, Sci. Rep. 9 (1) (2019) 4274, https://doi.org/10.1038/s41598-019-40850-w.
[28] S.V. Faraone, H. Larsson, Genetics of attention deficit hyperactivity disorder, Mol. Psychiatry 24 (4) (2019) 562–575, https://doi.org/10.1038/s41380-018-0070-0.
[29] S.D. Ostergaard, B.B. Trabjerg, T.D. Als, et al., Polygenic risk score, psychosocial environment and the risk of attention-deficit/hyperactivity disorder, Transl. Psychiatry 10 (1) (2020) 335, https://doi.org/10.1038/s41398-020-01019-6.
[30] N. Takahashi, T. Nishimura, T. Harada, et al., Polygenic risk score analysis revealed shared genetic background in attention deficit hyperactivity disorder and narcolepsy, Transl. Psychiatry 10 (1) (2020) 284, https://doi.org/10.1038/s41398-020-00971-7.
[31] S. Aguilar-Lacasana, N. Vilor-Tejedor, P.R. Jansen, et al., Polygenic risk for ADHD and ASD and their relation with cognitive measures in school children, Psychol. Med. (2020) 1–9, https://doi.org/10.1017/S0033291720003189.
[32] J. Martin, M.L. Hamshere, E. Stergiakouli, et al., Neurocognitive abilities in the general population and composite genetic risk scores for attention-deficit hyperactivity disorder, J. Child Psychol. Psychiatry 56 (6) (2015) 648–656, https://doi.org/10.1111/jcpp.12336.
[33] E. Du Rietz, J. Coleman, K. Glanville, et al., Association of polygenic risk for attention-deficit/hyperactivity disorder with co-occurring traits and disorders, Biol. Psychiatry Cogn. Neurosci. Neuroimag. 3 (7) (2018) 635–643, https://doi.org/10.1016/j.bpsc.2017.11.013.
[34] I. Brikell, H. Larsson, Y. Lu, et al., The contribution of common genetic risk variants for ADHD to a general factor of childhood psychopathology, Mol. Psychiatry 25 (8) (2020) 1809–1821, https://doi.org/10.1038/s41380-018-0109-2.
[35] T. Wimberley, E. Agerbo, H.T. Horsdal, et al., Genetic liability to ADHD and substance use disorders in individuals with ADHD, Addiction 115 (7) (2020) 1368–1377, https://doi.org/10.1111/add.14910.
[36] S. Vrshek-Schallhorn, C.B. Stroud, S. Mineka, et al., Additive genetic risk from five serotonin system polymorphisms interacts with interpersonal stress to predict depression, J. Abnorm. Psychol. 124 (4) (2015) 776–790, https://doi.org/10.1037/abn0000098.
[37] J.H. Moore, The ubiquitous nature of epistasis in determining susceptibility to common human diseases, Hum. Hered. 56 (1–3) (2003) 73–82, https://doi.org/10.1159/000073735.
[38] L. Qi, Y. Zhang, L. Wang, et al., Sex differences in psychotic and non-psychotic major depressive disorder in a Chinese Han population, J. Affect. Disord. 268 (2020) 55–60, https://doi.org/10.1016/j.jad.2020.03.003.
[39] N.M. Myer, J.R. Boland, S.V. Faraone, Pharmacogenetics predictors of methylphenidate efficacy in childhood ADHD, Mol. Psychiatry 23 (9) (2018) 1929–1936, https://doi.org/10.1038/mp.2017.234.
[40] Q. Wang, L.E. Limbird, Regulated interactions of the alpha 2A adrenergic receptor with spinophilin, 14–3-3zeta, and arrestin 3, J. Biol. Chem. 277 (52) (2002) 50589–50596, https://doi.org/10.1074/jbc.M208503200.
[41] E. Schlicker, M. Göthert, Interactions between the presynaptic alpha2-autoreceptor and presynaptic inhibitory heteroreceptors on noradrenergic neurones, Brain Res. Bull. 47 (2) (1998) 129–132, https://doi.org/10.1016/s0361-9230(98)00068-9.
[42] I.D. Waldman, J.T. Nigg, I.R. Gizer, et al., The adrenergic receptor alpha-2A gene (ADRA2A) and neuropsychological executive functions as putative endophenotypes for childhood ADHD, Cognitive Affect. Behav. Neurosci. 6 (1) (2006) 18–30.
[43] A.F. Arnsten, B.M. Li, Neurobiology of executive functions: catecholamine influences on prefrontal cortical functions, Biol. Psychiatry 57 (11) (2005) 1377–1384, https://doi.org/10.1016/j.biopsych.2004.08.019.
[44] R. Vidal-Estrada, R. Bosch-Munso, M. Nogueira-Morais, et al., Psychological treatment of attention deficit hyperactivity disorder in adults: A systematic review, Actas Esp Psiquiatr 40 (3) (2012) 147–154.
[45] K. Nishitomi, K. Yano, M. Kobayashi, et al., Systemic administration of guanfacine improves food-motivated impulsive choice behavior primarily via direct stimulation of postsynaptic α(2A)-adrenergic receptors in rats, Behav. Brain Res. 345 (2018) 21–29, https://doi.org/10.1016/j.bbr.2018.02.022.
[46] M. Calabrò, L. Mandelli, C. Crisafulli, et al., Psychiatric disorders and SLC6A4 gene variants: possible effects on alcohol dependence and alzheimer's disease, Mol. Biol. Rep. 47 (1) (2020) 191–200, https://doi.org/10.1007/s11033-019-05119-5.

[47] P.S. Ho, M.C. Shih, K.H. Ma, et al., Availability of the serotonin transporter in patients with alcohol dependence, World J. Biol. Psychiatry 12 (2) (2011) 134–142, https://doi.org/10.3109/15622975.2010.503813.
[48] K.L. Jones, R.M. Smith, K.S. Edwards, et al., Combined effect of maternal serotonin transporter genotype and prenatal stress in modulating offspring social interaction in mice, Int. J. Dev. Neurosci. 28 (6) (2010) 529–536, https://doi.org/10.1016/j.ijdevneu.2010.05.002.
[49] A. Kautzky, T. Vanicek, C. Philippe, et al., Machine learning classification of ADHD and HC by multimodal serotonergic data, Transl. Psychiatry 10 (1) (2020) 104, https://doi.org/10.1038/s41398-020-0781-2.