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ORIGINAL RESEARCH

Parallel Single-Tissue Summary-Data Mendelian Randomization and Functional Validation Identify the ARRDC1 Promoter SNP rs4494021 as a Genetic Marker Associated with Anxiety Disorders

Lesheng Wang[1,2,*], Zhipeng Xu[3,*], Gaomeng Luo[1,2], Wei Wei[1,2], Meimei Guo[4], Yunhe Yuan[1,2], Bei Shi[1,2], Haowen Guan[1,2], Sha Liu[5], Xiang Li[1,2,6–8]

[1]Department of Neurosurgery, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, 430071, People's Republic of China; [2]Brain Research Center, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, 430071, People's Republic of China; [3]Department of Neuropsychology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, 430071, People's Republic of China; [4]Department of Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, 325000, People's Republic of China; [5]Department of General Practice, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, 430071, People's Republic of China; [6]Medical Research Institute, Wuhan University, Wuhan, Hubei, 430071, People's Republic of China; [7]Frontier Science Center for Immunology and Metabolism, Wuhan University, Wuhan, Hubei, 430071, People's Republic of China; [8]Sino-Italian Ascula Brain Science Joint Laboratory, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, 430071, People's Republic of China

*These authors contributed equally to this work

Correspondence: Sha Liu, Department of General Practice, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, 430071, People's Republic of China, Email sha.liu@whu.edu.cn; Xiang Li, Department of Neurosurgery, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, 430071, People's Republic of China, Email li.xiang@whu.edu.cn

Purpose: Anxiety disorders are highly heritable, but their underlying genetic mechanisms remain poorly understood. This study aimed to identify and functionally characterize genes whose expression is causally linked to anxiety disorder risk by integrating the parallel single-tissue genetic data.

Methods: We applied a parallel single-tissue summary-data-based Mendelian randomization (SMR) approach. This integrated large-scale anxiety disorder GWAS statistics with expression quantitative trait loci (eQTL) data from peripheral blood and 13 brain regions. The functional impact of a prioritized SNP (rs4494021) was tested via dual-luciferase reporter assay. The behavioral consequences were investigated by performing stereotaxic, lentivirus-mediated overexpression of ARRDC1 specifically in the mouse cerebellar hemisphere, followed by comprehensive behavioral testing.

Results: The SMR analysis identified thirteen potential causal genes (ITIH3, ZKSCAN4, GNL3, SLC35C1, FNBP4, COPZ1, ATP5MC1, ITIH4, EHMT1, PTPMT1, ARRDC1, NEK4, and BTN3A2). Among them, ARRDC1 emerged as a significant gene, showing associations with anxiety disorders in blood, cerebellar hemisphere, and cerebellum. The risk allele of the promoter SNP rs4494021 significantly enhanced ARRDC1 transcriptional activity. Crucially, cerebellar overexpression of ARRDC1 in mice robustly induced anxiety-like behaviors, including reduced exploration in the open field test, decreased open-arm activity in the elevated plus maze, and increased avoidance in the light-dark box test.

Conclusion: By bridging genetic epidemiology with molecular and behavioral validation, this study identifies ARRDC1 promoter SNP rs4494021 as a genetically associated marker for anxiety disorders and functionally validates that elevated cerebellar ARRDC1 expression contributes to anxiety pathogenesis. These findings establish ARRDC1 as a functionally supported risk gene and highlight a novel component of anxiety-related neurocircuitry.

Keywords: anxiety disorder, genome-wide association study, summary data-based mendelian randomization, ARRDC1, expression quantitative trait loci, single nucleotide polymorphism

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Introduction

Anxiety disorders are a group of mental health conditions marked by an overwhelming and enduring sense of fear, worry, or avoidance behaviors that significantly impair daily functioning.[1] These disorders include generalized anxiety disorder, panic disorder, phobias, as well as other subtypes defined by clinical presentation. Anxiety disorders are among the most common mental illnesses globally, with a lifetime prevalence exceeding 20%.[2] Several meta-analyses of large epidemiological studies estimate that approximately 30–40% of individuals experience clinically significant anxiety symptoms during their lifetime.[3,4] This disorder frequently co-occurs with depression, insomnia, and physical health conditions like coronary artery disease, highlighting their broad impact on health.[5] Beyond their high prevalence, anxiety disorders impose a considerable burden on the quality of life and public health.[6] This underscores the urgent need to elucidate the underlying mechanisms of anxiety disorders and develop more effective prevention and treatment strategies. Current therapeutic strategies primarily involve psychotherapy and pharmacotherapy; however, these approaches often encounter limitations, including treatment resistance and adverse side effects. Accordingly, there is an urgent need to explore the pathogenesis of anxiety disorders, which may yield new insights into potential treatment avenues.

Previous twin and family studies estimate the heritability of anxiety disorders at 30–60%,[3,4] indicating a substantial genetic contribution. Genetic variation in anxiety disorders involves multiple loci and complex polygenic mechanisms. Genome-wide association studies (GWAS) have identified several anxiety-related risk loci, including rs1709393 in the region of chromosome 3q12.3 (located in the non-coding RNA region) and rs1067327 in the region of chromosome 2p21 (within the Calmodulin-Lysine N-Methyltransferase gene), as well as the Neurotrophic Receptor Tyrosine Kinase 2 gene encoding a brain-derived neurotrophic factor receptor.[1,7] A comprehensive multi-ancestry GWAS involving over 1.2 million participants revealed 51 novel anxiety-associated loci, and demonstrated polygenic risk scores that generalize across diverse populations.[7] Common genetic variants explain approximately 26–31% of the heritability for lifetime anxiety diagnoses and current symptoms.[3,7] Nonetheless, the biological implications of the recognized genetic variants are still predominantly ambiguous. Given that a significant number of the genetic variants identified in GWAS are situated within non-coding regions, these variants may influence diseases or disorders through mechanisms related to gene expression. Consequently, investigating the correlation between genetic variation and gene expression is crucial for a more comprehensive insight into the regulatory pathways that contribute to the onset of anxiety disorders.

The summary data-based mendelian randomization (SMR) methodology, utilizing genetic variants as instrumental variables (IVs), has emerged as a powerful framework for deducing causal connections between exposures (eg, biomarkers, behaviors) and disease outcomes while minimizing confounding bias compared with traditional GWAS.[8,9] Recent advancements integrate expression quantitative trait loci (eQTLs), methylation quantitative trait loci, and GWAS data into a unified analytical pipeline, enabling systematic dissection of gene regulatory mechanisms with pleiotropic effects across complex phenotypes.[10,11] This multi-omics SMR approach employs colocalization analyses to prioritize genetic variants jointly associated with molecular traits (such as gene expression and DNA methylation) and clinical endpoints,[12,13] thereby distinguishing direct causal effects from horizontal pleiotropy, suggesting its potential as a valuable instrument for investigating genes that are pleiotropically related to complex traits. Thus, SMR provides a principled way to leverage genetic variation to infer which genes have a causal role in disease pathogenesis.

While previous studies have established some links between genetic variants and anxiety,[14,15] previous efforts to characterize expression-trait relationships have been limited by reliance on single-tissue analyses that ignore neurobiological context, and inability to distinguish causal effects from mere correlations. To address these limitations, we adopted parallel SMR analyses across multiple tissues, integrating cis-expression quantitative trait loci (cis-eQTLs) across brain and blood with summary statistics derived from GWAS. Our study aimed to identify and prioritize genes whose expression levels are putatively causally associated with anxiety disorder risk across tissues. From the top candidates identified, we selected ARRDC1, a gene showing significant association in brain regions and blood, for downstream functional validation. We investigated the impact of an anxiety-associated single nucleotide polymorphism (SNP) - rs4494021 on ARRDC1 promoter activity using a dual-luciferase reporter assay and further examined the behavioral consequences of ARRDC1 overexpression in the mouse cerebellar hemisphere. This integrated approach from

computational prioritization to molecular and behavioral validation provides novel insights into the genetic architecture of anxiety disorders and elucidates the potential mechanistic role of ARRDC1.

Materials and Methods

Data Sources

GWAS Summary Statistics

GWAS were sourced from the FinnGen consortium,[16] which includes 35,875 cases of anxiety disorder alongside 444,414 healthy control subjects. To our knowledge, this dataset constitutes the latest GWAS results incorporating the highest number of anxiety disorder cases recorded thus far. The FinnGen initiative aims to compile and scrutinize genomic and national health register information from a cohort of 500,000 individuals in Finland.

eQTL Summary Data

In the context of eQTL analysis, we first utilized summary-level data concerning cis-eQTL data derived from blood samples of 31,684 participants from the eQTLGen Consortium, which are available at https://www.eqtlgen.org/cis-eqtls.html. Based on previous studies, multiple brain regions, including amygdala,[17,18] prefrontal cortex,[19,20] hippocampus,[21] and hypothalamus,[22] are closely associated with anxiety. Therefore, we also analyzed the different brain region data published by GTEx eQTL V8. Data from the V8 release of the GTEx eQTL summary pertaining to 13 different regions,[23] involving cortex, nucleus accumbens, caudate, substantia nigra, putamen, hypothalamus, amygdala, frontal cortex, hippocampus, cerebellum, anterior cingulate cortex, cerebellar hemisphere, spinal cord, is available for retrieval at https://yanglab.westlake.edu.cn/software/smr/#eQTLsummarydata. All summary data in this study are publicly available, as shown in Table 1.

SMR and HEIDI Analysis

The SMR analysis conducted aggregated statistics from eQTL and GWAS datasets to explore the relationship between gene expression and anxiety disorders. This investigation employed single nucleotide polymorphism (SNP) markers sourced from cis-eQTLs as IVs, wherein gene expression was treated as the exposure and anxiety disorder as the

Table 1 Details of the eQTL, GTEx_V8 and FinnGen_R10 Encompassed Within the SMR

Data Source	Total Cases
eQTL	
Blood	31684
FinnGen_R10	
Anxiety disorders	35875
GTEx_V8	
Cortex	205
Anterior cingulate cortex	147
Caudate	194
Nucleus accumbens	202
Putamen	170
Hypothalamus	170
Amygdala	129
Hippocampus	165
Substantia nigra	114
Cerebellum	209
Cerebellar hemisphere	175
Spinal cord	126
Frontal cortex	175

Abbreviations: eQTL, expression quantitative trait loci; GTEx_V8, genotype-tissue expression project V8.

resultant outcome. For the SMR to maintain its validity, the IVs must satisfy three critical conditions: 1) a robust correlation with gene expression; 2) the absence of correlation with any potential confounding factors; and 3) a direct influence on the outcome exclusively via gene expression, without the involvement of alternative pathways. The analysis was conducted using SMR software version 1.3.1, following the default settings, which entailed the exclusion of SNPs with a minor allele frequency below 0.01, the selection of cis-eQTLs at a significance threshold of $P < 5 \times 10^{-8}$, the removal of SNPs with linkage disequilibrium (LD) r-squared values among top SNPs exceeding 0.90 or falling below 0.05, and the exclusion of one SNP from each pair exhibiting LD r-squared values greater than 0.90. Gene loci of significance were identified with a P_SMR value lower than 0.05. Heterogeneity in dependent instruments (HEIDI) was conducted to ascertain the stability of the associations (with P_HEIDI greater than 0.05). To account for multiple testing across the candidate genes in the eQTLGen dataset, we applied the Benjamini-Hochberg false discovery rate (FDR) method. Genes with FDR < 0.05 and P_HEIDI > 0.05 were considered statistically significant. All instrumental variables for the significant genes demonstrated F statistics > 10, indicating robust instrument strength and minimizing the potential for weak instrument bias. The methodology for the SMR analysis has been previously documented.[24] The procedural flow of the SMR analysis is depicted in Figure 1.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis

To explore the potential biological functions and associated pathways, analyses were executed using RStudio version 4.4.1 within the clusterProfiler package.[25] The enrich GO function facilitated the execution of GO analysis, focusing on the sub-ontologies of biological process (BP), molecular function (MF), and cellular component (CC). This allowed us to identify significantly enriched GO terms among the genes of interest, with statistical significance determined by adjusting p-values using the Benjamini-Hochberg method. Additionally, KEGG pathway analysis was performed to clarify the roles of these genes within various biological pathways. The results highlighted several key pathways and processes that are potentially implicated in the underlying mechanisms of the studied traits. This integrative approach provided enhanced insights into the functional relevance of the genetic variants identified through SMR.

Data preprocessing and subsequent statistical and bioinformatics analyses were performed utilizing R version 4.4.1 (accessible at https://www.r-project.org/) and SMR (found at https://cnsgenomics.com/software/smr/).

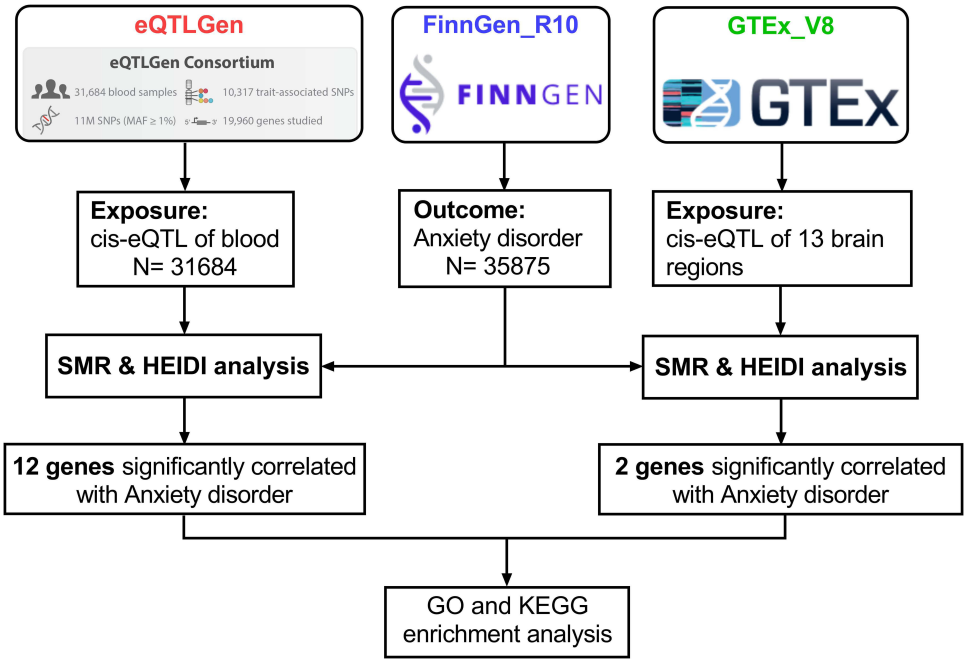

Figure 1 The procedural framework for SMR analysis employed in this investigation.

Dual-Luciferase Reporter Assay

HEK293T cells (Pricella, China) were plated in 24-well plates and maintained in high-glucose DMEM supplemented with 25 mM HEPES buffer, 1.0 mM sodium pyruvate (Servicebio, cat. no. G4517), 1% penicillin-streptomycin (Servicebio, cat. no. G4003), and 10% fetal bovine serum (Vazyme, cat. no. F103-03). Cells were cultured at 37 °C under 5% CO_2 until reaching 70–80% confluence. For transfection, Lipomaster 3000 reagent (Vazyme, cat. no. TL301-01) was used to deliver two plasmids: a promoterless pGL3-basic vector (Miaolingbio, cat. no. P0193) carrying the ARRDC1 promoter region (from 2000 bp upstream of the transcription start site) with either the C or T allele of rs4494021, which drives Firefly luciferase expression; and the pRL-TK control plasmid (Miaolingbio, cat. no. P0372) expressing Renilla luciferase constitutively. The plasmids were transfected at a 10:1 mass ratio (test:control). After 48 h, luciferase activity was measured with the Dual Luciferase Reporter Assay Kit (Vazyme, cat. no. DL101-01) on a luminometer. Firefly luciferase signal was recorded first, followed by quenching and measurement of Renilla luciferase activity. Promoter activity for each sample was expressed as the ratio of Firefly luminescence (FLU) to Renilla luminescence (RLU).

Lentiviral Production of ARRDC1 Overexpression

To generate lentiviral vectors for ARRDC1 overexpression, the full-length human ARRDC1 coding sequence (GenBank: NM_001162485.1) was cloned into a modified pCDH transfer plasmid. The following primers were designed for PCR amplification from a mouse cDNA template: Forward: 5’ -TTTTGACCTCCATAGAAGATTCTAGAGCCACCATGGGGAGGGTGCAGCTCTT - 3’; Reverse: 5’-TCTGGAACATCGTATGGGTAGGATCCTCAGCTTCCTGGGATCAGGCC – 3’. The PCR product and the pCDH-CMV-MCS-EF1-copGFP lentiviral backbone (System Biosciences) were digested with EcoRI and Xbal, gel-purified, and ligated to generate the recombinant plasmid pCDH-ARRDC1. The control lentiviral plasmid (pCDH-Empty) was prepared by excising the ARRDC1 insert from pCDH-ARRDC1 via restriction digestion, followed by self-ligation of the backbone, yielding an empty vector containing no exogenous coding sequence. All constructs were verified by Sanger sequencing. For lentivirus production, HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin until reaching 70–80% confluence in 10-cm dishes. Cells were co-transfected using Lipomaster 3000 transfection reagent (Vazyme, cat. no. TL301-01) with three plasmids: 5 μg of the transfer vector (pCDH-ARRDC1 or pCDH-Empty), 3.75 μg of the packaging plasmid psPAX2, and 1.25 μg of the envelope plasmid pMD2.G. The culture medium was replaced with fresh medium 6 h post-transfection. Supernatants containing viral particles were collected at 48 h and 72 h post-transfection, pooled, clarified by centrifugation at 3,000 × g for 10 min, and filtered through a 0.45-μm filter. Viral particles were concentrated by ultracentrifugation at 50,000 × g for 2 h at 4°C. The viral pellet was resuspended in sterile PBS, aliquoted, and stored at −80°C until use. Viral titers were consistently $>1 \times 10^9$ IU/mL for all preparations. All lentiviral work was performed according to protocols approved by the Institutional Biosafety Committee of Wuhan University. Overexpression efficiency was confirmed in N2A cells (Pricella, China) using quantitative real-time PCR.

Mice Used for Behavioral Tests

Male C57BL/6 mice, aged 9 weeks, were acquired from Whupenn Life Science Co., Ltd. (Wuhan, China), were housed in groups of four per cage with corncob bedding and nesting material. They were maintained on a 12-hour light/dark cycle with free access to food and water throughout the study. Mice were randomly assigned to either the Control (Con) or ARRDC1 overexpression (OE) group using a random number table, with no significant difference in initial body weight between groups. All behavioral experiments were performed during the light phase in dedicated testing rooms. The study protocol was reviewed and approved by the Animal Ethics Committee of Wuhan University (Approval No. ZN2023018) and conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. For euthanasia, mice were deeply anesthetized with pentobarbital sodium (100 mg/kg, intraperitoneal) and then euthanized by cervical dislocation. All procedures were performed in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (2020 edition).

Infusion of Lentiviral Constructs in Stereotaxic Surgery

For stereotaxic delivery of lentivirus, mice were anesthetized via intraperitoneal injection of 1% pentobarbital sodium (50 mg/kg). The depth of anesthesia was confirmed by the absence of a toe-pinch reflex. Upon loss of the toe-pinch reflex, each mouse was secured in a stereotaxic frame (RWD Life Science, Shenzhen, China). A midline scalp incision was made to expose the skull. Using a 10 μL micro syringe (Gaoge, Shanghai, China) attached to a micropump (KDS LEGATO, Scientific Holliston, USA), 500 nl of high-titer lentivirus (> 1×10^9 IU/mL) carrying either ARRDC1 (OE) or control (Con) constructs was slowly infused into each cerebellar hemisphere at a rate of 100 nl/min. The injection coordinates relative to bregma were: anterior-posterior (AP), −6.0 mm; dorsal-ventral (DV), −2.0 mm; and midline-lateral (ML), ±1.50 mm. The needle remained in place for 10 min before withdrawal to minimize backflow. Post-surgery, mice received carprofen (5 mg/kg) for analgesia over two days and were monitored daily for welfare. All surgical procedures were performed under anesthesia, and care was taken to minimize animal suffering in accordance with the American Veterinary Medical Association guidelines. All animals recovered without adverse events and were maintained at 25 °C on a 12 h light/dark cycle with free access to food and water. Behavioral testing commenced 7 days after surgery, performed by an experimenter blinded to group assignment, with the testing order randomized daily.

Quantitative RT-PCR

For cDNA synthesis, a total of 1 μg of RNA was subjected to reverse transcription utilizing the HiScript IV RT SuperMix for qPCR kit (Vazyme, China). Quantitative real-time PCR (qPCR) was performed on a Bio-Rad Real-Time PCR Detection System with the Rotor-Gene SYBR Green PCR Kit (Vazyme, China). Gene-specific primers were designed for the target gene ARRDC1 and the reference gene PGK1. The primer sequences were as follows: PGK1 Forward: 5' - TGCACGCTTCAAAAGCGCACG - 3', Reverse: 5' - AAGTCCACCCTCATCACGACCC - 3'; ARRDC1 Forward: 5' - CTTCCGAGCTATCCGGGTGA - 3', Reverse: 5' - GTCCCTCAAAAGATGTGGGTG - 3'. Each sample was run in duplicate, and the experiment was independently repeated at least twice. Relative expression levels were calculated using the ΔΔCT method.

Behavioral Test

Open-Field Test

To assess general anxiety-like behavior and locomotor activity, mice were tested in an open-field arena (Xinruan Information Technology Co., Ltd., model XR-XZ301, Shanghai, China). The test was conducted in a sound-attenuated room under dim white light (60 lux). Each mouse was placed in the center of a white plastic chamber (30 cm × 30 cm × 30 cm) and allowed to explore freely for 10 min while being recorded by an overhead camera. The recordings were analyzed with Xinruan VisuTrack software to quantify total distance traveled and the frequency and duration of entries into the central zone, defined as a 15×15 cm square in the middle of the arena.

Elevated Plus Maze

The elevated plus maze (EPM; Xinruan Information Technology Co., Ltd., model XR-XG201, Shanghai, China) was used to assess anxiety-related behavior. The apparatus consisted of two open arms (35 cm × 5 cm) and two enclosed arms (35 cm × 5 cm, with 15-cm-high walls) arranged in a plus shape and elevated 55 cm above the floor. Testing was performed during the early dark phase under dim indirect lighting (30 lux at maze level). Each mouse was placed on the central platform (6 cm × 6 cm) facing an open arm and allowed to explore freely for 5 min. Sessions were recorded via a ceiling-mounted camera. Between trials, the maze was wiped with 70% ethanol and dried to remove olfactory cues. Experimenters remained outside the testing room throughout the trial to avoid disturbing the animals.

Light-Dark Box Test

Anxiety-like behavior was evaluated in a light–dark box apparatus (Xinruan Information Technology Co., Ltd., model XR-XB110, Shanghai, China). The box comprises two equal-sized compartments (20 cm × 20 cm × 30 cm): a brightly lit chamber (white interior, ~400 lux) and a dark, enclosed chamber (black interior, <10 lux), connected by a small opening (5 cm × 5 cm) at floor level. Testing was conducted during the early active (dark) phase under low ambient light. Each mouse was placed gently in the center of the light compartment facing away from the opening and allowed to explore

freely for 5 min. Behavior was recorded via an overhead camera linked to Xinruan VisuTrack software. Between trials, the apparatus was cleaned thoroughly with 70% ethanol and dried to remove odor cues.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 9.0. Descriptive statistics are presented as mean ± standard deviation (SD). Differences between the Con and ARRDC1 OE groups were evaluated using two-tailed unpaired Student's t-tests. Where multiple comparisons were required, one-way ANOVA was applied, followed by Šídák's post hoc tests. A p-value < 0.05 was considered statistically significant.

To account for multiple comparisons across the eight behavioral parameters (open field test: total distance, center distance, center time, center entries; elevated plus maze: open arm time, open arm entries; light-dark box: transitions, time in dark), we applied Bonferroni correction. The significance threshold was adjusted to $\alpha=0.05/8=0.00625$. Results with $p < 0.00625$ were considered statistically significant after correction. Both uncorrected and Bonferroni-adjusted p-values are reported in the results section to ensure transparency.

Results

SMR Analysis Identifies Twelve Genes Associated with Anxiety Disorder in Blood

At the outset of our analysis, we conducted SMR analysis utilizing the eQTLGen dataset to pinpoint genes that exhibit significant associations with characteristics of anxiety disorders. Specifically, the eQTLGen dataset provided an extensive array of data, culminating in the identification of 14,075 candidate genes. After applying Benjamini-Hochberg FDR correction to control for multiple testing across 14,075 candidate genes, we identified 12 genes from the eQTLGen blood dataset that met the significance threshold (FDR < 0.05, P_HEIDI > 0.05). These genes were considered potentially causally related to anxiety disorders. Comprehensive information regarding these identified genes is illustrated in Figure 2 and Table 2.

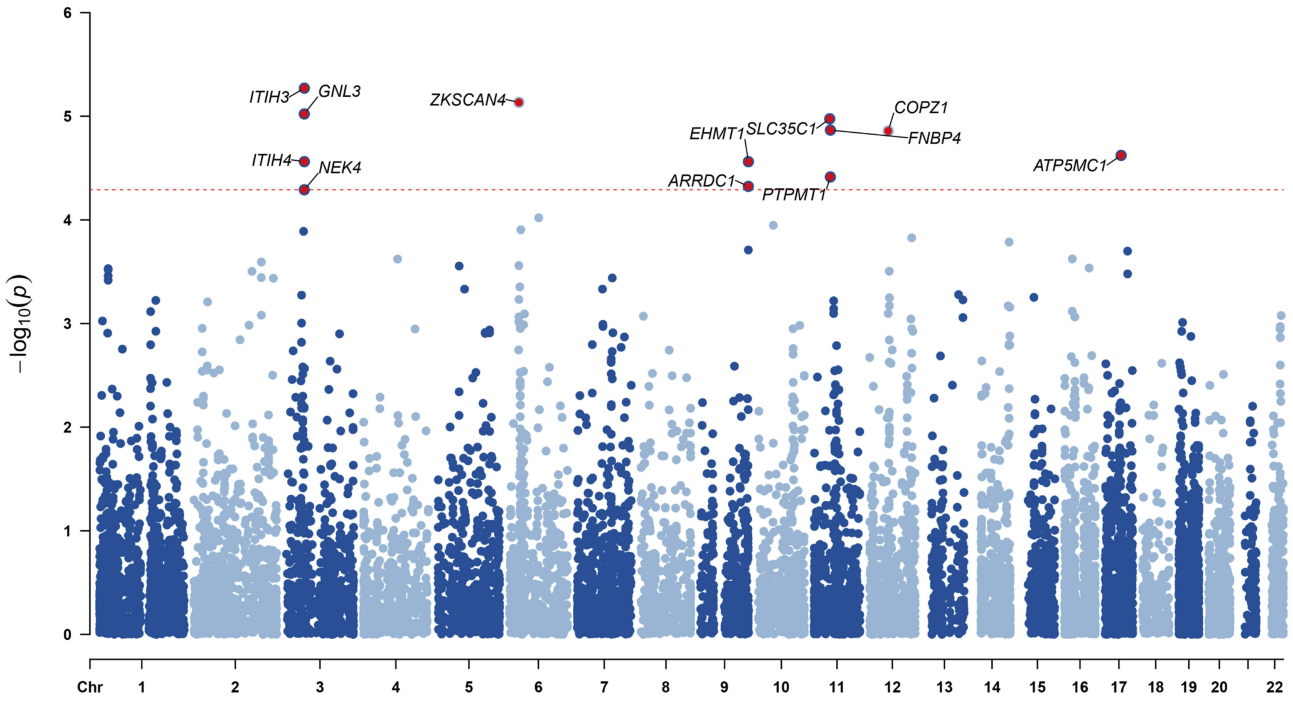

Figure 2 Manhattan plot for eQTL SMR analysis of blood. Genes associated with anxiety disorder are shown in red dots. The horizontal line represents the genome-wide significance threshold.

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Table 2 Details of the eQTL and GWAS Encompassed Within the SMR in Blood

Ensemble ID	Chr	Gene	Top SNP	SMR-Beta	SMR-P-value	HEIDI-P-value	FDR
ENSG00000162267	3	ITIH3	rs2710313	−0.360833	5.362×10^{-6}	0.166988	0.02432503
ENSG00000187626	6	ZKSCAN4	rs13200462	−0.233714	7.347×10^{-6}	0.1035367	0.02432503
ENSG00000163938	3	GNL3	rs2590838	0.267764	9.502×10^{-6}	0.1674218	0.02432503
ENSG00000181830	11	SLC35C1	rs3808976	0.125132	1.058×10^{-5}	0.1798001	0.02432503
ENSG00000109920	11	FNBP4	rs11039337	−0.280796	1.361×10^{-5}	0.6686161	0.02432503
ENSG00000111481	12	COPZ1	rs61614746	−0.317048	1.387×10^{-5}	0.4014014	0.02432503
ENSG00000159199	17	ATP5MC1	rs12948439	−0.117253	2.384×10^{-5}	0.141127	0.03207628
ENSG00000055955	3	ITIH4	rs13083728	−0.0625679	2.743×10^{-5}	0.1655019	0.03207628
ENSG00000181090	9	EHMT1	rs11137167	0.140015	2.744×10^{-5}	0.3275561	0.03207628
ENSG00000110536	11	PTPMT1	rs7107356	0.385977	3.852×10^{-5}	0.2713234	0.04096037
ENSG00000197070	9	ARRDC1	rs11137155	0.135815	4.752×10^{-5}	0.5718169	0.04096037
ENSG00000114904	3	NEK4	rs2577831	0.6045	5.125×10^{-5}	0.6122253	0.04096037

Abbreviations: Chr, chromosome; SNP, single nucleotide polymorphism; SMR, summary data-based mendelian randomization; HEIDI, heterogeneity in dependent instruments; FDR, false discovery rate (Benjamini-Hochberg adjusted).

Table 3 Summary of the SMR Analyses Across the Three Brain Regions

Regions	Ensemble ID	Chr	Gene	Top SNP	SMR-Beta	SMR-P-value	HEIDI-P-value	FDR
Cerebellar Hemisphere	ENSG00000197070	9	ARRDC1	rs4494021	0.071562	1.08×10^{-5}	0.474771	0.044385
Cerebellum	ENSG00000197070	9	ARRDC1	rs4494021	0.058956	7.52×10^{-6}	0.699557	0.023852
Cervical spinal cord	ENSG00000186470	6	BTN3A2	rs72841536	0.065191	2.70×10^{-5}	0.680261	0.046822

Abbreviations: Chr, chromosome; SNP, single nucleotide polymorphism; SMR, summary data-based mendelian randomization; HEIDI, heterogeneity in dependent instruments; FDR, false discovery rate (Benjamini-Hochberg adjusted).

Two Genes Identified Showing Association with Anxiety Across 13 Brain Regions

Then we identified two genes that exhibit a relationship with anxiety across various brain regions (Table 3 and Figure 3). Among the 13 brain regions examined, after FDR correction for multiple comparisons within each region (FDR < 0.05), arrestin domain containing 1 (ARRDC1), also contained in previously identified 12 genes (Figure 3A and B, Table 2), emerged as the most significant gene demonstrating association with anxiety in two specific regions, following adjustments for multiple comparisons. Additionally, the other gene, butyrophilin subfamily 3 member A2 (BTN3A2, ENSG00000186470), part of the human major histocompatibility complex, was also found to be the most prominent gene associated with anxiety in one distinct brain region (Figure 3C and Table 3).

The Putative Causal Relationship of Predicted Genes in Anxiety Disorder by Integrating GWAS and eQTL Data

To ascertain the causal relationship between predicted genes and anxiety disorder, a two-sample SMR analysis methodology was utilized, merging cis-eQTLs in blood with summary statistics from anxiety disorder GWAS. Within the eQTLGen dataset, the top six most significant genes identified are ENSG00000162267 (referred to as inter-alpha-trypsin inhibitor heavy chain 3 (ITIH3), P_SMR = 5.362×10^{-6}), ENSG00000187626 (referred to as zinc finger with KRAB and SCAN domains 4 (ZKSCAN4), P_SMR = 7.347×10^{-6}), ENSG00000163938 (referred to as G protein nucleolar 3 (GNL3), P_SMR = 9.502×10^{-6}), ENSG00000181830 (referred to as solute carrier family 35 member C1 (SLC35C1), P_SMR = 1.058×10^{-5}), ENSG00000109920 (referred to as formin binding protein 4 (FNBP4), P_SMR = 1.361×10^{-5}), and ENSG00000111481 (referred to as COPI coat complex subunit zeta 1 (COPZ1), P_SMR = 1.387×10^{-5}). An increased expression of GNL3 (Figure 4A) and SLC35C1 (Figure 4B) is associated with a heightened vulnerability to anxiety disorder, whereas an elevated expression of ITIH3 (Figure 4C), ZKSCAN4 (Figure 4D), FNBP4 (Figure 4E), and COPZ1 (Figure 4F) correlates

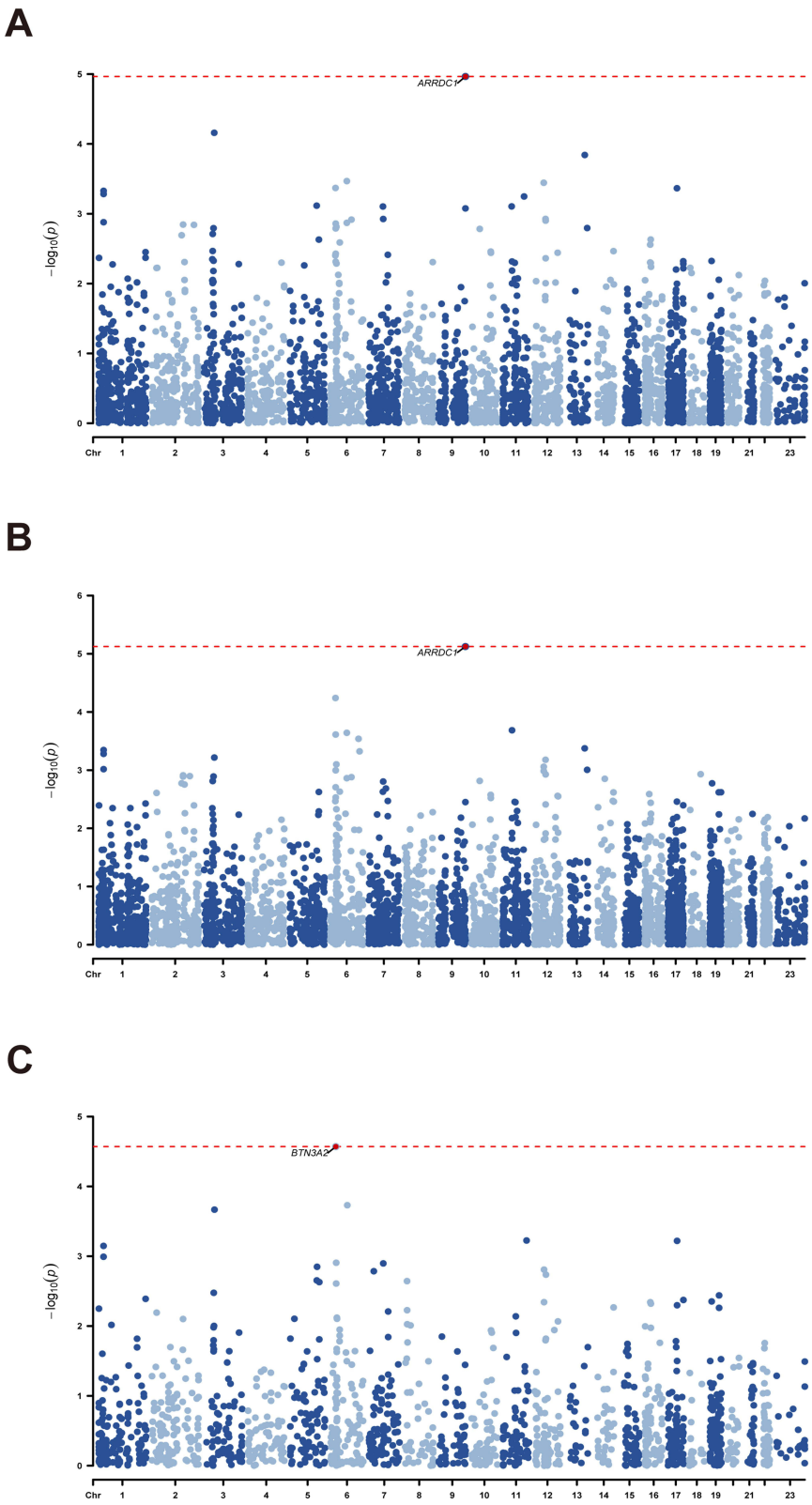

Figure 3 Manhattan plot for eQTL SMR analysis of different brain regions. (**A**) cerebellar hemisphere; (**B**) cerebellum; (**C**) cervical spinal cord. Red dots represent genes with significant association. The red dashed line indicates the FDR-corrected significance threshold.

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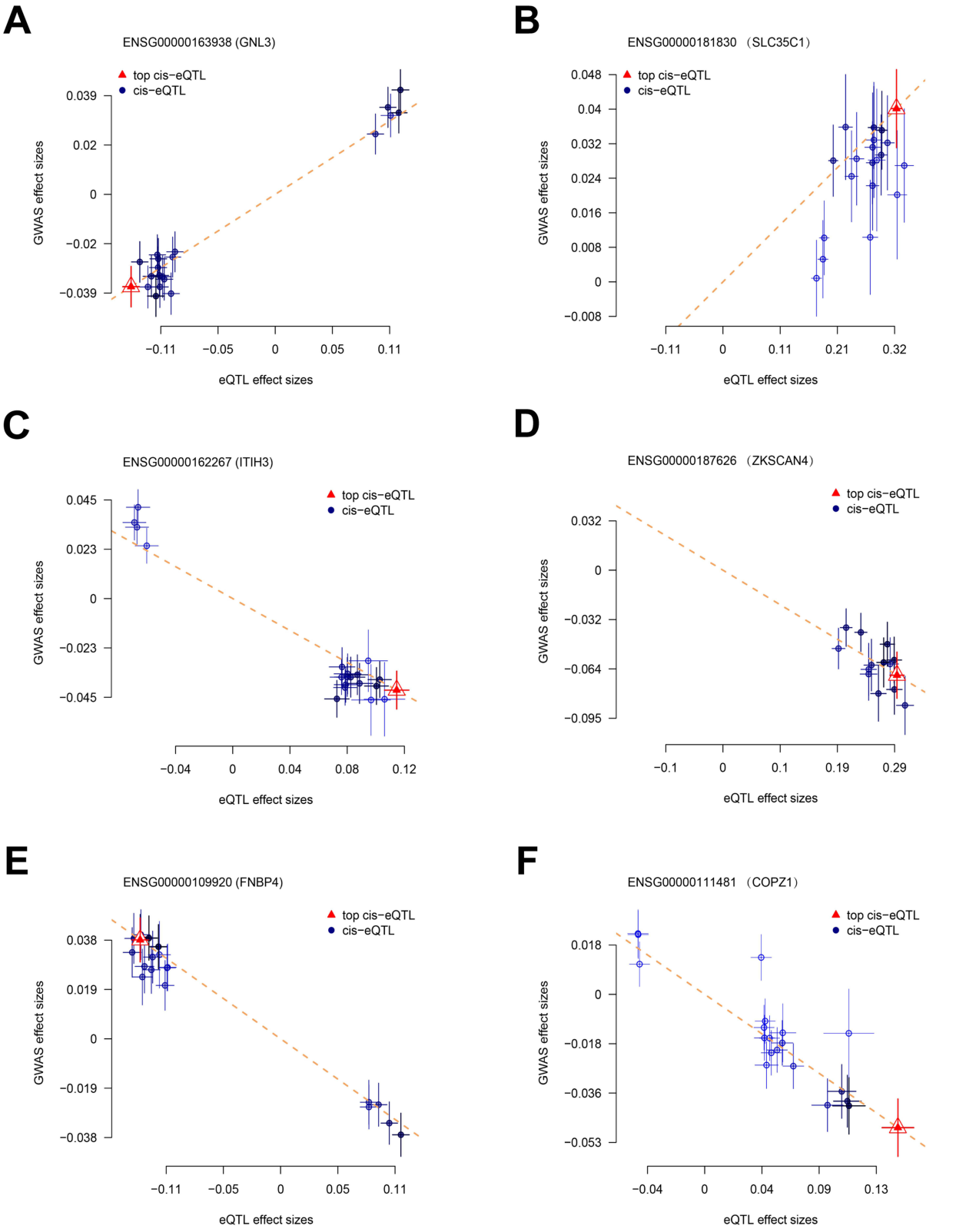

Figure 4 The scatterplots illustrate the relationship between individual genes involved in eQTL in blood and anxiety disorders. Specifically, the plots for genes (**A**) GNL3, (**B**) SLC35C1, (**C**) ITIH3, (**D**) ZKSCAN4, (**E**) FNBP4, and (**F**) COPZ1 are presented. The horizontal axis represents the effect sizes of single nucleotide polymorphisms (SNPs) within the eQTL genes, while the vertical axis displays the effect sizes of SNPs associated with anxiety disorders. Each blue circle with cross symbol denotes a SNP, and the red triangles signify the most significant SNPs. Yellow dashed lines illustrate the regression line, which indicates the directionality of the association between the effect sizes of eQTL and those of GWAS. Ascending segments from left to right reveal a positive correlation, suggesting that increased gene expression is associated with a heightened risk of anxiety disorders. In contrast, descending segments indicate a negative correlation, where elevated gene expression correlates with a diminished risk of anxiety disorders.

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with a reduced risk of this mental health issue. To illustrate the findings of our SMR analysis, locus plots were generated for the aforementioned six novel discoveries: ITIH3 (Figure S1A), ZKSCAN4 (Figure S1B), GNL3 (Figure S1C), SLC35C1 (Figure S1D), FNBP4 (Figure S1E), and COPZ1 (Figure S1F). These six figures emphasize the importance of the SMR and eQTL P-values in highlighting the relevance of these genes to anxiety disorder specifically. Furthermore, data from additional six genes, including ATP synthase membrane subunit c locus 1 (ATP5MC1), inter-alpha-trypsin inhibitor heavy chain 4 (ITIH4), euchromatic histone lysine methyltransferase 1 (EHMT1), protein tyrosine phosphatase mitochondrial 1 (PTPMT1), ARRDC1, and NIMA related kinase 4 (NEK4), were specifically isolated and illustrated on scatter plots and locus plots to showcase the associations between these genes and the disorders (Figures S2 and S3).

In order to achieve a more thorough and direct insight into the influence of gene expression regulation on disease outcomes, we utilized cis-eQTL data derived from databases encompassing 13 distinct brain regions for multi-tier validation (Tables S1–S14). Through the application of SMR analysis and the HEIDI test within the GTEx v8 database, we identified significant correlations between specific gene loci, namely ARRDC1 and BTN3A2, and the risk of anxiety in particular brain tissues. Specifically, the expression of the ARRDC1 gene in two distinct regions of the brain, namely the cerebellar hemisphere and the cerebellum, exhibited a significant positive correlation with the risk of anxiety (Figure 5A and B). Likewise, the expression of the BTN3A2 gene in the cervical spinal cord also demonstrated a positive association with anxiety risk (Figure 5C). Moreover, ARRDC1 and BTN3A2 genes were meticulously extracted and represented through scatter plots and locus plots to demonstrate the relationships between these genes and the disorders (Figure S4).

GO and KEGG Enrichment Analysis

Next, we performed the GO enrichment analysis for 13 genes to elucidate the interactions between these genes and their associated terms, and the KEGG enrichment analysis to demonstrate the connections between the genes and their corresponding functional pathways. Through GO enrichment analysis, a total of 54 BPs, 19 CCs, and 27 MFs were identified. The top five pathways in the BP category were all associated with hyaluronan metabolic process, negative regulation of Notch signaling pathway, mucopolysaccharide metabolic process, regulation of Notch signaling pathway, and glycosaminoglycan metabolic process (Figure 6A). The top five CCs include platelet dense granule lumen, platelet dense granule, mitochondrial proton-transporting ATP synthase complex, coupling factor F(o), COPI vesicle coat, and ciliary rootlet (Figure 6B). The top five MFs comprise serine-type endopeptidase inhibitor activity, endopeptidase inhibitor activity, peptidase inhibitor activity, histone methyltransferase activity (H3-K9 specific), and endopeptidase regulator activity (Figure 6C). Moreover, KEGG enrichment analysis mainly identified ten pathways, including lysine degradation, longevity regulating pathway, oxidative phosphorylation, ribosome biogenesis in eukaryotes, diabetic cardiomyopathy, chemical carcinogenesis - reactive oxygen species, thermogenesis, Parkinson disease, prion disease, and Huntington disease (Figure 6D). Comprehensive information regarding GO and KEGG enrichment results is available in Tables S15–S18.

The Anxiety-Associated ARRDC1 SNP Alters Promoter Activity in a Dual-Luciferase Reporter Assay

Among the multiple candidate genes identified by the SMR analysis, we selected ARRDC1 for further functional validation based on the following rationales. First, tissue-specific relevance: ARRDC1 was the only gene that showed significant, cross-regional associations specifically within brain tissues (cerebellar hemisphere and cerebellum), the primary organ of action for anxiety disorders, thereby enhancing its biological plausibility. Second, the association of ARRDC1 with anxiety was detected in both blood and brain eQTL datasets, suggesting its utility as a potential cross-tissue consistency and biomarker. Third, the cis-eQTL rs4494021 is located within the promoter region of ARRDC1, providing a direct and testable target for molecular functional validation. To determine whether this SNP directly affects transcriptional regulation, we performed a dual-luciferase reporter assay. We cloned the ARRDC1 promoter region containing either the mutant (C) or wild-type (T) allele of rs4494021 into a promoterless luciferase vector (Figure 7A). The construct carrying the mutant (C) allele drove significantly higher luciferase activity compared to the wild-type (T) allele construct (Figure 7B), showing approximately a 1.5-fold increase in

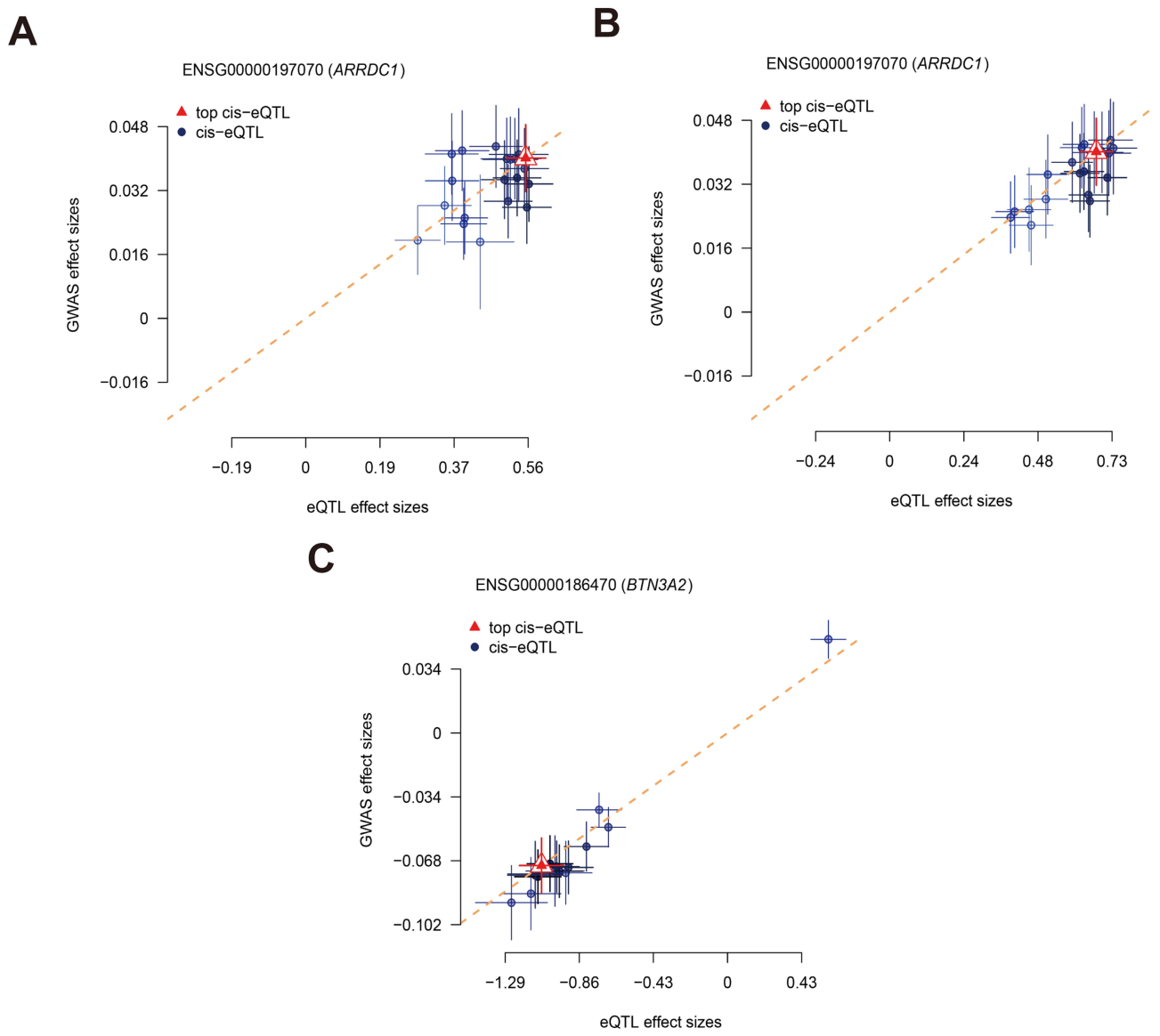

Figure 5 The scatterplot illustrates the correlation findings of specific genes identified in expression quantitative trait loci (eQTL) derived from various brain regions in relation to anxiety disorders. (**A**) ARRDC1 in cerebellar hemisphere; (**B**) ARRDC1 in cerebellum; (**C**) BTN3A2 in cervical spinal cord. The horizontal axis represents the effect sizes of single nucleotide polymorphisms (SNPs) within the eQTL genes, while the vertical axis displays the effect sizes of SNPs associated with anxiety disorders. Each blue circle with cross symbol denotes a SNP, and the red triangles signify the most significant SNPs. Yellow dashed lines illustrate the regression line, which indicates the directionality of the association between the effect sizes of eQTL and those of GWAS. Ascending segments from left to right reveal a positive correlation, suggesting that increased gene expression is associated with a heightened risk of anxiety disorders.

promoter activity. This result confirms that the anxiety-predisposing SNP rs4494021 can directly enhance ARRDC1 transcriptional activity in vitro.

ARRDC1 Overexpression Resulted in Anxiety-Like Behaviors in Mice

Building on the finding that the anxiety-associated SNP rs4494021 enhances ARRDC1 promoter activity in vitro, we next sought to determine whether increased ARRDC1 expression in a relevant brain region could causally influence anxiety-like behavior. Since our SMR analysis specifically implicated the cerebellum and cerebellar hemisphere, we targeted this region for functional validation in vivo, followed by a series of behavioral tests including the OFT, EPM, and LDB.

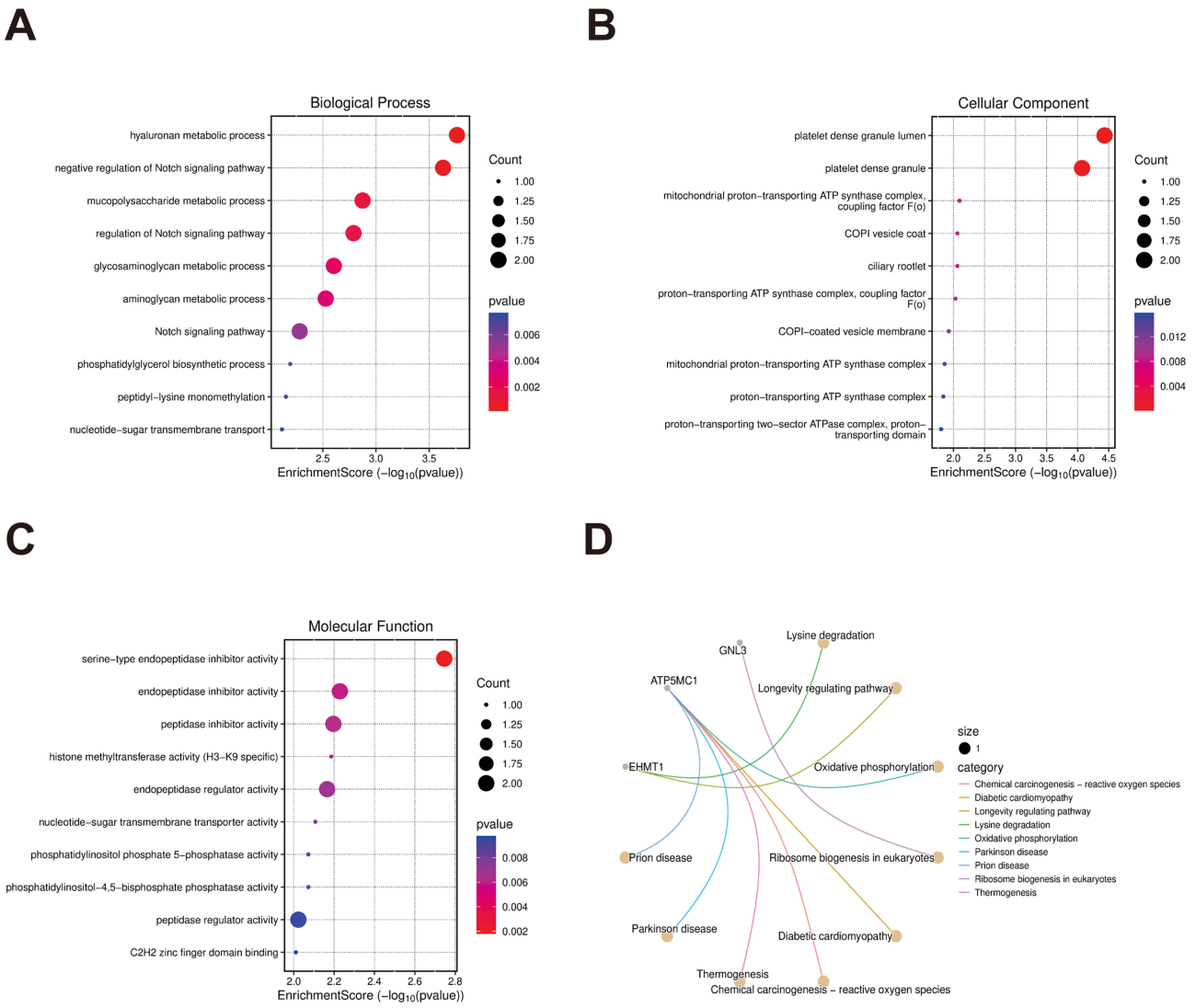

Figure 6 GO and KEGG enrichment results. (**A**) BPs; (**B**) CCs; (**C**) MFs; (**D**) KEGG enrichment results.

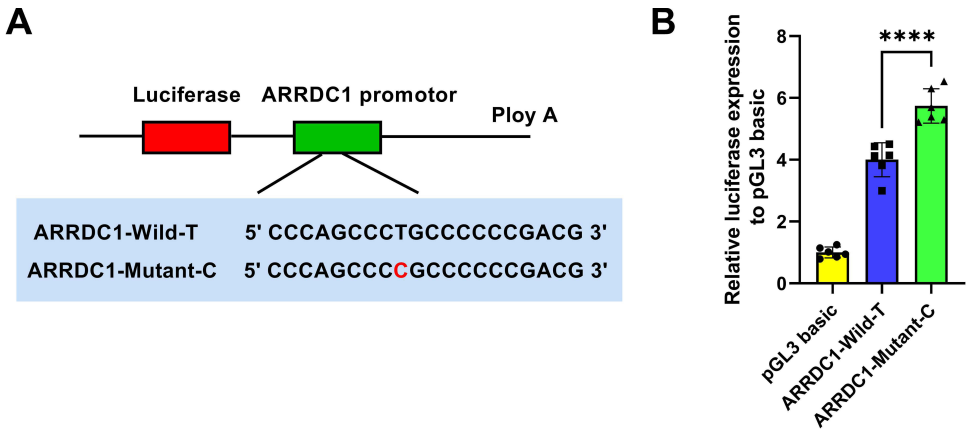

Figure 7 SNP rs4494021 regulates ARRDC1 promoter activity in a dual-luciferase reporter assay. (**A**) Schematic of the pGL3-based reporter constructs carrying the ARRDC1 promoter sequence with either the wild-type (T) or mutant (C). (**B**) Relative luciferase activity in HEK293 cells. The construct containing the C allele drove significantly higher transcriptional activity (~1.5-fold) compared to the T allele. The promoter-less pGL3-basic vector served as a negative control. n = 6 biological replicates per group. One-way ANOVA, $F(2, 15) = 160.8$, Dunnett's post-hoc test; $p < 0.0001$. Error bars represent SD; ****$p < 0.0001$.

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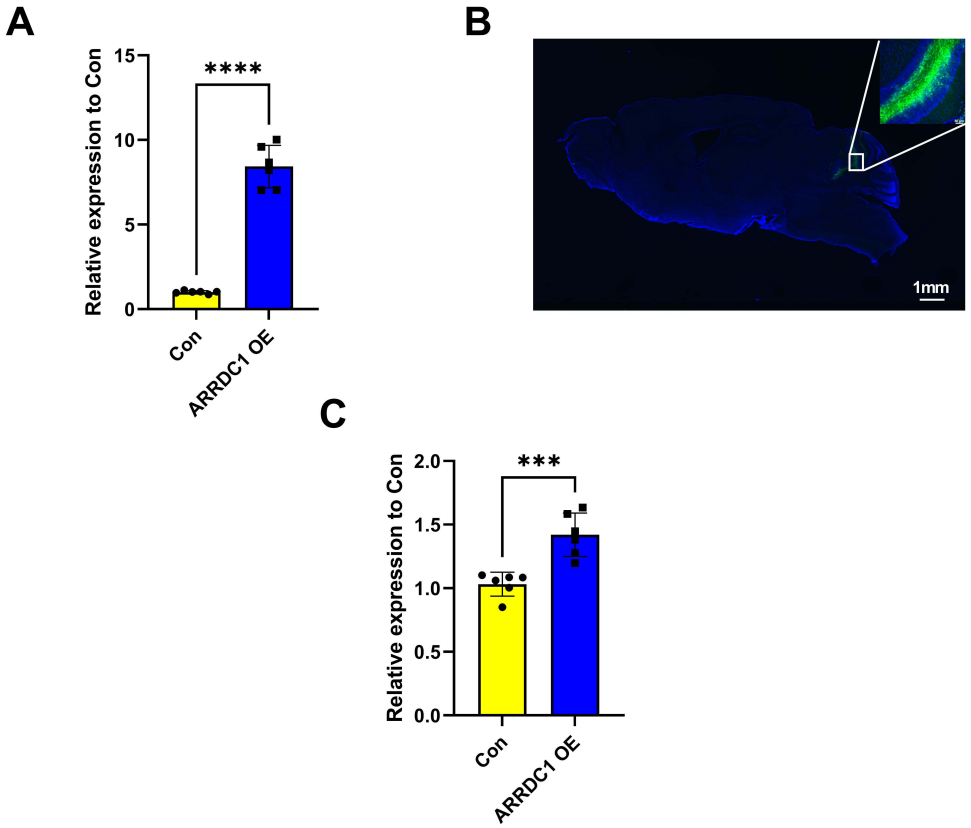

Figure 8 Validation of ARRDC1 overexpression in vitro and in vivo. (**A**) ARRDC1 mRNA levels were significantly elevated in N2A cells transduced with ARRDC1-OE lentivirus compared to control (Con) virus (n = 6; unpaired two-tailed t-test, p < 0.0001). (**B**) Representative fluorescence image showing lentiviral-mediated gene delivery into the cerebellar hemisphere. (**C**) qPCR analysis confirmed successful ARRDC1 overexpression in the cerebellar hemisphere of mice injected with ARRDC1-OE lentivirus (n = 6; unpaired two-tailed t-test, p = 0.0006). ***p < 0.001, ****p < 0.0001.

First, the overexpression efficiency of the previously designed lentivirus was validated in vitro (Figure 8A). Successful OE of ARRDC1 in the cerebellar hemispheres (Figure 8B) was confirmed by qPCR analyses. Mice injected with ARRDC1 OE lentivirus showed a significant increase in ARRDC1 mRNA compared to the control group injected with Con lentivirus (Figure 8C).

Subsequent behavioral analyses revealed a consistent anxiety-like phenotype in ARRDC1-OE mice. After Bonferroni correction for multiple comparisons, ARRDC1-OE mice showed significantly reduced distance in the center (unadjusted threshold p = 0.0042), and fewer entries into the center (unadjusted threshold p = 0.0024) in the OFT, as well as reduced time spent in open arms (unadjusted threshold p = 0.0045) in the EPM, and increased time spent in dark (unadjusted threshold p = 0.0052) in LDB compared to controls. Other parameters (total distance: unadjusted p = 0.0082; time spent in center: unadjusted p = 0.0327; entries into the open arms: unadjusted p = 0.0376; light–dark transitions: unadjusted p = 0.0086) showed consistent directional trends but did not reach statistical significance after correction (Figure 9). Together, these results indicate that elevated ARRDC1 expression, which is potentiated by the risk-associated SNP rs4494021, is sufficient to elicit anxiety-like behaviors in mice.

Discussion

This study aims to investigate the relationship between gene expression and anxiety disorder as the phenotype of interest, utilizing a two-sample SMR analysis methodology to integrate cis-eQTLs with summary statistics from GWAS data. We prioritized 13 genes (ITIH3, ZKSCAN4, GNL3, SLC35C1, FNBP4, COPZ1, ATP5MC1, ITIH4, EHMT1, PTPMT1, ARRDC1, NEK4, and BTN3A2) from blood and brain analyses, which are different from those reported in previous studies,[26–28] with twelve genes showing strong associations in blood and two (ARRDC1, BTN3A2) demonstrating consistent significance in specific brain regions. Follow-up experiments confirmed that an anxiety-linked SNP,

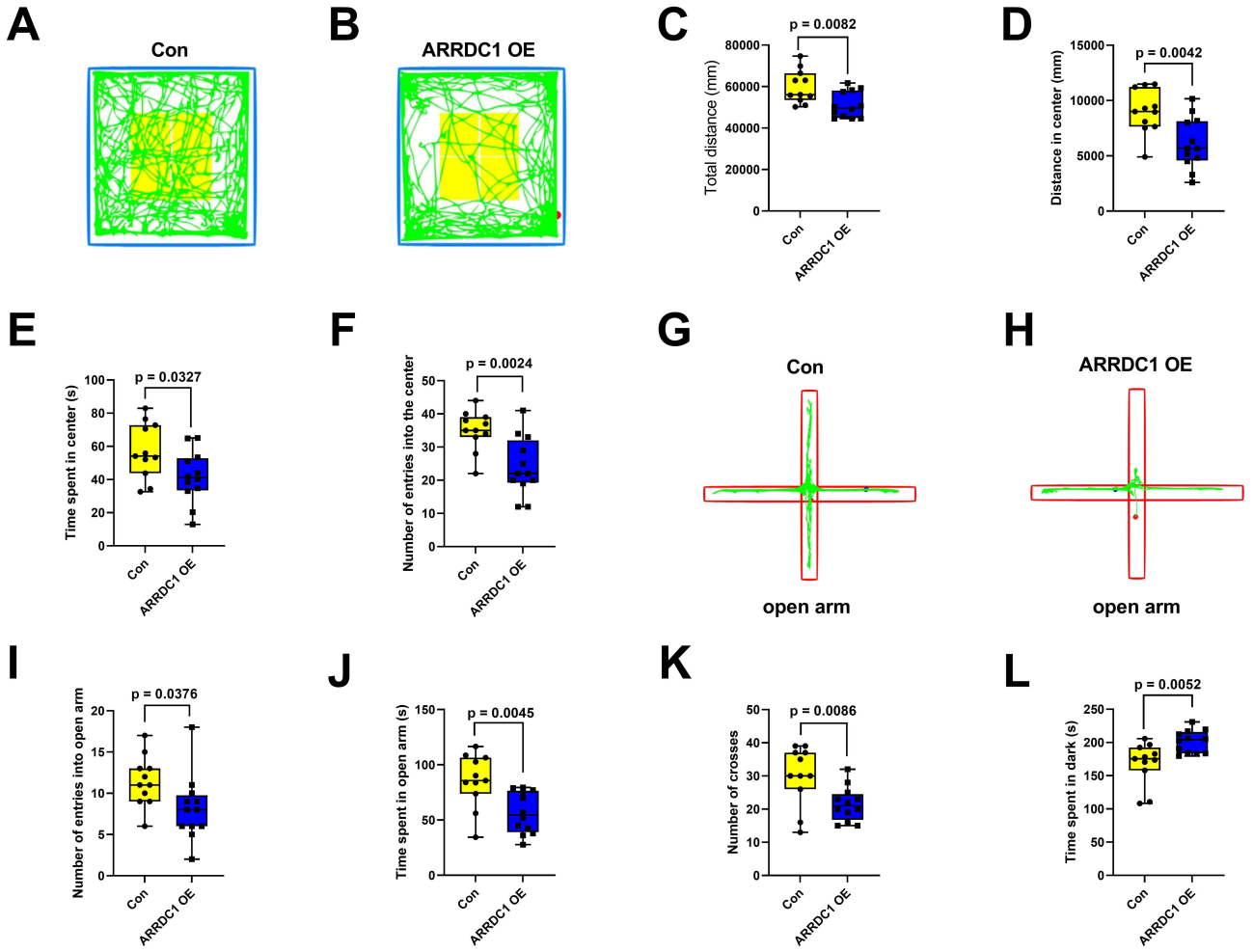

Figure 9 ARRDC1 overexpression in the cerebellar hemisphere induces anxiety-like behaviors in mice. (**A** and **B**) Representative movement traces of control (Con) and ARRDC1-OE mice in the OFT. (**C**–**F**) Quantification of OFT parameters: total distance traveled (**C**), distance in the center (**D**), time in center (**E**), and number of center entries (**F**). ARRDC1-OE mice showed significant reductions in all measures. (**G** and **H**) Representative traces in the EPM. (**I** and **J**) ARRDC1-OE mice spent less time in the open arms (**I**) and entered them less frequently (**J**) compared to controls. (**K** and **L**) In the LDB test, ARRDC1-OE mice made fewer light–dark transitions (**K**) and spent more time in the dark compartment (**L**). n = 11–12; unpaired two-tailed t-test; data are presented as mean ± SD; p value: unadjusted.

rs4494021, functionally regulates the promoter activity of ARRDC1. Furthermore, targeted overexpression of ARRDC1 in the mouse cerebellar hemisphere was sufficient to induce a pronounced anxiety-like behavioral phenotype. Collectively, these results contribute novel genetic insights into anxiety disorders and highlight specific molecular candidates for future mechanistic and therapeutic studies.

A pivotal aspect of our study was the extension of SMR analysis to brain-specific expression data. The identification of ARRDC1 as a significant gene in the cerebellar hemisphere and cerebellum is of particular interest. Beyond its classical motor functions, the cerebellum is increasingly recognized for its role in cognitive and affective regulation.[29,30] Our experimental finding that ARRDC1 overexpression within the cerebellar hemisphere recapitulates anxiety-like behaviors provides direct functional support for the genetic association and points to a previously underappreciated cerebellar component in anxiety neurocircuitry. Mechanistically, ARRDC1 has multiple important functions in cell biology, such as intercellular communication,[31,32] neurotoxicity protection,[33] and tumor development.[34] Although there is currently no direct evidence that ARRDC1 has a clear causal relationship with mental disorders, its role in intercellular communication may indirectly affect the pathogenesis of such diseases. It is worth mentioning that this study found that the expression of ARRDC1 was detected in both blood and brain, and it was positively correlated with the risk of anxiety disorders. The reproducibility of the ARRDC1 signal across tissues, combined with its functional SNP validation, enhances its potential as a biomarker. Therefore, ARRDC1 may serve as a novel non-invasive biomarker for anxiety disorders, although this needs to be verified through large-scale clinical samples.

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Alongside ARRDC1, our SMR analysis concurrently nominated several other candidate genes from peripheral blood, collectively delineating broader systemic mechanisms implicated in anxiety susceptibility. These genes coalesce around three intersecting biological axes: neuroimmune modulation, neurodevelopmental regulation, and fundamental cellular homeostasis. The inflammatory component is highlighted by ITIH3, which regulates extracellular matrix stability and neuroinflammation, potentially influencing anxiety-relevant brain regions like the prefrontal cortex and amygdala,[27,35,36] and by FNBP4, a regulator of cytoskeletal dynamics with links to immune cell function and neuroinflammatory pathways.[37] Neurodevelopmental processes are implicated through ZKSCAN4, a transcription factor crucial for chromatin remodeling and genomic stability during neural development,[38,39] and GNL3, a nucleolar protein governing ribosome biogenesis, neuronal differentiation, and synaptic plasticity.[40,41] Finally, core cellular metabolic and homeostatic functions are represented by SLC35C1, a Golgi transporter essential for protein glycosylation whose deficiency leads to neurodevelopmental disorders featuring anxiety,[42,43] and COPZ1, a regulator of vesicular trafficking and iron metabolism linked to ferroptosis and neuronal vulnerability.[44–47] While these six genes, primarily identified from blood-based analyses, underscore the heterogeneous and systemic pathophysiological landscape of anxiety involving immune, developmental, and metabolic disturbances, the present study's functional focus on ARRDC1 distinguishes it by providing direct, tissue-specific causal evidence within a defined brain circuit, thereby offering a precise mechanistic anchor within this complex polygenic framework.

The identification of rs4494021 as a functional regulatory variant in the ARRDC1 promoter has potential translational implications. The integration of genetic markers into clinical practice could facilitate personalized risk assessment and early screening for anxiety disorders. For instance, such markers could be incorporated into Artificial Intelligence-driven predictive models that integrate genetic, behavioral, and environmental data to identify at-risk individuals and guide treatment selection.[48] If validated in prospective cohorts, these genetic markers may contribute to polygenic risk scores that identify at-risk individuals before symptom onset, enabling early preventive strategies—a key public health approach to reducing the burden of anxiety disorders. Furthermore, the causal involvement of ARRDC1 in anxiety-like behaviors suggests that pathways regulating its expression, such as the promoter variant identified here, could serve as novel therapeutic targets. Future studies integrating genetic data with environmental and lifestyle factors may further refine personalized treatment strategies,[49] moving beyond symptom-based approaches toward more individualized interventions.

The present study employed GO and KEGG enrichment analyses to elucidate the functional interactions and pathways associated with the identified gene set. GO enrichment analysis showed significant associations in BPs, CCs, and MFs, revealing the genes' roles in cellular regulation and metabolism. In BPs, pathways linked to hyaluronan metabolism and Notch signaling suggest involvement in extracellular matrix remodeling and cell fate. Hyaluronan is crucial for cell proliferation, migration, and tissue repair. Key CCs like platelet dense granules and COPI vesicle coat indicate roles in intracellular trafficking and organelle function, with implications for hemostasis and thrombosis. In MFs, serine-type endopeptidase inhibitor activity suggests regulation of proteolytic processes, vital for protein homeostasis. Histone methyltransferase activity points to roles in chromatin remodeling and gene expression regulation, highlighting the genes' multifunctionality. Notably, previous studies have revealed that GO enrichment analysis of anxiety disorder consistently highlights terms such as neurotransmitter receptor activity (eg, serotonin, dopamine, and norepinephrine receptors), G protein-coupled neurotransmitter receptor activity, and synaptic signaling as central to anxiety pathophysiology.[50–52] These findings align with clinical observations of dysregulated neurotransmitter levels (eg, reduced serotonin and elevated corticosterone) in anxiety models.[51,53] Our findings and previous results suggest systemic and structural contributions to anxiety.

The KEGG enrichment analysis in the present study identified ten major pathways, including lysine degradation, longevity regulation, oxidative phosphorylation, and pathways associated with neurodegenerative diseases such as Parkinson's disease, prion disease, and Huntington's disease. The involvement of these genes in lysine degradation and oxidative phosphorylation highlights their potential role in energy metabolism and cellular respiration. The association with longevity regulation suggests that these genes may influence cellular aging and lifespan. The enrichment of pathways linked to neurodegenerative diseases indicates that these genes may contribute to the pathogenesis of these conditions, possibly through mechanisms involving oxidative stress, protein aggregation, and mitochondrial dysfunction.

In addition to our findings, previous studies have highlighted significant enrichments in neuroactive ligand-receptor interactions, serotonergic synapse, and PI3K-AKT/mTOR signaling, which are critical for synaptic plasticity and neuronal survival.[51,52,54] Pathways such as MAPK signaling, calcium signaling, and cAMP-mediated cascades further underscore the role of intracellular signaling in anxiety modulation.[52,54] Notably, inflammatory pathways like IL-17 and TNF signaling are recurrently linked to anxiety, particularly in comorbid conditions such as glioblastoma or rheumatoid arthritis, where neuroinflammation exacerbates anxiety symptoms.[55–57] Current and previous studies collectively reveal that key pathways involving energy metabolism, cellular aging, and neurodegenerative processes converge with neuroinflammatory signaling, synaptic plasticity mechanisms, and stress-related cascades to drive anxiety disorders through shared mechanisms like oxidative stress, mitochondrial dysfunction, and protein aggregation.

Limitations

Admittedly, while our SMR analyses integrating GWAS and eQTL data have advanced the discovery of novel susceptibility genes for anxiety disorders, several methodological and conceptual limitations warrant critical consideration. First, the validity of SMR relies on key assumptions about IVs, and horizontal pleiotropy, where genetic variants influence anxiety through pathways independent of gene expression remains a potential confounder. Violations of these assumptions are common in anxiety research. For instance, horizontal pleiotropy, where IVs affect outcomes via pathways independent of the exposure, is poorly addressed in many studies. Anxiety-associated eQTLs often exhibit pleiotropic effects on neurodevelopmental and immune pathways, complicating causal interpretation. Second, tissue and context specificity of eQTLs pose significant challenges. Our included eQTL datasets derive from peripheral blood and brain regions, while anxiety-related gene expression is tightly regulated in brain-specific cell types (eg, cortical glutamatergic neurons, microglia). Furthermore, the sample size for the animal experiments was determined based on common practice in the field rather than a formal a priori power analysis. Future studies should incorporate power calculations to further validate these findings. Also, the study used only male mice, which limits the generalizability of our findings to females, given the known sex differences in anxiety disorders. Additionally, dynamic context-dependent regulation (eg, stress-induced epigenetic changes) is ignored in static eQTL datasets, further limiting translational relevance. Third, statistical power and heterogeneity across GWAS and eQTL cohorts reduce robustness. Weak instrument bias arises when cis-eQTLs explain < 1% of expression variance, leading to Type II errors. Conversely, sample overlap between GWAS and eQTL datasets may inflate Type I errors due to cryptic relatedness. Moreover, this study included biological interpretation gaps. SMR identifies statistical associations but rarely validates mechanisms experimentally. Multi-omics integration (eg, splicing QTLs, proteomics) and cross-species validation are essential to bridge this gap. Finally, although we performed functional validation for ARRDC1, the precise molecular and circuit-level mechanisms through which ARRDC1 and other candidate genes influence anxiety require further elucidation. Future studies should prioritize multi-omics integration (eg, splicing QTLs, proteomics), employ larger and more diverse cohorts, and utilize cell-type-specific and perturbation-based models in relevant neural circuits to establish definitive causal mechanisms.

Conclusion

Taken together, our findings establish ARRDC1 as a new and functionally validated risk gene for anxiety disorders. Through parallel single-tissue SMR analysis, we identified the promoter SNP rs4494021 as a genetic marker associated with increased ARRDC1 expression and disease risk. Critically, elevating ARRDC1 levels in the cerebellar hemisphere of mice was sufficient to drive a suite of anxiety-like behaviors, providing causal evidence that ARRDC1 overexpression contributes to anxiety pathogenesis. It thereby uncovers ARRDC1 as a point of convergence in the neurobiology of anxiety for future mechanistic and translational studies. While the integrated evidence strongly supports ARRDC1's role, future studies in diverse cohorts and with cell-type-specific manipulations will be needed to fully establish its pathogenic mechanism and therapeutic potential.

Abbreviations

GWAS, genome-wide association studies; SMR, summary data-based Mendelian randomization; eQTL, expression quantitative trait loci; GTEx, genotype-tissue expression project; cis-eQTLs, cis-expression quantitative trait loci;

FDR, false discovery rate; IVs, instrumental variables; SNP, single nucleotide polymorphism; LD, linkage disequilibrium; HEIDI, Heterogeneity in dependent instruments; GO, Gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; BP, biological process; MF, molecular function; CC, cellular component; ARRDC1, arrestin domain containing 1; BTN3A2, butyrophilin subfamily 3 member A2; ITIH3, inter-alpha-trypsin inhibitor heavy chain 3; ZKSCAN4, zinc finger with KRAB and SCAN domains 4; GNL3, G protein nucleolar 3; SLC35C1, solute carrier family 35 member C1; FNBP4, formin binding protein 4; COPZ1, COPI coat complex subunit zeta 1; ATP5MC1, ATP synthase membrane subunit c locus 1; ITIH4, inter-alpha-trypsin inhibitor heavy chain 4; EHMT1, euchromatic histone lysine methyltransferase 1; PTPMT1, protein tyrosine phosphatase mitochondrial 1; NEK4, NIMA related kinase 4; Chr, chromosome; OE, overexpression; RT, reverse transcription; qPCR, quantitative real-time PCR; OFT, open-field test; EPM, elevated plus maze; LDB, light-dark box.

Data Sharing Statement

The datasets supporting the behavioral and qPCR results of this study are available from the corresponding author (Xiang Li) upon reasonable request.

The original data and summary statistics for anxiety disorders can be available at https://finngen.gitbook.io/documentation/data-download.

Ethical Statement

We utilized summary-level information derived from publicly accessible GWAS that have obtained ethical clearance from their respective institutional review boards, as well as informed consent from all individuals involved in the studies. The present study involved no direct contact with human subjects, no collection of individual-level data, and no access to personally identifiable information. According to Article 32, items 1 and 2 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (National Health Commission of China, February 18, 2023), research using publicly available, anonymized data without intervention or interaction with human subjects is exempt from institutional ethical review. Therefore, formal approval from our Institutional Review Board was not required. Moreover, all animal procedures were performed in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (2020 edition) and were approved by the Experimental Animal Welfare Ethics Committee, Zhongnan Hospital of Wuhan University (Approval code ZN2023018 and March 6th, 2023).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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