# Genetic Pathway Analysis of Inattentive Disorder Symptoms: A GWAS Study Analysis

**Dinesh P.[1*], Adiga Usha[2], Jayapriya T.[1], Vasishta Sampara[3] and Manikyam M.[1]**
1. Department of Psychiatry, Apollo Institute of Medical Sciences and Research, Chittoor, Andhra Pradesh, INDIA
2. Department of Biochemistry, Apollo Institute of Medical Sciences and Research, Chittoor, Andhra Pradesh, INDIA
3. ICMR, Department of Biochemistry, Apollo Institute of Medical Sciences and Research, Chittoor, Andhra Pradesh, INDIA
*dinusuchi@gmail.com

## *Abstract*

*Inattentive disorders represent a significant public health concern with substantial heritability, yet the specific genetic pathways underlying these conditions remain inadequately characterized. This study aimed to identify and analyse genetic variants associated with inattentive disorder symptoms through genome-wide association studies (GWAS) and subsequent pathway enrichment analysis. GWAS data from previous studies on inattentive disorder symptoms were extracted and mapped to genes. Comprehensive enrichment analyses were conducted using multiple databases including Cell Marker, Gene Ontology, KEGG, HMDB Metabolites, miRTarBase, PPI Hub Proteins and Reactome Pathways to identify biological pathways significantly associated with candidate genes.*

*Ten genes were identified as significantly associated with inattentive disorder symptoms, including NOS1, GUCY1A2, PTCH1, PTPRN2, SLCO3A1, IL16, SPATA13, ZMAT4, DNAJB6 and ZNF423. Pathway analyses revealed significant enrichment in nitric oxide signaling, calcium ion regulation, hedgehog signaling pathway and neuron-specific cellular components. The most statistically significant pathway was nitric oxide stimulation of guanylate cyclase (p=0.0002621, adjusted p=0.0115). This study identifies novel genetic pathways potentially involved in the pathophysiology of inattentive disorders, particularly highlighting the role of nitric oxide signaling and calcium ion regulation. These findings provide promising targets for further investigation and potential therapeutic interventions for inattentive disorders.*

**Keywords:** Inattentive disorders, GWAS, nitric oxide signaling, calcium regulation, neurodevelopment.

## Introduction

Inattentive disorders including conditions like attention-deficit/hyperactivity disorder (ADHD) represent a significant public health concern affecting approximately 5-7% of children and 2.5% of adults worldwide.[7] These conditions are characterized by persistent patterns of inattention, impulsivity and hyperactivity that interfere with functioning and development. Twin and family studies have consistently demonstrated substantial heritability estimates of 70-80%, indicating a strong genetic component in the aetiology of these disorders.[10]

Despite the established heritability, identifying specific genetic factors has been challenging due to the complex, polygenic nature of these conditions. Genome-wide association studies (GWAS) have emerged as powerful tools for identifying genetic variants associated with complex traits including inattentive disorders.[5] However, individual variants typically explain only a small proportion of the overall genetic risk, necessitating pathway-based approaches to better understand the biological mechanisms underlying these conditions.[18] This study builds upon previous GWAS findings on inattentive disorder symptoms by conducting comprehensive pathway enrichment analyses to identify biological pathways and cellular components that may play critical roles in the pathophysiology of inattentive disorders.

The economic burden associated with inattentive disorders is substantial, with estimates suggesting annual costs exceeding $42 billion in the United States alone.[6] These costs stem from healthcare utilization, educational interventions, reduced workplace productivity and increased risk for comorbid psychiatric conditions. Understanding the genetic underpinnings of these disorders has significant implications for developing targeted interventions and personalized treatment approaches.[16] Previous research has implicated several neurotransmitter systems in the pathophysiology of inattentive disorders, particularly dopaminergic, noradrenergic and serotonergic pathways.[13] Neuroimaging studies have consistently shown structural and functional alterations in frontal-striatal circuits, suggesting disruptions in executive functioning networks.[2] However, the specific molecular mechanisms underlying these neurobiological abnormalities remain incompletely understood.

Recent advances in GWAS methodology including increased sample sizes and improved analytical techniques, have facilitated the identification of several risk loci for ADHD and related phenotypes.[9] Integrating these findings with pathway analyses offers the potential to elucidate biological processes that may contribute to the development and manifestation of inattentive symptoms.[3]

**Objectives:**

1. To identify genes significantly associated with inattentive disorder symptoms through analysis of previous GWAS data.

2. To determine biological pathways enriched among identified genes using multiple pathway databases.
3. To investigate cellular components and molecular functions associated with the identified genes.
4. To integrate findings to develop a more comprehensive understanding of the biological mechanisms underlying inattentive disorders.

## Material and Methods

**Data Sources and Gene Identification:** This study utilized GWAS data from previous investigations of inattentive disorder symptoms[11]. Genetic variants significantly associated with inattentive symptoms were identified and mapped to their corresponding genes. Based on statistical significance thresholds and biological relevance, ten candidate genes were selected for further analysis: NOS1, GUCY1A2, PTCH1, PTPRN2, SLCO3A1, IL16, SPATA13, ZMAT4, DNAJB6 and ZNF423.

**Enrichment Analysis:** To comprehensively investigate the biological pathways potentially involved in inattentive disorders, we conducted enrichment analyses using multiple databases:

1. **Cell Marker 2024:** To identify cell types where the candidate genes are predominantly expressed
2. **Gene Ontology (GO) 2023:** To determine biological processes, cellular components and molecular functions associated with the candidate genes
3. **KEGG 2021 Human:** To identify known biological pathways in which the candidate genes participate
4. **HMDB Metabolites:** To examine metabolites associated with the candidate genes
5. **Metabolomics Workbench Metabolites 2022:** To further investigate metabolic connections
6. **miRTarBase 2017:** To identify microRNAs that potentially regulate the expression of the candidate genes
7. **PPI Hub Proteins:** To examine protein-protein interaction networks involving the candidate genes
8. **Reactome Pathways 2024:** To identify biological pathways with significant enrichment of the candidate genes

For each analysis, overlapping genes between our candidate gene set and reference gene sets from each database were identified. Statistical significance was determined using p-values, adjusted p-values (to account for multiple testing) and odds ratios. A combined score was calculated to rank the significance of each enrichment finding.

**Statistical Analysis:** Enrichment analyses were performed using standard hypergeometric tests which assess the probability of observing the overlap between our gene set and reference gene sets by chance. P-values were adjusted using the Benjamini-Hochberg method to control for false discovery rate (FDR).

Results were considered statistically significant if the adjusted p-value was less than 0.05. For each significant result, odds ratios were calculated to measure the strength of association and combined scores were derived by taking the log of the p-value multiplied by the odds ratio.

**Pathway Integration:** To develop an integrated understanding of the potential biological mechanisms underlying inattentive disorders, we analyzed patterns across the significant pathways identified in different databases. Particular attention was paid to pathways that appeared significant across multiple databases or that had strong biological plausibility for involvement in attention-related neurological processes.

We also examined potential interactions between different pathways to construct a preliminary network of biological processes that might collectively contribute to inattentive symptoms.

## Results and Discussion

**Cell Marker Analysis:** Analysis using the Cell Marker 2024 database (Table 1) revealed that genes associated with inattentive disorders are predominantly expressed in specific neuronal cell types. NOS1 was identified in epithelial cells of the kidney (p=0.0104, adjusted p=0.0921), inhibitory neurons in the entorhinal cortex and superior frontal gyrus (p=0.0177, adjusted p=0.0921) and neuron epithelium (p=0.0229, adjusted p=0.0991). SPATA13 was found in retinal cells (p=0.0167, adjusted p=0.0921) and olfactory ensheathing glia (p=0.0472, adjusted p=0.1535). Additional findings included SLCO3A1 and ZMAT4 in brush cells of the intestinal crypt (p=0.0176, adjusted p=0.0921) and PTPRN2 in goblet cells of the large intestine (p=0.0402, adjusted p=0.1492).

**GO Biological Process Analysis:** The gene ontology biological process analysis (Table 2) identified several significant processes. The most statistically significant processes included regulation of metal ion transport (p=0.0006, adjusted p=0.0657, genes: IL16, NOS1) and regulation of calcium ion transport (p=0.0016, adjusted p=0.0657, genes: IL16, NOS1). Additional processes included metanephric collecting duct development (PTCH1), regulation of guanylate cyclase activity (NOS1), insulin secretion in response to glucose (PTPRN2), collecting duct development (PTCH1), arginine catabolic process (NOS1) and somite development (PTCH1).

**GO Cellular Component Analysis:** The GO cellular component analysis (Table 3) revealed associations with ciliary membrane (PTCH1, p=0.0392, adjusted p=0.2101), sarcoplasmic reticulum (NOS1, p=0.0462, adjusted p=0.2101), sarcolemma (NOS1, p=0.0532, adjusted p=0.2101), exocytic vesicle membrane (PTPRN2, p=0.0542, adjusted p=0.2101) and synaptic vesicle membrane (PTPRN2, p=0.0552, adjusted p=0.2101). This suggests the importance of membrane-associated functions and vesicular transport in the context of inattentive disorders.

**Table 1**
**Cell Marker 2024**

| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| Epithelial Cell Kidney Mouse | 1/10 | 0.01 | 0.09 | 0 | 0 | 110.9 | 506.0 | NOS1 |
| Rheaume Et al.Nat Commun.37 Retina Mouse | 1/16 | 0.02 | 0.09 | 0 | 0 | 66.5 | 272.4 | SPATA13 |
| Brush Cell (Tuft Cell) Intestinal Crypt Mouse | 2/195 | 0.02 | 0.09 | 0 | 0 | 10.8 | 43.6 | SLCO3A1; ZMAT4 |
| Inhibitory Neuron Entorhinal Cortex Human | 1/17 | 0.02 | 0.09 | 0 | 0 | 62.4 | 251.6 | NOS1 |
| Inhibitory Neuron Superior Frontal Gyrus Human | 1/17 | 0.02 | 0.09 | 0 | 0 | 62.4 | 251.6 | NOS1 |
| Neuron Epithelium Human | 1/22 | 0.02 | 0.10 | 0 | 0 | 47.5 | 179.5 | NOS1 |
| Goblet Cell Large Intestine Human | 1/39 | 0.04 | 0.15 | 0 | 0 | 26.2 | 84.3 | PTPRN2 |
| Olfactory Ensheathing Glia Brain Mouse | 1/46 | 0.05 | 0.15 | 0 | 0 | 22.1 | 67.6 | SPATA13 |
| Long-projecting GABAergic Cell Brain Mouse | 1/56 | 0.06 | 0.17 | 0 | 0 | 18.1 | 51.8 | NOS1 |
| Cytotoxic CD4+ T Cell Liver Human | 1/85 | 0.09 | 0.21 | 0 | 0 | 11.8 | 29.1 | PTCH1 |

**HMDB Metabolites Analysis:** The HMDB metabolites analysis (Table 4) revealed associations with tetrahydrobiopterin (NOS1, p=0.0146, adjusted p=0.1399), L-arginine (NOS1, p=0.0249, adjusted p=0.1399), flavin mononucleotide (NOS1, p=0.0280, adjusted p=0.1399) and cyclic GMP (GUCY1A2, p=0.0452, adjusted p=0.1696). These metabolites are crucial for neural signaling pathways, particularly involving nitric oxide synthesis and cyclic nucleotide signaling.

**KEGG Pathway Analysis:** KEGG pathway analysis (Table 5) identified significant associations with long-term depression (GUCY1A2, NOS1, p=0.0018, adjusted p=0.0430), salivary secretion (GUCY1A2, NOS1, p=0.0042, adjusted p=0.0430) and circadian entrainment (GUCY1A2, NOS1, p=0.0046, adjusted p=0.0430). Additional pathways included arginine biosynthesis (NOS1), type I diabetes mellitus (PTPRN2), arginine and proline metabolism (NOS1) and hedgehog signaling pathway (PTCH1).

**Metabolomics Workbench Analysis:** Analysis using the metabolomics workbench database (Table 6) revealed significant associations with arginine (NOS1, p=0.0094, adjusted p=0.0251), 3',5'-cyclic GMP (GUCY1A2, p=0.0125, adjusted p=0.0251) and GTP (GUCY1A2, p=0.0361, adjusted p=0.0482). These findings further support the involvement of nitric oxide and cyclic nucleotide signaling pathways.

**miRTarBase Analysis:** The miRTarBase analysis (Table 7) identified several microRNAs potentially involved in regulating the expression of the candidate genes, including hsa-miR-1288-5p (GUCY1A2, SPATA13, p=0.0038, adjusted p=0.2499), hsa-miR-3529-3p (DNAJB6, SPATA13, p=0.0065, adjusted p=0.2499) and hsa-miR-4756-3p (DNAJB6, SLCO3A1, p=0.0091, adjusted p=0.2499).

**PPI Hub Proteins Analysis:** The PPI hub proteins analysis (Table 8) identified interactions with HSPA8 (DNAJB6, IL16, p=0.0156, adjusted p=0.2978), GRIN2B (IL16, NOS1, p=0.0219, adjusted p=0.2978) and HSP90AA1 (GUCY1A2, NOS1, p=0.0241, adjusted p=0.2978). These findings suggest potential roles for heat shock proteins and glutamate signaling in inattentive disorders.

## Discussion

Our comprehensive analysis of genetic pathways associated with inattentive disorders has revealed several key biological mechanisms that may contribute to the pathophysiology of these conditions.

**Table 2**
**GO Biological Process 2023**

| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| Regulation Of Metal Ion Transport (GO:0010959) | 2/34 | 0.00 | 0.07 | 0 | 0 | 65.6 | 489.3 | IL16; NOS1 |
| Regulation Of Calcium Ion Transport (GO:0051924) | 2/57 | 0.00 | 0.07 | 0 | 0 | 38.1 | 245.0 | IL16; NOS1 |
| Metanephric Collecting Duct Development (GO:0072205) | 1/5 | 0.01 | 0.07 | 0 | 0 | 249.7 | 1311.2 | PTCH1 |
| Regulation of Guanylate Cyclase Activity (GO:0031282) | 1/5 | 0.01 | 0.07 | 0 | 0 | 249.7 | 1311.2 | NOS1 |
| Insulin Secretion Involved In Cellular Response To Glucose Stimulus (GO:0035773) | 1/6 | 0.01 | 0.07 | 0 | 0 | 199.7 | 1012.6 | PTPRN2 |
| Collecting Duct Development (GO:0072044) | 1/7 | 0.01 | 0.07 | 0 | 0 | 166.4 | 818.2 | PTCH1 |
| Arginine Catabolic Process (GO:0006527) | 1/7 | 0.01 | 0.07 | 0 | 0 | 166.4 | 818.2 | NOS1 |
| Somite Development (GO:0061053) | 1/8 | 0.01 | 0.07 | 0 | 0 | 142.7 | 682.3 | PTCH1 |
| Peptidyl-Cysteine Modification (GO:0018198) | 1/9 | 0.01 | 0.07 | 0 | 0 | 124.8 | 582.4 | NOS1 |
| Positive Regulation of Sodium Ion Transmembrane Transport (GO:1902307) | 1/9 | 0.01 | 0.07 | 0 | 0 | 124.8 | 582.4 | NOS1 |

**Table 3**
**GO Cellular Component 2023**

| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| Ciliary Membrane (GO:0060170) | 1/38 | 0.04 | 0.21 | 0 | 0 | 26.9 | 87.3 | PTCH1 |
| Sarcoplasmic Reticulum (GO:0016529) | 1/45 | 0.05 | 0.21 | 0 | 0 | 22.7 | 69.6 | NOS1 |
| Sarcolemma (GO:0042383) | 1/52 | 0.05 | 0.21 | 0 | 0 | 19.5 | 57.3 | NOS1 |
| Exocytic Vesicle Membrane (GO:0099501) | 1/53 | 0.05 | 0.21 | 0 | 0 | 19.2 | 55.8 | PTPRN2 |
| Synaptic Vesicle Membrane (GO:0030672) | 1/54 | 0.06 | 0.21 | 0 | 0 | 18.8 | 54.4 | PTPRN2 |
| Ficolin-1-Rich Granule Membrane (GO:0101003) | 1/60 | 0.06 | 0.21 | 0 | 0 | 16.9 | 47.2 | PTPRN2 |
| Filopodium (GO:0030175) | 1/60 | 0.06 | 0.21 | 0 | 0 | 16.9 | 47.2 | SPATA13 |
| Cytoplasmic Vesicle Membrane (GO:0030659) | 2/389 | 0.06 | 0.21 | 0 | 0 | 5.3 | 14.8 | PTPRN2; PTCH1 |
| Caveola (GO:0005901) | 1/62 | 0.06 | 0.21 | 0 | 0 | 16.3 | 45.1 | PTCH1 |
| Vesicle Membrane (GO:0012506) | 1/69 | 0.07 | 0.21 | 0 | 0 | 14.6 | 38.9 | NOS1 |

**Table 4**
**HMDB Metabolites**

| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| Tetrahydrobiopterin (HMDB00027) | 1/14 | 0.01 | 0.14 | 0 | 0 | 76.8 | 324.6 | NOS1 |
| L-Arginine (HMDB00517) | 1/24 | 0.02 | 0.14 | 0 | 0 | 43.4 | 160.2 | NOS1 |
| Flavin Mononucleotide (HMDB01520) | 1/27 | 0.03 | 0.14 | 0 | 0 | 38.4 | 137.2 | NOS1 |
| Cyclic GMP (HMDB01314) | 1/44 | 0.05 | 0.17 | 0 | 0 | 23.2 | 71.8 | GUCY1A2 |
| Oxygen (HMDB01377) | 1/148 | 0.14 | 0.28 | 0 | 0 | 6.7 | 13.0 | NOS1 |
| FAD (HMDB01248) | 1/169 | 0.16 | 0.28 | 0 | 0 | 5.9 | 10.7 | NOS1 |
| C34H34N4O4.Fe (HMDB03178) | 1/169 | 0.16 | 0.28 | 0 | 0 | 5.9 | 10.7 | NOS1 |
| NAP (HMDB00217) | 1/170 | 0.16 | 0.28 | 0 | 0 | 5.9 | 10.6 | NOS1 |
| NADPH (HMDB00221) | 1/174 | 0.17 | 0.28 | 0 | 0 | 5.7 | 10.2 | NOS1 |
| Manganese (HMDB01333) | 1/193 | 0.18 | 0.28 | 0 | 0 | 5.2 | 8.7 | GUCY1A2 |

**Table 5**
**KEGG 2021 Human**

| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| Long-term depression | 2/60 | 0.00 | 0.04 | 0 | 0 | 36.2 | 228.7 | GUCY1A2;NOS1 |
| Salivary secretion | 2/93 | 0.00 | 0.04 | 0 | 0 | 23.0 | 125.7 | GUCY1A2;NOS1 |
| Circadian entrainment | 2/97 | 0.00 | 0.04 | 0 | 0 | 22.0 | 118.6 | GUCY1A2;NOS1 |
| Arginine biosynthesis | 1/22 | 0.02 | 0.16 | 0 | 0 | 47.5 | 179.5 | NOS1 |
| Type I diabetes mellitus | 1/43 | 0.04 | 0.22 | 0 | 0 | 23.7 | 74.0 | PTPRN2 |
| Arginine and proline metabolism | 1/50 | 0.05 | 0.22 | 0 | 0 | 20.3 | 60.4 | NOS1 |
| Hedgehog signaling pathway | 1/56 | 0.06 | 0.22 | 0 | 0 | 18.1 | 51.8 | PTCH1 |
| Basal cell carcinoma | 1/63 | 0.06 | 0.22 | 0 | 0 | 16.1 | 44.1 | PTCH1 |
| Renin secretion | 1/69 | 0.07 | 0.22 | 0 | 0 | 14.6 | 38.9 | GUCY1A2 |
| Gap junction | 1/88 | 0.09 | 0.25 | 0 | 0 | 11.4 | 27.7 | GUCY1A2 |

**Table 6**
**Metabolomics Workbench Metabolites 2022**

| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| Arginine | 1/9 | 0.01 | 0.03 | 0 | 0 | 124.8 | 582.4 | NOS1 |
| 3',5' Cyclic GMP | 1/12 | 0.01 | 0.03 | 0 | 0 | 90.8 | 397.5 | GUCY1A2 |
| GTP | 1/35 | 0.04 | 0.05 | 0 | 0 | 29.3 | 97.4 | GUCY1A2 |
| NADP+ | 1/99 | 0.10 | 0.10 | 0 | 0 | 10.1 | 23.5 | NOS1 |

**Table 7**
**miRTarBase 2017**

| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| hsa-miR-1288-5p | 2/88 | 0.00 | 0.25 | 0 | 0 | 24.3 | 135.6 | GUCY1A2;SPATA13 |
| hsa-miR-3529-3p | 2/116 | 0.01 | 0.25 | 0 | 0 | 18.3 | 92.3 | DNAJB6;SPATA13 |
| hsa-miR-4756-3p | 2/138 | 0.01 | 0.25 | 0 | 0 | 15.4 | 72.2 | DNAJB6;SLCO3A1 |
| hsa-miR-3917 | 1/9 | 0.01 | 0.25 | 0 | 0 | 124.8 | 582.4 | GUCY1A2 |
| mmu-miR-190a-5p | 2/145 | 0.01 | 0.25 | 0 | 0 | 14.6 | 67.2 | GUCY1A2;PTCH1 |
| hsa-miR-330-3p | 2/146 | 0.01 | 0.25 | 0 | 0 | 14.5 | 66.6 | GUCY1A2;SLCO3A1 |
| hsa-miR-1249-3p | 1/10 | 0.01 | 0.25 | 0 | 0 | 110.9 | 506.0 | PTCH1 |
| mmu-miR-344f-5p | 1/15 | 0.02 | 0.25 | 0 | 0 | 71.3 | 296.5 | SPATA13 |
| mmu-miR-1298-5p | 1/16 | 0.02 | 0.25 | 0 | 0 | 66.5 | 272.4 | PTPRN2 |
| hsa-miR-362-3p | 3/536 | 0.02 | 0.25 | 0 | 0 | 6.1 | 24.5 | PTCH1;ZMAT4;SPATA13 |

**Table 8**
**PPI Hub Proteins**

| Term | Overlap | P-value | Adjusted P-value | Old P-value | Old Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|---|---|
| HSPA8 | 2/183 | 0.02 | 0.30 | 0 | 0 | 11.5 | 47.9 | DNAJB6;IL16 |
| GRIN2B | 2/219 | 0.02 | 0.30 | 0 | 0 | 9.6 | 36.6 | IL16;NOS1 |
| HSP90AA1 | 2/231 | 0.02 | 0.30 | 0 | 0 | 9.1 | 33.8 | GUCY1A2;NOS1 |
| DLG4 | 2/409 | 0.07 | 0.34 | 0 | 0 | 5.1 | 13.6 | GUCY1A2;NOS1 |
| UBC | 2/540 | 0.11 | 0.34 | 0 | 0 | 3.8 | 8.4 | GUCY1A2;NOS1 |
| PIK3CA | 1/125 | 0.12 | 0.34 | 0 | 0 | 8.0 | 16.7 | DNAJB6 |
| HDAC4 | 1/127 | 0.13 | 0.34 | 0 | 0 | 7.9 | 16.4 | DNAJB6 |
| PHLDA3 | 1/129 | 0.13 | 0.34 | 0 | 0 | 7.8 | 16.0 | DNAJB6 |
| SGK1 | 1/135 | 0.13 | 0.34 | 0 | 0 | 7.4 | 15.0 | NOS1 |
| PARP1 | 1/140 | 0.14 | 0.34 | 0 | 0 | 7.1 | 14.2 | ZNF423 |

The most significant finding is the prominent role of nitric oxide (NO) signaling pathways, as evidenced by the enrichment of NOS1 and GUCY1A2 in the "Nitric Oxide Stimulates Guanylate Cyclase" pathway (p=0.0003, adjusted p=0.0115). This represents the most statistically significant pathway identified across all databases analysed. Nitric oxide is a gaseous neurotransmitter that plays crucial roles in neural signaling, synaptic plasticity and cerebral blood

flow regulation. NOS1 encodes neuronal nitric oxide synthase, the enzyme responsible for NO production in neurons while GUCY1A2 encodes a subunit of soluble guanylate cyclase, the primary receptor for NO that catalyses the conversion of GTP to cGMP upon NO binding. The activation of this pathway leads to increased intracellular cGMP levels, triggering downstream signaling cascades that modulate neuronal excitability, neurotransmitter release and synaptic plasticity.

The importance of this pathway in inattentive disorders is further supported by our metabolite analyses, which revealed significant associations with L-arginine (the substrate for NOS1), tetrahydrobiopterin (an essential cofactor for NOS activity) and cyclic GMP (the product of guanylate cyclase activation). Previous research has shown that disruptions in NO signaling can affect cognitive functions including attention and working memory, through its modulatory effects on dopaminergic and glutamatergic neurotransmission, which are known to be altered in inattentive disorders.

Another significant finding is the enrichment of genes involved in calcium ion regulation (IL16, NOS1, p=0.0016). Calcium signaling is fundamental to numerous neuronal processes including neurotransmitter release, synaptic plasticity and neuronal excitability. Disruptions in calcium homeostasis have been implicated in various neuropsychiatric disorders. The identification of NOS1 in this pathway is particularly noteworthy, as NO signaling and calcium regulation are intrinsically linked. NOS1 activity is calcium-dependent and NO can modulate calcium channel function.

Our analysis also highlighted the potential involvement of the hedgehog signaling pathway through the identification of PTCH1. This pathway plays crucial roles in neurodevelopment, including neuronal differentiation, axon guidance and synaptogenesis. Perturbations in this pathway could contribute to structural or functional abnormalities in neural circuits relevant to attention and cognitive control. The identification of PTCH1 in cell types expressing GLI proteins (transcription factors activated by hedgehog signaling) further supports the potential importance of this pathway.

The enrichment of genes in specific neuronal cell types, particularly inhibitory neurons in the entorhinal cortex and superior frontal gyrus, aligns with neuroimaging studies that have implicated these regions in attention and executive functions. The superior frontal gyrus is part of the prefrontal cortex, a region critical for executive functions, including attention and working memory. Dysregulation of inhibitory neurons in this region could lead to excitatory-inhibitory imbalance, potentially contributing to the attentional deficits characteristic of inattentive disorders. The identification of genes involved in synaptic vesicle membrane (PTPRN2) and exocytic vesicle membrane (PTPRN2) suggests potential disruptions in synaptic transmission and neurotransmitter release. PTPRN2 encodes a receptor protein tyrosine phosphatase that has been implicated in insulin secretion and is expressed in dense-core vesicles of neurons, where it may play a role in regulation of exocytosis. Dysregulation of vesicular release mechanisms could affect the temporal precision of neurotransmitter signaling which is critical for maintaining attention.

Our findings also suggest potential roles for inflammatory and immune-related processes, as evidenced by the enrichment of IL16 in pathways related to CD4 receptor binding and interleukin signaling. Neuroinflammation has increasingly been recognized as a potential contributor to various neuropsychiatric disorders including ADHD. IL16 encodes a proinflammatory cytokine that functions as a chemoattractant for CD4+ cells and has been shown to be expressed in neurons and glial cells in the brain.

The enrichment of genes in pathways related to circadian entrainment (GUCY1A2, NOS1) is particularly intriguing given the well-documented sleep disturbances and circadian rhythm abnormalities in individuals with inattentive disorders. The NO-cGMP pathway has been implicated in the regulation of sleep-wake cycles, potentially providing a mechanistic link between genetic variations in this pathway and the sleep disturbances commonly observed in these disorders. Interestingly, our analysis also identified associations with KEGG pathways related to long-term depression (LTD), a form of synaptic plasticity characterized by decreased synaptic strength. Aberrant synaptic plasticity mechanisms could contribute to the cognitive and behavioural manifestations of inattentive disorders by affecting the efficiency of information processing within neural networks responsible for attention and cognitive control.

The identification of multiple microRNAs potentially regulating our candidate genes suggests an additional layer of regulatory complexity. MicroRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally and have been implicated in various neurodevelopmental processes and neuropsychiatric disorders. Further investigation of these microRNA-gene interactions could provide insights into the molecular mechanisms underlying inattentive disorders and potentially identify novel therapeutic targets.

Our findings support the hypothesis that inattentive disorders arise from dysregulation of multiple interrelated biological pathways rather than from discrete genetic lesions. The significant enrichment of synaptic transmission pathways aligns with prevailing theories regarding altered neurotransmitter signaling in ADHD.[4] Particularly noteworthy is the enrichment of genes involved in catecholamine metabolism, which corresponds with the known efficacy of stimulant medications that target dopaminergic and noradrenergic systems.[17]

The identification of neuronal development pathways suggests that inattentive disorders may partly result from aberrant neurodevelopmental processes occurring early in life.[14] This hypothesis is supported by longitudinal neuroimaging studies demonstrating altered trajectories of cortical development in individuals with ADHD.[15] The enrichment of circadian rhythm regulation pathways represents a novel finding that warrants further investigation. Recent research has documented high rates of sleep disturbances in individuals with inattentive disorders, suggesting a potential mechanistic link between circadian rhythm disruption and attention regulation.[8]

## Limitations and Future Directions

Despite the strengths of our comprehensive pathway analysis approach, several limitations should be acknowledged. First, GWAS data predominantly captures common genetic variants, potentially missing rare variants that may contribute substantially to individual risk.[19] Second, pathway analyses are constrained by the current state of knowledge regarding gene function and pathway architecture.[1] Future research should integrate these findings with data from other omics platforms including transcriptomics, proteomics and metabolomics to provide a more comprehensive understanding of the biological mechanisms underlying inattentive disorders. Additionally, investigating how identified genetic pathways interact with environmental risk factors, may elucidate important gene-environment interactions contributing to disorder heterogeneity.[12]

This study represents a significant advance in understanding the genetic architecture of inattentive disorders by identifying enriched biological pathways that may contribute to their aetiology. The convergence of evidence on synaptic transmission, catecholamine metabolism, neuronal development and circadian rhythm regulation provides a framework for future mechanistic studies and potential therapeutic targets. By illuminating the biological underpinnings of these conditions, our findings contribute to the long-term goal of developing more effective, personalized interventions for individuals affected by inattentive disorders.

## Conclusion

This comprehensive analysis of genetic pathways associated with inattentive disorder symptoms has identified several key biological mechanisms that may contribute to the pathophysiology of these conditions. The most significant finding is the enrichment of genes involved in nitric oxide signaling, particularly NOS1 and GUCY1A2, suggesting that disruptions in NO-cGMP signaling may play a central role in the aetiology of inattentive disorders.

Additional important pathways identified include calcium ion regulation, hedgehog signaling, synaptic vesicle function and circadian entrainment. The cellular component analysis revealed associations with specific neuronal structures including inhibitory neurons in brain regions critical for attention and executive functions.

These findings provide novel insights into the biological underpinnings of inattentive disorders and identify promising targets for further investigation. Future studies should focus on validating these findings in larger cohorts and exploring the functional consequences of variations in the identified genes and pathways. Additionally, the development of animal models targeting these pathways could facilitate the testing of potential therapeutic interventions.

The integration of genetic findings with neuroimaging, neurophysiological and behavioural data will be essential for developing a more comprehensive understanding of the complex pathophysiology of inattentive disorders. Such an integrated approach may ultimately lead to more precise diagnostic methods and targeted therapeutic strategies for individuals with these conditions.

## References

1. Cantor R.M., Lange K. and Sinsheimer J.S., Prioritizing GWAS results: A review of statistical methods and recommendations for their application, *Am J Hum Genet*., **86(1)**, 6-22 **(2010)**

2. Cortese S. et al, Toward systems neuroscience of ADHD: a meta-analysis of 55 fMRI studies, *Am J Psychiatry*, **169(10)**, 1038-1055 **(2012)**

3. de Leeuw C.A. et al, MAGMA: generalized gene-set analysis of GWAS data, *PLoS Comput Biol.*, **11(4)**, e1004219 **(2015)**

4. Del Campo N. et al, The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/ hyperactivity disorder, *Biol Psychiatry*, **69(12)**, e145-157 **(2011)**

5. Demontis D. et al, Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder, *Nat Genet.*, **51(1)**, 63-75 **(2019)**

6. Doshi J.A. et al, Economic impact of childhood and adult attention-deficit/hyperactivity disorder in the United States, *J Am Acad Child Adolesc Psychiatry*, **51(10)**, 990-1002 **(2012)**

7. Faraone S.V. et al, Attention-deficit/hyperactivity disorder, *Nat Rev Dis Primers*, **1**, 15020 **(2015)**

8. Hvolby A., Associations of sleep disturbance with ADHD: implications for treatment, *Atten Defic Hyperact Disord*., **7(1)**, 1-18 **(2015)**

9. Klein M. et al, Brain imaging genetics in ADHD and beyond - mapping pathways from gene to disorder at different levels of complexity, *Neurosci Biobehav Rev*., **80**, 115-155 **(2017)**

10. Larsson H. et al, The heritability of clinically diagnosed attention deficit hyperactivity disorder across the lifespan, *Psychol Med*., **44(10)**, 2223-2229 **(2014)**

11. Lasky-Su J., Neale B.M., Franke B., Anney R.J., Zhou K., Maller J.B., Vasquez A.A., Chen W., Asherson P., Buitelaar J.,

Banaschewski T., Ebstein R., Gill M., Miranda A., Mulas F., Oades R.D., Roeyers H., Rothenberger A., Sergeant J., Sonuga-Barke E., Steinhausen H.C., Taylor E., Daly M., Laird N., Lange C. and Faraone S.V., Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations, *Am J Med Genet B Neuropsychiatr Genet*, **147B(8)**, 1345-54 **(2008)**

12. Nigg J., Nikolas M. and Burt S.A., Measured gene-by-environment interaction in relation to attention-deficit/ hyperactivity disorder, *J Am Acad Child Adolesc Psychiatry*, **49(9)**, 863-873 **(2010)**

13. Sharma A. and Couture J., A review of the pathophysiology, etiology and treatment of attention-deficit hyperactivity disorder (ADHD), *Ann Pharmacother*, **48(2)**, 209-225 **(2014)**

14. Shaw P. et al, Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation, Proc Natl Acad Sci USA, **104(49)**, 19649-19654 **(2007)**

15. Shaw P. et al, Development of cortical surface area and gyrification in attention-deficit/hyperactivity disorder, *Biol Psychiatry*, **72(3)**, 191-197 **(2012)**

16. Thapar A. and Cooper M., Attention deficit hyperactivity disorder, *Lancet*, **387(10024)**, 1240-1250 **(2016)**

17. Volkow N.D. et al, Evaluating dopamine reward pathway in ADHD: clinical implications, *JAMA*, **302(10)**, 1084-1091 **(2009)**

18. Wang K., Li M. and Hakonarson H., Analysing biological pathways in genome-wide association studies, *Nat Rev Genet*., **11(12)**, 843-854 **(2010)**

19. Zuk O. et al, Searching for missing heritability: designing rare variant association studies, Proc Natl Acad Sci USA, **111(4)**, E455-464 **(2014)**.

(Received 25th April 2025, accepted 20th June 2025)