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Review

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Addiction Risk Polymorphisms Links Autism Spectrum Disorder with Reward Deficiency Syndrome: Insights from GWAS and Pharmacogenomic Meta Analyses

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Review

Addiction Risk Polymorphisms Links Autism Spectrum Disorder with Reward Deficiency Syndrome: Insights from GWAS and Pharmacogenomic Meta Analyses

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Abstract

Autism Spectrum Disorder (ASD) is a complex syndrome with a plethora of clinical manifestations. Although such variability may be attributable to diverse genetic and epigenetic factors, mesolimbic dopaminergic reward pathways are consistently implicated in the illness' course and neuropathobiology pointing to a need in an updated perspective on such a potential overlap area. To that end, we introduce a structured framework dissecting ASD's pathophysiology into stages while emphasizing the involvement of the classical reward structures. Through data mining of Genome-Wide Association Studies (GWAS) and *in silico* analyses, we uncovered genetic and epigenetic markers pointing to Reward Deficiency Syndrome (RDS) component of ASD. We employ innovative approaches, including protein-protein interactions, gene regulatory networks, and systems biology analyses, to identify pathways and ASD gene ontologies. The multi-omics analysis integrates findings across genomics, proteomics, metabolomics, and phenomics, reinforcing the concept of ASD as an endophenotype of RDS. This comprehensive approach offers new insights into the genetic architecture and molecular mechanisms of ASD, paving the way for personalized medicine strategies thus yielding novel therapeutic interventions.

Keywords: Autism Spectrum Disorder; Genome-Wide Association Studies; pharmacogenomics; Reward Deficiency Syndrome; genetic addiction risk severity

Introduction

Autism spectrum disorder (ASD) is a relatively common neurodevelopmental condition afflicting approximately 2% of children in the US [1]. Lifetime societal costs are estimated to be $7 trillion owing to healthcare, special education and support services expenditures along with lost productivity, caregiving responsibilities, and long-term care needs; these costs are estimated to double by 2029 [2]. The fact that the ASD prevalence is rising over the past few decades, albeit partially due to refinement of psychodiagnostic tools, increased awareness, and expanded eligibility for services [3], calls for novel neurobiological insights that may inform improved prevention, diagnosis, and treatment [4].

As the "spectrum" term implies, ASD is a complex syndrome encompassing a wide range of symptoms and severity levels manifested in various life areas challenges [5]. Nonetheless, the diagnostic shift by the American Psychiatric Association from diagnostic categories to a unified spectrum [5] reflects advances in research demonstrating the overlap in seeming heterogeneity of symptoms among individuals previously diagnosed with different autism-related disorders (28213805). Although ASD's central features are commonly understood to include social communication challenges, repetitive behaviors, and sensory sensitivities [5] it may be also postulated to involve alterations in reward processing [6,7].

Clinically, in addition to heightened prevalence of addictive disorders [8,9], ASD patients frequently exhibit hyper- or hypo-responsivity to sensory input [10–12], potentially affecting perception of pleasurable stimuli and their hedonic processing [13]. Moreover, a core feature of ASD, repetitive behaviors, may be a form of reinforcement [14,15] responding to altered sensory experiences [16,17] or problematic social interactions [18,19]. These behaviors could function as

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alternative sources of reward [20,21]. Repeated artificial dopamine enhancement in the neurophysiologic reward system by such repetitive behaviors [22] may lead, akin to stress [23] or addictive substances [24], to a dysfunctional hypodopaminergic circuitry [24,25] that renders it less responsive to social interactions and other natural reinforcers [20,26,27], that is to say, reward deficiency syndrome (RDS) [6,28].

At the neurochemical level, dysregulation in neurotransmitter systems playing key roles in modulating reward function, motivation, and social behavior including dopamine, glutamate, serotonin, and oxytocin [29,30], have been observed in patients with ASD [31,32]. From a neuroanatomical perspective, structural and functional abnormalities in brain reward regions [33], such as the nucleus accumbens, prefrontal cortex, and amygdala [34,35] have been reported to be abnormal in ASD [36–39].

Genetic variations and environmental factors have likewise been implicated in the ASD etiology and pathophysiology [40,41]. Some of these factors may influence the development and functioning of brain circuits involved in reward processing, predisposing individuals with ASD to dysregulation in this domain. With regard to the genetic makeup, mutations of the dopamine transporter alter dopaminergic signaling which leads to ASD manifestations [42–44] as dysregulated nigrostriatal dopaminergic neurotransmission promotes stereotyped movements and habitual behaviors [13,45]. Besides, large number of single gene abnormalities are associated with ASD, and a substantial proportion of these encode synaptic molecules [46] including mTOR dependent increases in neuronal spine density and excess synapses in Tsc2 haplo insufficient mice linked with cortico-striatal hyperconnectivity and resultant stereotypies [47]. ASD patients similarly display such hyperconnectivity [48,49] in conjunction with the dysregulated genes engaging with mTOR [50–53].

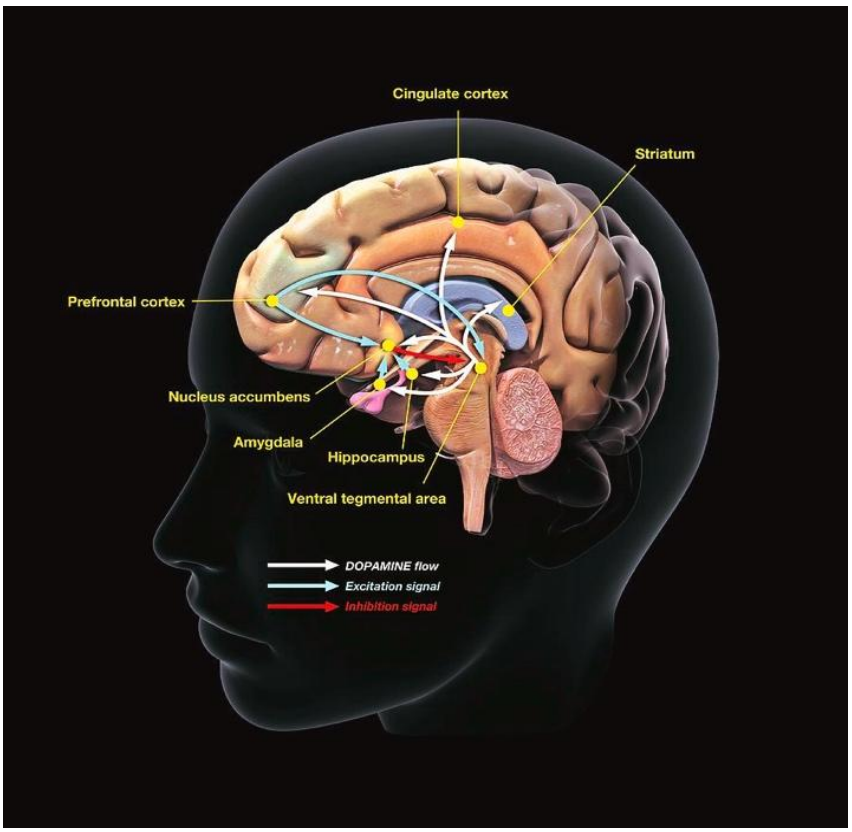

Figure 1. Simplified schematic of mesolimbic reward pathways. Dopaminergic neurons projecting from the ventral tegmental area (VTA) innervate the prefrontal cortex and the nucleus accumbens which is a central mesolimbic reward structure. The VTA is populated by dopaminergic projecting neurons, and it also receives and sends GABAergic projections, receives glutaminergic projections, and it includes cholinergic (Ach) interneurons. The VTA accepts inputs from many brain loci. Interneurons play crucial roles in transducing and modulating VTA inputs and outputs. Free internet use.

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In sum, dysregulated reward system could be an overlapping area underlying diverse aspects of ASD's symptomatology. Understanding the role of reward and its interface with neurobiological, environmental, and genetic factors could open novel vistas for the development of ASD therapeutic interventions. Here we report on data mining via Genome-Wide Association Studies (GWAS) and deep *in silico* pharmacogenomic analyses to uncover and validate specific ASD associated neurogenetic and epigenetic signatures. We hypothesized that ASD shares common underlying mechanisms with RDS, suggesting the former as a subset of the latter. Additionally, we strived to investigate whether ASD presets with gene loci associated with genetic addiction risk severity (GARS), serving as a marker for dysregulated reward function [53].

Methods

In summary, according to our previous published papers [54–60], the strategy of analysis for this investigation was built based on including all phenotypes of GWAS reports related to ASD [obtained from GWAS atlas], then filtering these annotations according to some cutoffs, prioritizing them, and finally use them to find potential interactions in a Multi-Omics perspective (Genomics, Proteomics, Transcriptomics, Metabolomics, and Phenomics) and also systems biology investigations for a deeper and more reliable predictions. Clearly, we theorized that the variants interplay in a sub-genomic level and as such make a larger impact on the phenotypes by Genes, Proteins, miRNAs, TFs, and other signaling pathways playing undiscovered and unknown roles. What we have accomplished relies on mining related to big data (from many GWAS reports) and refining subsequent outcomes based on the best p-values. It also involves the most impacting variant functionalities (Structural and Regulatory vs. non-coding and synonymous), and reliable strong associations among the genes and their proteins based on curated clues from literature. Lastly, our unique strategy also explored a system biological approach to help frame the important instructiveness between the refined genes and ASD manifestations and pathogenesis. In the following, we described the main steps and their details for more clarifications.

SNP-Trait Associations Through Data Mining of GWAS

Our data mining procedure we performed for assessing the ASD phenotype was designed on the basis of the GWAS catalog database (https://www.ebi.ac.uk/gwas/home) [61]. Utilizing this database was employed to help identify all related loci thought various Catalog IDs (CIDs). It is noteworthy to mention that every CID consists of both single and multiple GWA studies. Following extracting the mapped genes from each CID, these genes were combined together. A massive database was then built containing duplications, RNA genes, Pseudogenes, and Protein-coding Genes. Discarding the duplications and deleting the RNA genes and Pseudogenes, the remaining protein-coding genes was a refinement of the resultant data. Importantly following this refinement procedure, they remained. Thus, all further analyses were carried out on this final refined file.

Primary and Deep In Silico Databases

The PGx potential of the GARS panel (*DRD1, DRD2, DRD3, DRD4, MAOA, COMT, DAT1, SLC6A4, OPRM1,* and *GABRA3*) in ASD was assessed using our previously published strategy [56–60]. Primary analyses included Protein-Protein Interactions (PPIs); Gene Regulatory Networks (GRNs); Disease, drugs and chemicals (DDCs); and Gene Coexpression Networks (GCNs). PPIs performed by STRING-MODEL and Signaling checked by Cytoscape. GRN is done by Gene-miR Interactions (GMIs) (miRTarBase), and DDC applied Protein-Drug Interactions (PDI) through DrugBank, and Protein-Chemical Interactions (PCI) via Comparative Toxicogenomics Database (CTD). Deep in silico analyses performed in a systems biology analysis by Enrichr for pathway, gene ontology (GO), and Disease-Drugs Associations, and also a Meta-analysis by Metascape. GWAS catalog (https://www.ebi.ac.uk/gwas/home) [61] searched for ASD and all of its related Phenotypes. Each Phenotypes with a unique catalog ID (CID) was obtained separately and after

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multiple cut-offs including removing the duplicated studies and studies with missing main information, the outputs were then combined together. All details and references are summarized in Table 1 [54,55,61–70].

Table 1. Contents, validations, and references of all primary and deep in silico databases and systems biology approaches utilized in the current paper.

Level	Database	Site	Software (version)	References
GWAS data mining	GWAS catalog	https://www.ebi.ac.uk/gwas/home	EMBL-EBI 2024	61
PPIs	STRING-MODEL	https://string-db.org/	STRING (12.0)	54
GRNs	GMIs (miRTarBase)	https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php	NetworkAnalyst (3.0)	62
	Signaling	https://cytoscape.org/	Cytoscape (3.10.1)	63
DDCs	PDIs	https://go.drugbank.com/	NetworkAnalyst (3.0)	64
	PCIs	https://ctdbase.org/	NetworkAnalyst (3.0)	65
EA	Pathway Analysis	https://maayanlab.cloud/Enrichr/	Enrichr	66
	GO	https://maayanlab.cloud/Enrichr/	Enrichr	67
	DDA	https://maayanlab.cloud/Enrichr/	Enrichr	68
Multi-Omics	Genomics, Proteomics, Transcriptomics, Metabolomics, and Phenomics	https://maayanlab.cloud/enrichr-kg	Enrichr-KG	69
MA	CEO	https://metascape.org/gp/index.html#/main/step1	Metascape	70
PGx	VAA	https://www.pharmgkb.org/	PharmGKB	55

Abbreviations: PPIs: Protein-Protein Interactions; GRNs: Gene Regulatory Networks; GMIs: Gene-miRNA Interactions; DDCs: Diseases, Drugs & Chemicals; PDIs: Protein-Drug Interactions; PCIs: Protein-Chemical Interactions; EA: Enrichment Analysis; GO: Gene Ontology; DDA: Diseases Drugs Assessment; MA: Meta Analysis; CEO: Clustering Enriched Ontology. PGx: Pharmacogenomics; VAA: Variant Annotation Assessment.

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Clustering Enriched Ontology (CEO) and Meta-Analyses

Zhou et al employed the accumulative hypergeometric test (or Fisher's exact test) to calculate the p-values and enrichment factors for each ontology category. According to the Metascape research, [70] "ontology terms found in GO form a hierarchical structure of increasing granularity, making the terms inherently redundant." Terms from several ontology sources, like GO, KEGG, and MSigDB, might be closely connected as well. As a result, functional enrichment analysis might reveal duplicated or related phrases, making it difficult to select non-redundant and representative processes for reporting in the analysis output." A flowchart represented in Figure 2 elucidates step-by-step our strategy pipeline with a brief description of each step. This figure illustrates the input, prioritized, and output data for this in deep silica PGx and GWAS investigation.

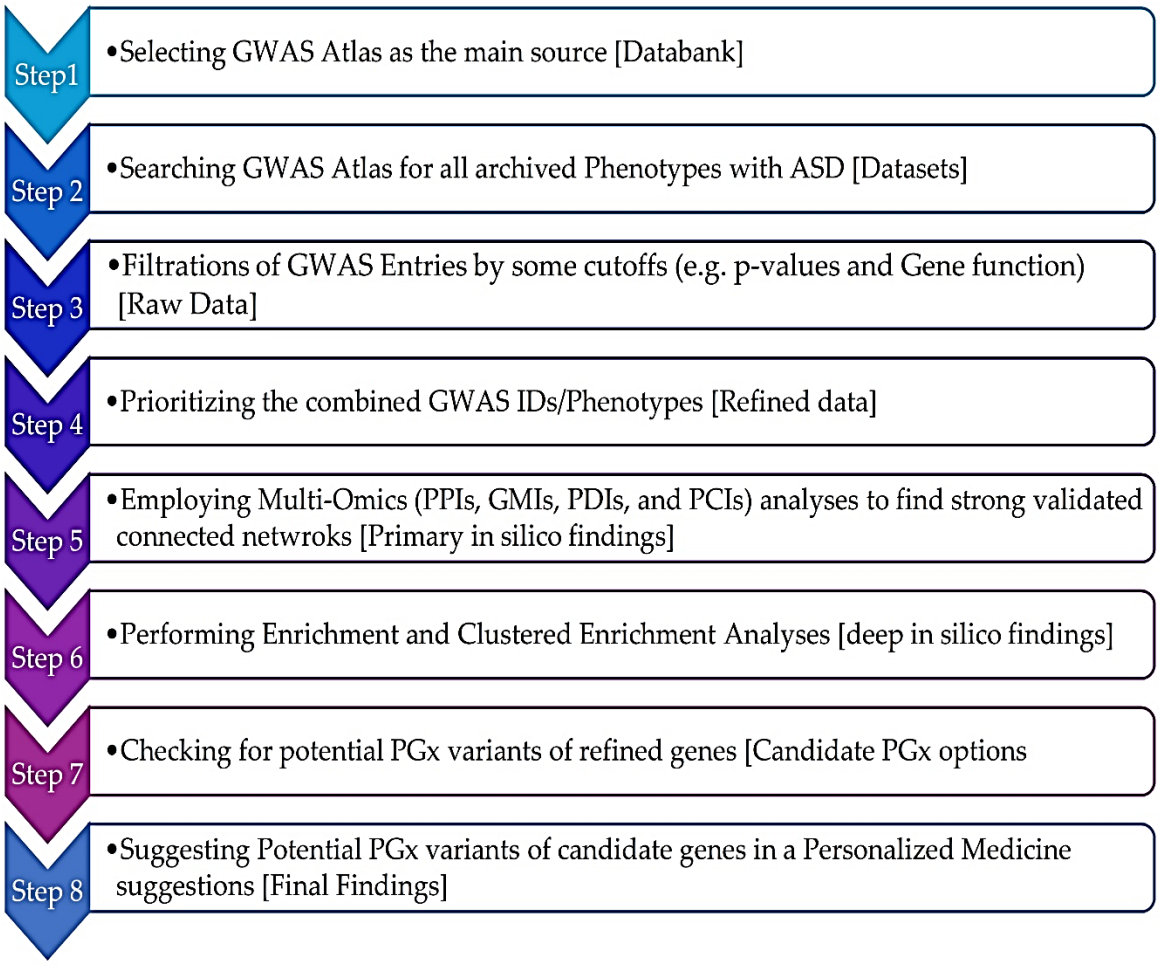

Figure 2. Flowchart scheme of strategy used in the current study from Databank selection to Final findings through 8 specific steps. GWAS: Gnome-Wide Association Studies; ASD: Autism Spectrum Disorder; PPIs: Protein-Protein Interactions; GMIs: Gene-miRNA Interactions; PDIs: Protein-Drug Interactions; PCIs: Protein-Chemical Interactions; and PGx: Pharmacogenomics'.

Statistical Evaluations

In the STRING-MODEL, the expected number of edges represents how many connections would be predicted if nodes (proteins) were selected randomly. A very small PPI enrichment p-value suggests that the actual number of observed edges is much higher than expected by chance, indicating that the proteins are biologically connected rather than randomly distributed. NetworkAnalyst provides two key centrality metrics—degree and betweenness. Nodes with a high degree (i.e., many connections) are considered hubs of the network. Betweenness, on the other hand, reflects how often a node lies on the shortest path between other nodes. Interestingly, a node can

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have a low degree but still have high betweenness if it connects densely interconnected clusters, serving as a bridge within the network. During module detection, NetworkAnalyst incorporates gene expression values as edge weights. For the enrichment analysis, Enrichr provides several statistical outputs: p-value, q-value, Z-score (rank), and combined score. In this study, we focused on the p-value, q-value, and odds ratio (OR), which are commonly reported in biological research, and did not include the combined score. The p-values were calculated using standard methods such as the hypergeometric test or Fisher's exact test. These tests assume a binomial distribution and independence in the probability of any gene being part of a specific set. The q-value represents a multiple testing-corrected p-value, adjusted using the Benjamini-Hochberg method to control for false discovery.

Results

GWAS-Based Deep In Silico PGx Investigations

Following a SNP-Trait Association Analysis by GWAS raw data, a primary and a deep *in silico* PGx analyses successfully carried out on ASD and GARS genes. A pioneering strategy designed starting from raw data of massive GWAS information and followed by a logical pipeline to find novel insights into uncovered mechanisms of actions playing roles in ASD. Statistically, the *p*-value threshold was <1.E-06 and the input data are summarized in Table 2.

Table 2. The included raw data from all GWAS related to ASD.

Phenotype	**GWAS CID**	**Associations (N)**	**Studies (N)**
Autism Spectrum Disorder	EFO_0003756	1321	55
Autism Spectrum Disorder Symptom	EFO_0005426	40	12
Autism	EFO_0003758	44	18
Social Communication Impairment	EFO_0005427	21	2
Asperger Syndrome	EFO_0003757	5	2
Obsessive-compulsive Disorder	EFO_0004242	258	25
Anorexia Nervosa	MONDO_0005351	268	25
Tourette syndrome	EFO_0004895	220	14
Attention Deficit Hyperactivity Disorder	EFO_0003888	1838	85
Schizophrenia	MONDO_0005090	5049	159
Intelligence	EFO_0004337	3846	41
Behavior or Behavioral Disorder Measurement	EFO_0004782	14	17
Bipolar Disorder	MONDO_0004985	1592	127
Unipolar Depression	EFO_0003761	2668	299

CID means GWAS Catalog ID and N refers to Numbers.

Totally, 17,184 associations and 881 studies included. Following combining the CID, replicated genes were refined (2,980 genes remained). Duplicated genes were removed (990 genes remained) and in the next, based on separated search for each mapped gene in GeneCArds

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(http://www.genecards.org/), RNA genes and Pseudogenes removed (667 remained). To find the most important curated genes, a PPIs performed by STRING-MODEL and the top 20 genes based on the degree of betweenness (connections) selected for further steps. The top 20 genes extracted from 881 GWAS which were filtered, refined, and prioritized in this study are as follows: *CTNNB1, ESR1, RHOA, CDC42, FOXO3, MAP3K7, PRKCD, ERBB4, UBE2D3, MAPT, CTNND1, HDAC4, BRAF, ESR2, PPP2R5C, ERBB3, TLR4, HTT, SRC*, and *GRIN2B*. Next, these 20 GWAS-mined genes were combined with 10 GARS genes in a final list to find any potential relationships. This candidate genes list was tested by another STRING-MODEL (Figure 3).

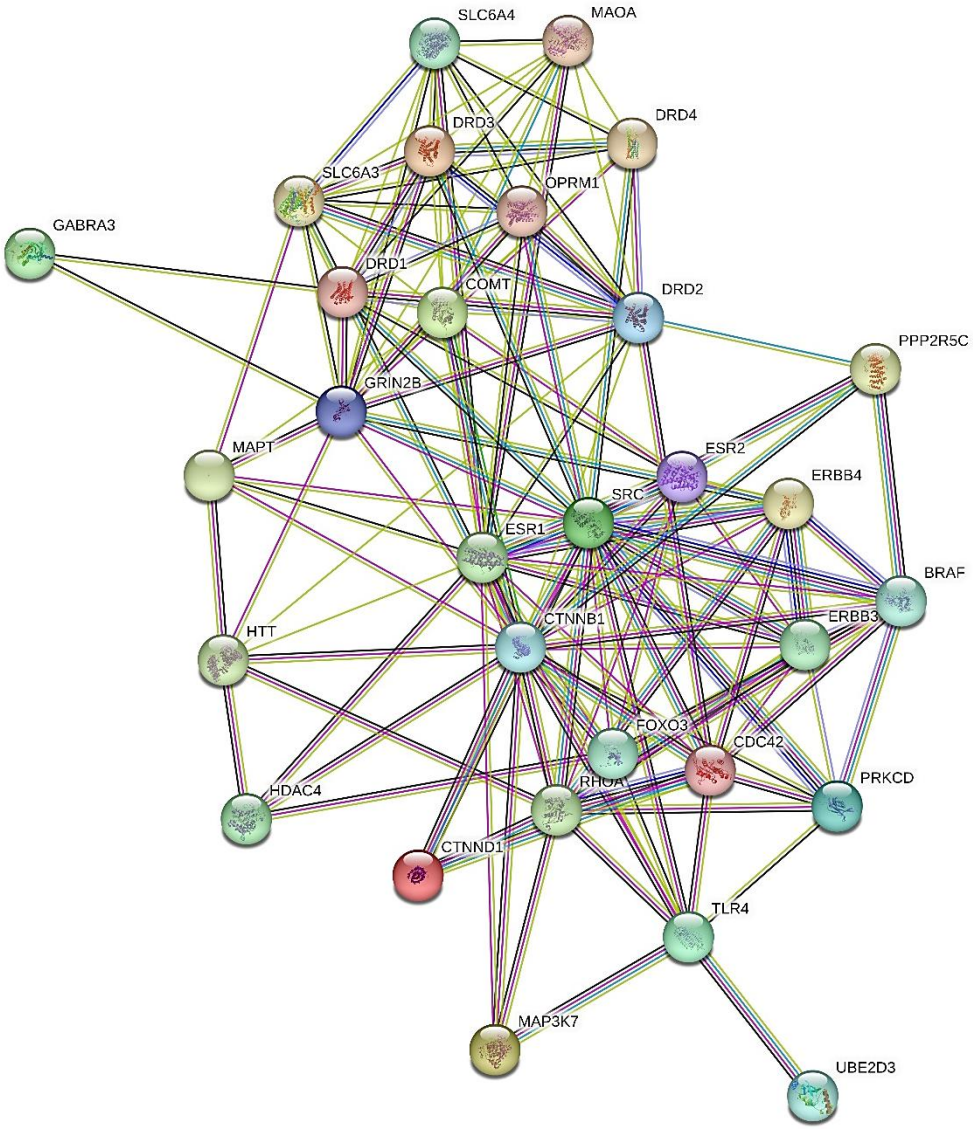

Figure 3. STRING-MODEL included 20 genes mined from 881 GWA studies and 10 GARS genes. Each protein contains various colored lines which refer to a specific interaction including blue as obtained from curated databases; pink as experimentally determined; green as gene neighborhood; red as gene fusions; dark blue as gene co-occurrence; light green as textmining; black as co-expression; and pale purple as protein homology.

Signaling

Cytoscape [71] identified dopaminergic synapse through Signor (https://signor.uniroma2.it/) as the most significant curated pathway among the genes listed in this study. *DRD1, DRD2, DRD3, DRD4,* and *GRIN2B* with a similarity score of 0.19 and a p-value of 8.68E-6 involved in dopaminergic synapse (Figure 4).

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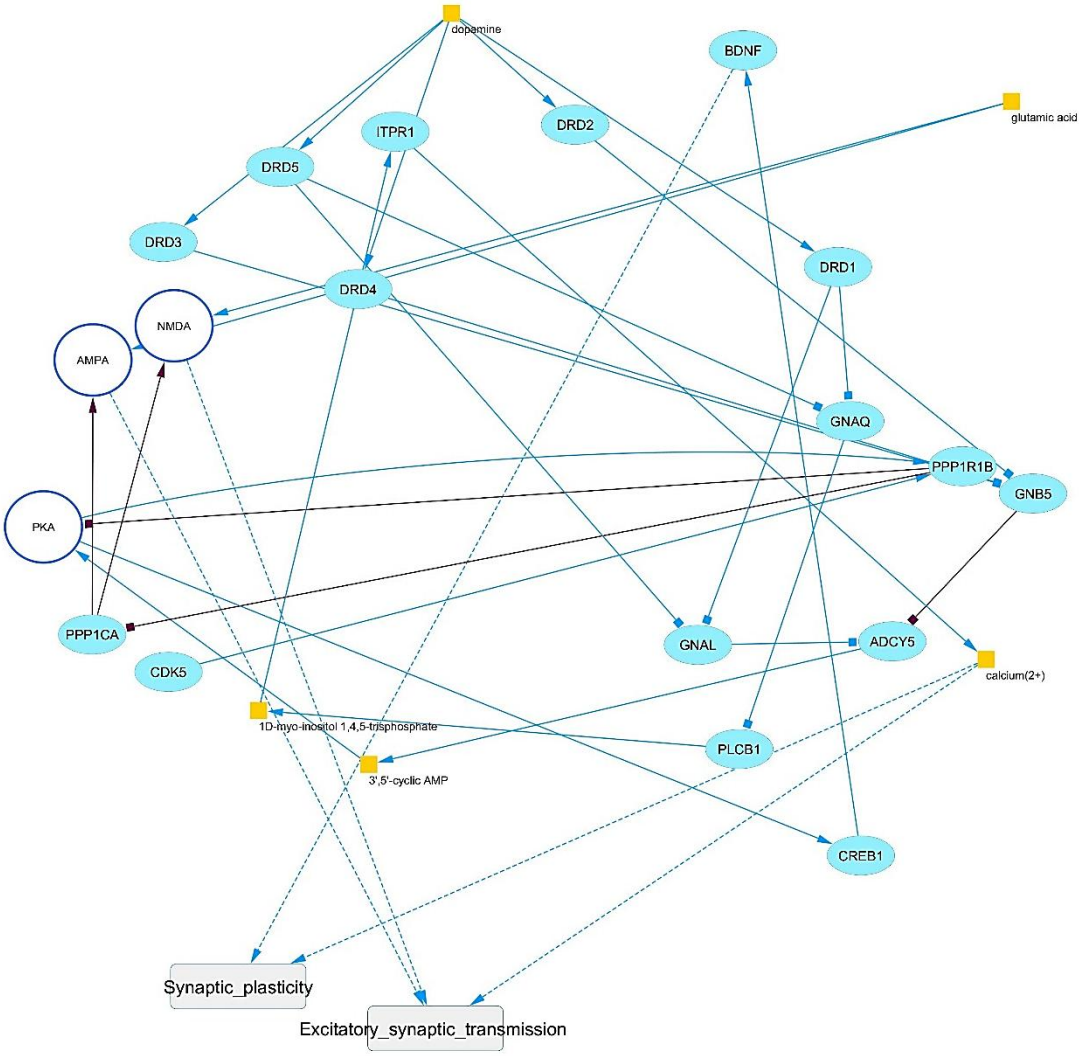

Figure 4. Dopaminergic synapse signaling network found by Cytoscape highlighting on Dopamine receptor family genes and GRIN2B triggering by Dopamine leading to Synaptic plasticity and excitatory synaptic transmission.

GMIs

A Linear Bi/Tripartite model visualized by NetworkAnalyst revealed multiple Gene-miRNA associations, among them, hsa-miR-16-5p found most-interacted with a linking degree of 7 with *COMT*, *OPRM1*, *MAP3K7*, *PPP2R5C*, *PRKCD*, *HTT*, and *SLC6A4* genes (Figure 5).

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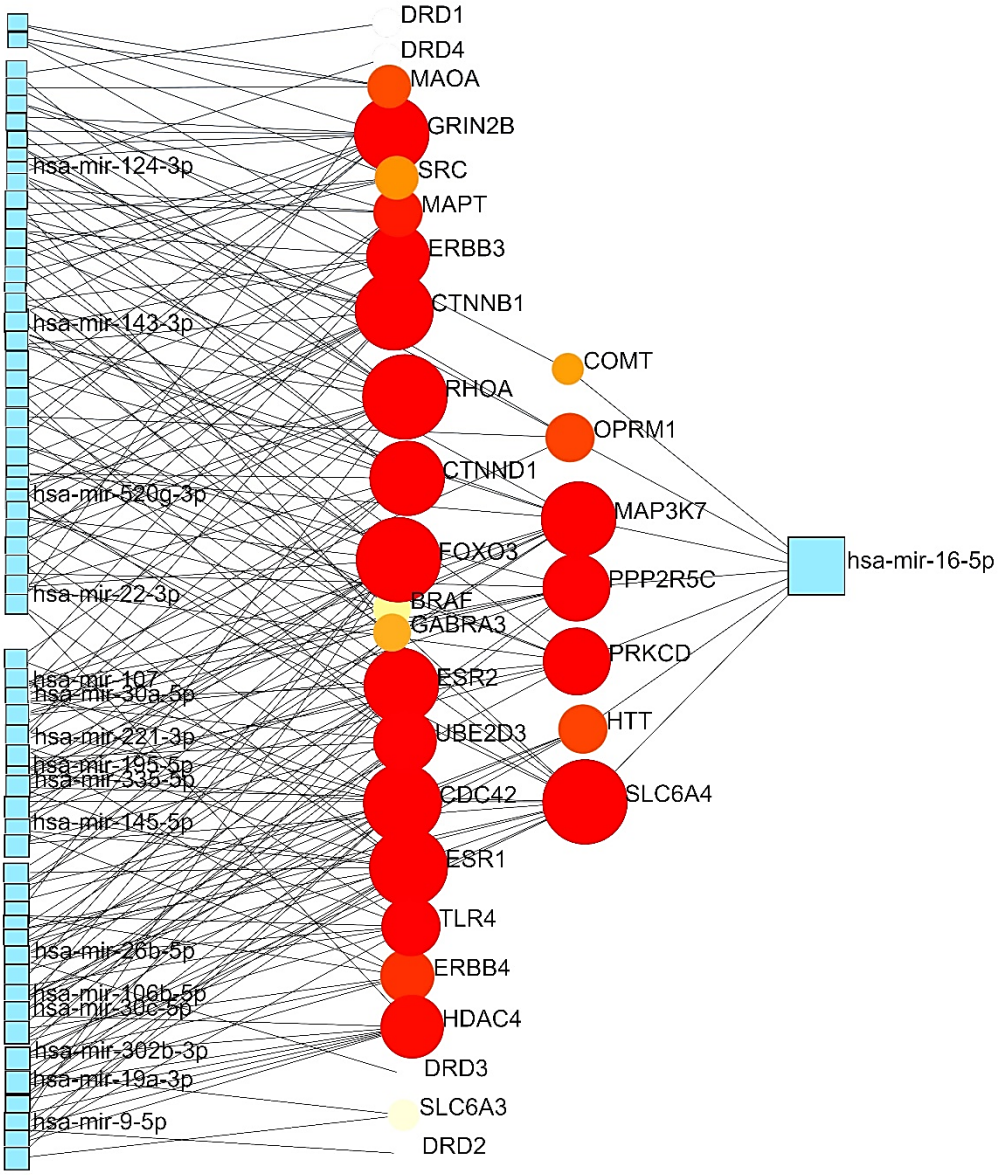

Figure 5. Linear Bi/Tripartite model of GMIs highlights hsa-miR-16-5p as the most interacted miRNA with candidate gene list emphasizing on hsa-miR-16-5p interacting with *COMT, OPRM1, MAP3K7, PPP2R5C, PRKCD, HTT*, and *SLC6A4* genes.

PDIs

Through a Fruchterman-Rheingold model of PDIs, Networkanalyst outputs displayed that there are connections among the FDA-Approved drugs from Drugbank [72] and genes of interest. There are important axes which demonstrate the associations of ASD with RDS; the important axes might be as follows: 1) COMT> [Nialamide]>MAOA>[Minaprine]>DRD2>[Amoxapine]>GABRA3. 2) PRKCD>[Tamoxifen]>ESR2>[Dehydroepiandrosterone]>GABRA3>[Amoxapine]>DRD4. 3) PRKCD>[Tamoxifen]>ESR2>[Dehydroepiandrosterone]>GRIN2B>[Haloperidol]>DRD1. Results indicate that Amoxapine has a central role with strong connections (DRD1, DRD2, DRD4, SLC6A4. SLC6A3, and GABRA3). Interestingly, Dehydroepiandrosterone correlates with three important genes ESR2, GRIN2B (both genes are in ASD group), and GABRA3 (GARS genes); and also, Haloperidol links with DRD1, DRD2 (both GARS genes), and GRIN2B (ASD gene) (Figure 6).

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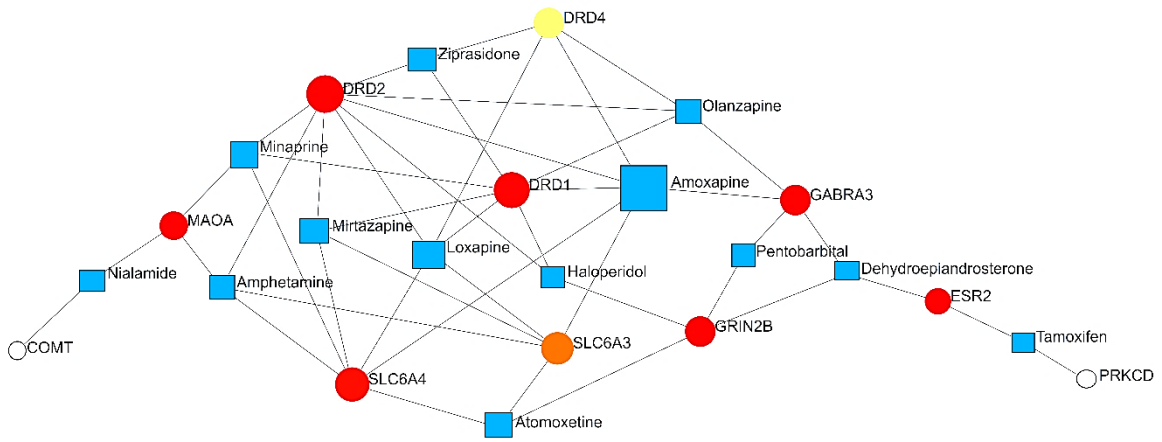

Figure 6. Fruchterman-Rhengold model of PDIs showing important relationships among FDA-approved drugs with genes of both ASD and GARS groups. Interestingly, *DRD2* has 8 interactions with both genes and drugs of this network.

PCIs

According to the CTD [73], Figure 7 illustrated PCIs for ASD and GARS genes (candidate gene list) in a Sugiyama model which clearly shows ESR1 as the most connected gene with several chemicals and Aflatoxin B1 as the most important chemical with a linking degree of 17.

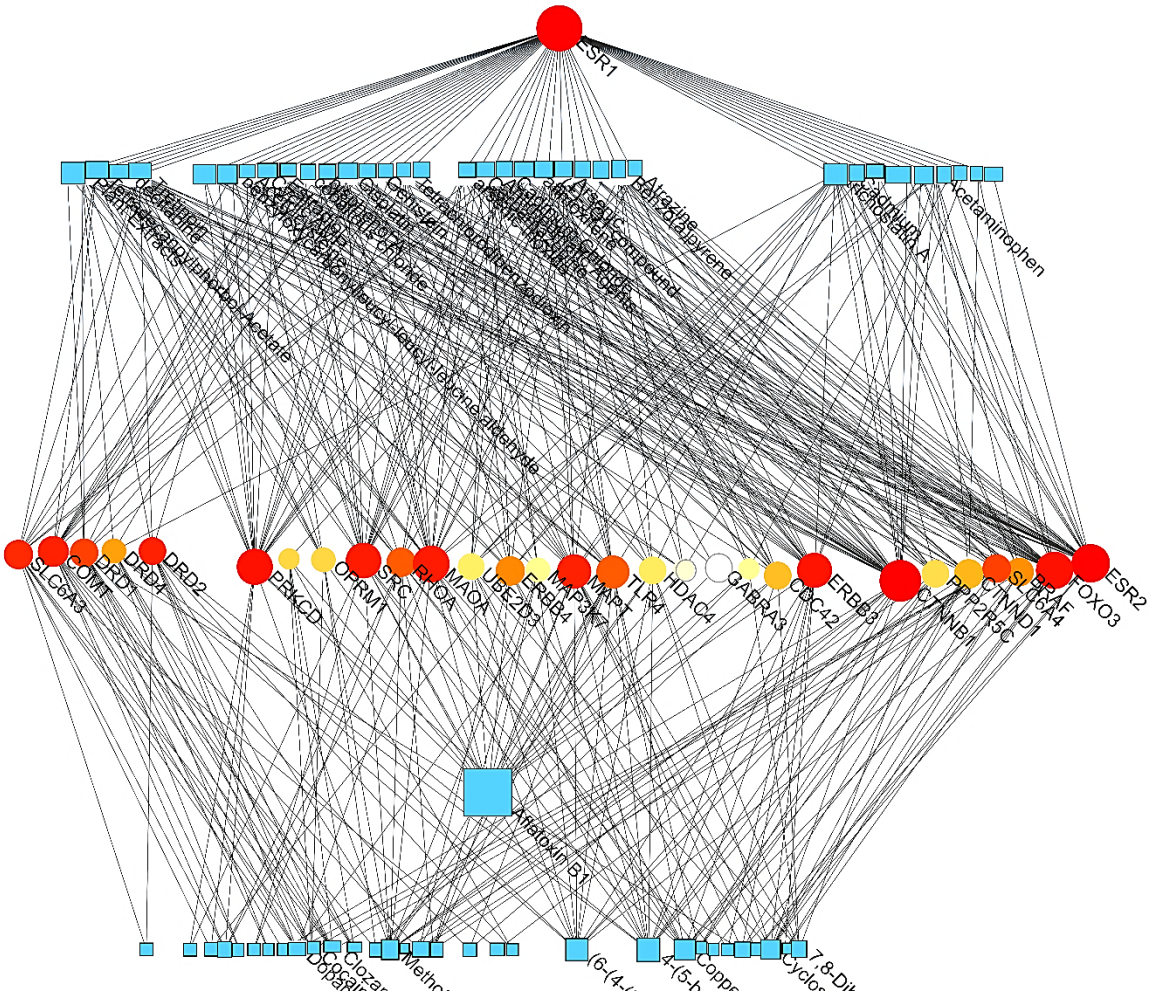

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Figure 7. Sugiyama model of PCIs confirming the tight interactions of selected proteins with known chemicals, as the most interacted chemical is Aflatoxin B1 in this figure which has interactions with 17 proteins. Importantly, ESR1 is the major protein predicted in this network with the highest interactions.

Enrichr Analysis (EA) Results

Following primary *in silico* analyses (STRING-MODEL, Signaling, GMIs, PDIs, and PCIs), EA, Multi-Omics, and Meta-analysis performed for the candidate gene list mined and prioritized form GWA studies which described earlier.

1. Pathways

Reactome 2022, KEGG 2021, and Panther 2016 were employed to find the potential pathways related to the candidate gene list. By combining and categorizing all three databases' results, Table 3 was generated showing Dopaminergic synapse with a q-value of 3.6E-11 as the most significant pathway predicted by KEGG. Noteworthy, the top-scored pathway of PANTHER is Dopamine receptor mediated signaling pathway Homo sapiens (P05912). In addition, following Signal Transduction R-HAS-162582, Reactome also predicted Dopamine Receptors R-HAS-390651.

Table 3. The combined results of Reactome, KEGG, and PANTHER databases are based on a candidate gene list.

N	Index	Name	P-value	q-value	OR
1	KEGG	Dopaminergic synapse	2.29E-13	3.65E-11	69.15
2	Reactome	Signal Transduction R-HSA-162582	3.50E-13	1.50E-10	16.73
3	KEGG	Proteoglycans in cancer	1.24E-11	9.88E-10	43.24
4	Reactome	Dopamine Receptors R-HSA-390651	2.05E-11	4.38E-09	3072.15
5	Reactome	Disease R-HSA-1643685	3.34E-11	4.76E-09	13.88
6	Panther	Dopamine receptor mediated signaling pathway Homo sapiens P05912	1.30E-11	6.49E-09	108.28
7	Reactome	Transmission Across Chemical Synapses R-HSA-112315	6.36E-11	6.79 E-09	35.68
8	Reactome	Diseases Of Signal Transduction By Growth Factor Receptors And Second Messengers R-HSA-5663202	3.39E-10	2.90 E-09	23.62
9	KEGG	Rap1 signaling pathway	6.20 E-10	3.29E-08	35.59
10	KEGG	Adherens junction	8.95E-10	3.56 E-08	76.56
11	Reactome	Neurotransmitter Clearance R-HSA-112311	8.58E-10	6.11 E-08	511.9
12	KEGG	Shigellosis	2.17 E-09	6.89 E-08	30.15
13	Reactome	Signaling By Receptor Tyrosine Kinases R-HSA-9006934	1.55 E-09	9.44 E-08	20.05
14	Panther	CCKR signaling map ST Homo sapiens P06959	3.97 E-09	9.93 E-08	38.16
15	Reactome	Neuronal System R-HSA-112316	3.39 E-09	1.81E-07	22.27
16	KEGG	Alcoholism	9.13 E-09	2.21 E-07	33.65
17	KEGG	Cocaine addiction	9.74 E-09	2.21 E-07	90.57
18	Reactome	PI3K/AKT Signaling In Cancer R-HSA-2219528	9.71 E-09	3.99 E-07	50.18
19	Reactome	Signaling By ERBB2 R-HSA-1227986	9.74 E-09	3.99 E-07	90.57
20	KEGG	Chemokine signaling pathway	3.55 E-07	7.05E-06	26.59

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21	Panther	Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway Homo sapiens P00027	4.88 E-07	8.13E-06	39.34
22	KEGG	Neuroactive ligand-receptor interaction	5.72 E-07	1.01E-05	17.89
23	KEGG	Neurotrophin signaling pathway	8.67 E-07	1.38 E-05	34.84
24	Panther	Angiogenesis Homo sapiens P00005	2.08 E-06	2.6 E-05	28.95
25	Panther	Cadherin signaling pathway Homo sapiens P00012	2.72 E-06	2.72 E-05	27.34
26	Panther	Adrenaline and noradrenaline biosynthesis Homo sapiens P00001	6.85 E-06	5.71 E-05	100.75
27	Panther	EGF receptor signaling pathway Homo sapiens P00018	2.05 E-05	0.000147	29.11
28	Panther	Integrin signaling pathway Homo sapiens P00034	8.33E-05	0.000521	20.06
29	Panther	Ras Pathway Homo sapiens P04393	0.000149	0.000829	33.51
30	Panther	Wnt signaling pathway Homo sapiens P00057	0.000753	0.003648	11.06

N, q-value, and OR refer to Numbers, Adjusted p-value, and Odds Ration, respectively.

Metabolomics Predictions

As an episodic analysis, Metabolomics Workbench Metabolites 2022 indicated that the most impacted metabolite playing a key role among ASD and RDS was Dopamine (q-value=3.25×10^{-4}), followed by Pyruvaldehyde, N4-Acetylaminobutanal, 3,4-Dihydroxyphenylglycol, Indoleacetaldehyde, and Serotonin.

Metabolomics Workbench Metabolites 2022

Index	Name	P-value	Adjusted p-value	Odds Ratio
1	Dopamine	0.00003250	0.0003250	356.54
2	Pyruvaldehyde	0.008967	0.01492	137.69
3	N4-Acetylaminobutanal	0.008967	0.01492	137.69
4	3,4-Dihydroxyphenylglycol	0.008967	0.01492	137.69
5	Indoleacetaldehyde	0.01045	0.01492	114.74
6	Serotonin	0.01045	0.01492	114.74
7	5-Hydroxyindoleacetaldehyde	0.01045	0.01492	114.74
8	3,4-Dihydroxymandelaldehyde	0.01194	0.01492	98.34
9	S-Adenosylhomocysteine	0.06679	0.07239	15.27
10	S-Adenosylmethionine	0.07239	0.07239	14.02

2. Gene Ontologies (GO)

Integral GO analyses were conducted including Biological Process, Cellular Component, and Molecular Function indexes. The results of each process were then merged and summarized in Table 4. Table 4 clearly shows that the most significant GO process is Dopamine Metabolic Process (GO: 0042417) with a q-value of 1.72×10^{-10}. Furthermore, the top-scored processes in Cellular Component and Molecular Function were Neuron Projection (GO: 0043005) and Kinase Binding (GO: 0019900), respectively.

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Table 4. Merged information of three GO biological indexes related to the candidate gene list.

Index	Name	P-value	q-value	OR
GO Biological Process 2023	Dopamine Metabolic Process (GO:0042417)	1.79E-13	1.72E-10	383.79
GO Biological Process 2023	Catecholamine Metabolic Process (GO:0006584)	6.82E-12	3.29E-09	499.05
GO Biological Process 2023	Response To Ethanol (GO:0045471)	4.52E-11	1.45E-08	307.03
GO Cellular Component 2023	Neuron Projection (GO:0043005)	2.40E-10	1.80E-08	20.6
GO Cellular Component 2023	Dendrite (GO:0030425)	1.18E-07	4.43E-06	22.81
GO Biological Process 2023	Response To Organic Cyclic Compound (GO:0014070)	2.32E-08	5.58E-06	75.16
GO Biological Process 2023	Prepulse Inhibition (GO:0060134)	3.04E-08	5.85E-06	1109.33
GO Biological Process 2023	Response To Cocaine (GO:0042220)	6.07E-08	9.75E-06	739.52
GO Cellular Component 2023	Axon (GO:0030424)	5.21E-07	1.3E-05	24.84
GO Biological Process 2023	Regulation Of Dopamine Uptake Involved In Synaptic Transmission (GO:0051584)	1.06E-07	1.46E-05	554.61
GO Biological Process 2023	Phospholipase C-activating G Protein-Coupled Receptor Signaling Pathway (GO:0007200)	1.43E-07	1.72E-05	51.01
GO Biological Process 2023	Positive Regulation Of Neuron Death (GO:1901216)	1.85E-07	1.88E-05	102.26
GO Biological Process 2023	Regulation Of Postsynaptic Membrane Potential (GO:0060078)	2.08E-07	1.88E-05	98.95
GO Molecular Function 2023	Kinase Binding (GO:0019900)	4.19E-06	0.000624	13.11
GO Cellular Component 2023	Membrane Raft (GO:0045121)	0.000114	0.00213	18.47
GO Cellular Component 2023	Focal Adhesion (GO:0005925)	0.000253	0.003474	10.26
GO Cellular Component 2023	Cell-Substrate Junction (GO:0030055)	0.000278	0.003474	10.04
GO Molecular Function 2023	Estrogen Response Element Binding (GO:0034056)	9.72E-05	0.004419	178.23
GO Molecular Function 2023	Monoamine Transmembrane Transporter Activity (GO:0008504)	0.000119	0.004419	158.42

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GO Molecular Function 2023	Dynactin Binding (GO:0034452)	0.000119	0.004419	158.42
GO Molecular Function 2023	Sodium: Chloride Symporter Activity (GO:0015378)	0.000168	0.00486	129.6
GO Molecular Function 2023	Alkali Metal Ion Binding (GO:0031420)	0.000196	0.00486	118.8
GO Molecular Function 2023	Postsynaptic Neurotransmitter Receptor Activity (GO:0098960)	0.000366	0.007792	83.84
GO Molecular Function 2023	Protein Tyrosine Kinase Activity (GO:0004713)	0.000433	0.008073	23
GO Molecular Function 2023	G Protein-Coupled Receptor Activity (GO:0004930)	0.000506	0.008374	12.34
GO Cellular Component 2023	Catenin Complex (GO:0016342)	0.000803	0.008598	54.79
GO Cellular Component 2023	Non-Motile Cilium (GO:0097730)	0.000922	0.008641	50.87
GO Cellular Component 2023	Bounding Membrane Of Organelle (GO:0098588)	0.001183	0.009858	5.89
GO Cellular Component 2023	Ciliary Membrane (GO:0060170)	0.001479	0.01109	39.55
GO Molecular Function 2023	Protein Kinase Binding (GO:0019901)	0.000897	0.01336	7.69

GO, q-value, and OR refer to Gene Ontology, Adjusted p-value, and Odds Ration, respectively.

3. Diseases Drugs Associations

To reach a confidential clinical prediction, the list containing 20 GWAS-mined and 10 GARS genes were checked through three clinical disease/disorder databases (i.e. DisGeNET, GeDiPNet 2023, and Jensen DISEASES). Table 5 shows the output of combining and categorizing all three databases based on the best *q*-value. According to this table, the most significant phenotype related to the candidate gene list which was predicted by DisGeNET is Obsessive-Compulsive Disorder (q-value=1.49×10^{-16}). Additionally, substance abuse disorder (q-value= 6.38×10^{-15}) and Mental depression (q-value= 1.06×10^{-14}) were the best-scored diseases assessed by Jensen DISEASES and GeDiPNet, respectively.

Table 5. Diseases/Disorders related to the candidate gene list predicted by DisGeNET, GeDiPNet, and Jensen DISEASES.

Index	Name	P-value	q-value	OR
DisGeNET	**Obsessive-Compulsive Disorder**	**5.14E-20**	**1.49E-16**	**129.86**
DisGeNET	Mental Depression	4.20E-19	6.07E-16	45.49
DisGeNET	Abnormal behavior	1.00E-18	8.45E-16	54.94
DisGeNET	Addictive Behavior	1.17E-18	8.45E-16	77.55
DisGeNET	Nonorganic psychosis	2.45E-18	1.42E-15	73.01
DisGeNET	Gambling, Pathological	6.32E-18	2.70E-15	1215.26
DisGeNET	Cognition Disorders	6.54E-18	2.70E-15	67.41

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DisGeNET	Impulsive character (finding)	8.69E-18	3.14E-15	237.31
DisGeNET	Hyperactive behavior	1.42E-17	4.57E-15	36.43
DisGeNET	Nicotine Dependence	1.64E-17	4.73E-15	101.74
Jensen DISEASES	**Substance abuse**	**3.59E-17**	**6.38E-15**	**198.61**
GeDiPNet	**Mental Depression**	**3.40E-17**	**1.06E-14**	**30.01**
GeDiPNet	Mood Disorder	3.44E-17	1.06E-14	58.89
Jensen DISEASES	Alcohol dependence	2.31E-16	2.06E-14	106.87
GeDiPNet	Schizophrenia	6.51E-15	1.34E-12	22.03
Jensen DISEASES	Heroin dependence	3.57E-13	2.12E-11	332.58
GeDiPNet	Bipolar Disorder	2.97E-13	4.57E-11	24.71
Jensen DISEASES	Nicotine dependence	1.18E-12	5.25E-11	131.82
Jensen DISEASES	Gilles de la Tourette Syndrome	7.85E-11	2.80E-09	118.62
Jensen DISEASES	Cocaine dependence	1.38E-10	4.10E-09	234.74
GeDiPNet	Cognitive Disorder	6.88E-11	8.46E-09	121.52
Jensen DISEASES	Dementia	4.01E-10	1.02E-08	53.96
GeDiPNet	Status Marmoratus	1.77E-10	1.81E-08	221.69
Jensen DISEASES	obsessive-compulsive disorder	1.23E-09	2.74E-08	142.44
Jensen DISEASES	Major depressive disorder	1.72E-09	3.41E-08	68.14
Jensen DISEASES	Oppositional defiant disorder	7.39E-09	1.20E-07	255.87
GeDiPNet	Age-Related Memory Disorders	4.95E-09	4.29E-07	104.91
GeDiPNet	Memory Disorders	5.57E-09	4.29E-07	102.21
GeDiPNet	Autism	2.05E-08	0.000001399	15.1
GeDiPNet	Minimal Brain Dysfunction	2.95E-08	0.000001677	170.53

*q-value and OR mean adjusted p-value and Odds Ratio.

Multi-Omics Analysis by Enrichr-KG

Using Enrichr-KG [74], we selected 5 libraries to set a Multi-Omics analysis including GWAS catalog (Genomics), KEGG (Proteomics), Gene Ontology (Metabolomics), TRRUST (Transcriptomics), and DisGeNET (Phenomics). Multi-Omics results supported the role of ASD as endophenotype and RDS as true phenotype (Table 6 and Figure 8).

Table 6. Multi-Omics results of candidate genes of ASD and GARS.

Term	Library	p-value	q-value	z-score	combined score
Obsessive-Compulsive Disorder	DisGeNET	5.14E-20	1.49E-16	129.9	5767
Mental Depression	DisGeNET	4.20E-19	6.07E-16	45.49	1925
Abnormal behavior	DisGeNET	1.00E-18	8.45E-16	54.94	2277
Addictive Behavior	DisGeNET	1.17E-18	8.45E-16	77.55	3202
Nonorganic psychosis	DisGeNET	2.45E-18	1.42E-15	73.01	2961
dopamine metabolic process (GO:0042417)	GO_Biological_Process_2021	1.79E-13	1.82E-10	383.8	11260

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Dopaminergic synapse	KEGG_2021_Human	2.29E-13	3.65E-11	69.15	2013
Proteoglycans in cancer	KEGG_2021_Human	1.24E-11	9.88E-10	43.24	1086
catecholamine metabolic process (GO:0006584)	GO_Biological_Process_2021	1.59E-11	8.09E-09	399.2	9926
regulation of dopamine uptake involved in synaptic transmission (GO:0051584)	GO_Biological_Process_2021	2.87E-10	9.73E-08	767.9	16870
Rap1 signaling pathway	KEGG_2021_Human	6.20E-10	3.29E-08	35.59	754.4
Adherens junction	KEGG_2021_Human	8.95E-10	3.56E-08	76.56	1595
Shigellosis	KEGG_2021_Human	2.17E-09	6.89E-08	30.15	601.5
positive regulation of neuron death (GO:1901216)	GO_Biological_Process_2021	7.85E-09	2E-06	94.9	1771
regulation of protein kinase B signaling (GO:0051896)	GO_Biological_Process_2021	1.91E-08	3.9E-06	30.08	534.7
Neuroticism	GWAS_Catalog_2019	0.000057824	0.006743	22.11	215.7
Bone mineral density (hip)	GWAS_Catalog_2019	0.000071735	0.006743	43.4	414.1
BMI (adjusted for smoking behaviour)	GWAS_Catalog_2019	0.0002067	0.008483	29.87	253.5
Extremely high intelligence	GWAS_Catalog_2019	0.0002147	0.008483	29.47	248.9
Personality dimensions	GWAS_Catalog_2019	0.0002256	0.008483	109.7	920.7

The items with q-value more than 0.05 are deleted.

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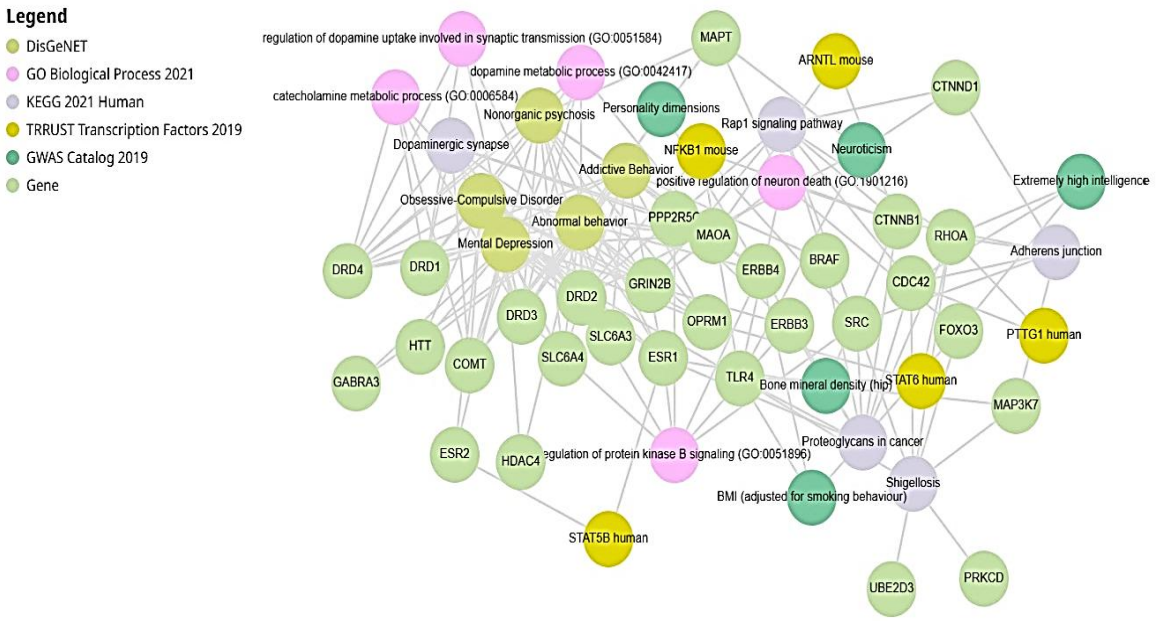

Figure 8. Multi-Omics results of 5 databases visualized by Enrichr-KG highlighting on Dopaminergic synapse processes, Mental Depression, Addictive behavior, Obsessive-Compulsive Disorder, dopamine receptor gene family, and GRIN2B gene as the high-scored connection zone.

Meta-Analysis by Metascape

Pathway and Process Enrichment Analysis

For each gene list, pathway and process enrichment analyses were performed using the following ontology sources: KEGG Pathway, GO Biological Processes, Reactome Gene Sets, Canonical Pathways, CORUM, and WikiPathways. Terms having a p-value < 0.01, a minimum count of 3, and an enrichment factor > 1.5 (the ratio between observed and predicted counts) are sorted into clusters based on membership similarities. More specifically, p-values were determined using the cumulative hypergeometric distribution [75], whereas q-values are derived using the Benjamini-Hochberg process to account for multiple testing . When doing hierarchical clustering on enriched phrases, Kappa scores [76] are used as the similarity measure, and sub-trees with a similarity greater than 0.3 are deemed clusters. The most statistically significant phrase inside a cluster is selected to represent the cluster (Table 7).

Table 7. Top 20 clusters with their representative enriched terms (one per cluster).

GO	Category	Description	Log10(P)	Log10(q)
GO:0071407	GO Biological Processes	cellular response to organic cyclic compound	-15.64	-11.30
GO:0042417	GO Biological Processes	dopamine metabolic process	-14.94	-10.90
GO:0007610	GO Biological Processes	Behavior	-14.11	-10.54

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hsa05205	KEGG Pathway	Proteoglycans in cancer	-12.50	-9.24
R-HSA-112315	Reactome Gene Sets	Transmission across Chemical Synapses	-11.42	-8.34
GO:0060322	GO Biological Processes	head development	-11.37	-8.34
R-HSA-5663202	Reactome Gene Sets	Diseases of signal transduction by growth factor receptors and second messengers	-11.13	-8.21
GO:0033674	GO Biological Processes	positive regulation of kinase activity	-10.95	-8.07
GO:0043410	GO Biological Processes	positive regulation of MAPK cascade	-10.74	-7.87
GO:0070201	GO Biological Processes	regulation of establishment of protein localization	-10.23	-7.44
M237	Canonical Pathways	PID VEGFR1 2 PATHWAY	-10.20	-7.44
WP399	WikiPathways	Wnt signaling pathway and pluripotency	-9.16	-6.54
R-HSA-5683057	Reactome Gene Sets	MAPK family signaling cascades	-9.11	-6.52
WP710	WikiPathways	DNA damage response only ATM dependent	-8.93	-6.38
GO:0097305	GO Biological Processes	response to alcohol	-8.30	-5.82
GO:0071363	GO Biological Processes	cellular response to growth factor stimulus	-7.74	-5.34
GO:0001775	GO Biological Processes	cell activation	-7.67	-5.29
R-HSA-1280215	Reactome Gene Sets	Cytokine Signaling in Immune system	-7.35	-5.02
GO:0010001	GO Biological Processes	glial cell differentiation	-7.21	-4.91

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WP4312	WikiPathways	Rett syndrome causing genes	-6.83	-4.63

"Log10(P)" is the p-value in log base 10. "Log10(q)" is the multi-test adjusted p-value in log base 10.

To further capture the relationships between the terms, a subset of enriched terms was selected and rendered with the best p-values from each of the 20 clusters, with the constraint that there are no more than 15 terms per cluster and no more than 250 terms in total. The network is envisaged by Cytoscape, where each node characterizes an enriched term and is colored first by its cluster ID and then by its p-value (Figure 9).

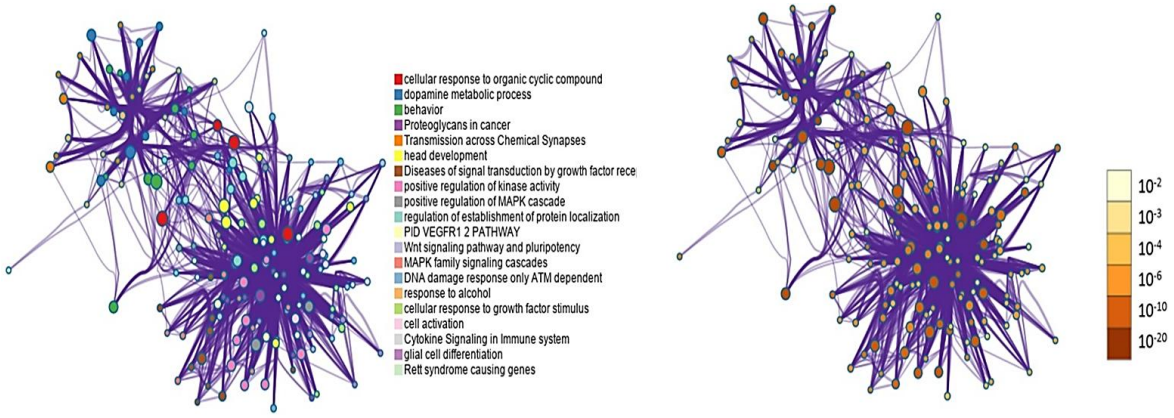

Figure 9. Network of enriched terms: (a) colored by cluster ID, where nodes that share the same cluster ID are typically close to each other; (b) colored by p-value, where terms containing more genes tend to have a more significant p-value.

PPI Enrichment Analysis

For the given gene list, PPI enrichment analysis was performed with the following databases: STRING, BioGrid [77], and OmniPath [78]. In WebIM. Only physical interactions in STRING (physical score > 0.132) and BioGrid were utilized. The resultant network consists of the subset of proteins form physical interactions with at least one other member in the list [79]. The MCODE networks [80] detected for the gene list have been collected and are shown in Figure 8. Pathway and process enrichment analysis used to each MCODE component independently, and the three best-scoring terms by p-value retained as the functional description of the corresponding components, described in the legend of Figure 10.

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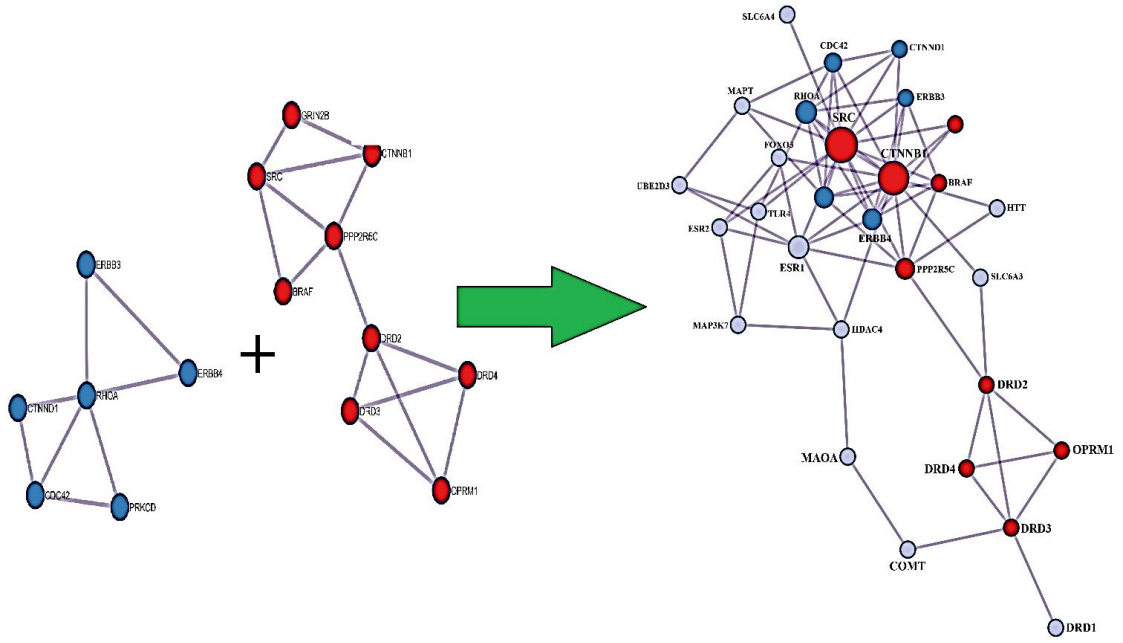

Figure 10. PPI network and MCODE components identified in the candidate genes list. The red circles represent MCODE_1 gene which stand for synaptic signaling [Log(P)= -11.2], Behavior [Log(P)= -10.3], and Dopaminergic synapse [Log(P)= -9.7]. The blue circles represent MCODE_2 genes which refer to Signaling by Receptor Tyrosine Kinases [Log(P)= -10.6], Signaling by ERBB2 [Log(P)= -10.0], and VEGFA-VEGFR2 Pathway [Log(P)= -8.8]. The right model resulted from the combination of MCODE_1 and MCODE_2 are as follows: cellular response to organic cyclic compound (GO:0071407) with a Log10(P) of -15.9, dopamine metabolic process (GO:0042417) with a Log10(P) of -15.1, and cellular response to organonitrogen compound (GO:0071417) with a Log10(P) of -14.5. .

PGx Variant Annotation Assessment (PGx-VAA)

PGx-VAA was conducted to find the strength and gaps of the gene towards the development of a novel personalized medicine treatment for patients undergoing ASD and to detect the risk alleles of ASD by an updated GARS test. To reach this goal, a wide screening was conducted on all 30 genes of interest and 1,419 annotations found. Among these annotations 571 were significant and reliable to follow for drug prescribing and risk allele genotyping. Notably, no significant PGx annotations were found for these genes: *CDC42, FOXO3, MAP3K7, PRKCD, ERBB4, UBE2D3, HDAC4, PPP2R5C, BRAF, HTT, GABRA3,* and *SRC*. According to the findings of the present investigation, there are major PGx suggestions which might signify these evidence-based in silico results and reveal the clinical gaps between ASD and RDS. These gaps can strongly be the PGx variations need to be tested in the future studies. For example, PPP2R5C indicated a powerful Clustered PPI contact with DRD2 and HDAC4 is link MAOA to ESR1 and CTNNB1. Concordantly with GWAS mining results, CTNNB1 was the top-scored gene of ASD. Regarding Figure 8, there are two axes which should be considered between ASD and RDS; The first axis is DRD1>DRD3>DRD2>PPP2R5C>CTNNB1>>>other genes and the second axis is DRD3>COMT>MAOA>HDAC4>[MAP3K7]-[ESR1]-[CTNNB1]>>other genes. Both of these axes are important and are highly recommended to be investigated in future studies; but the second axis contains interesting genes with epigenetic impacts such as COMT (a methyl transferase) and HDAC4 (a Histone deacetylase influences on Histone modifications).

Discussion

Our research elucidated the genetic architecture of Autism Spectrum Disorder (ASD) by leveraging GWAS. Utilizing the GWAS catalog, our goal was to comprehensively identify entries linked to ASD. This initial step involved aggregating genes listed under each relevant catalog ID (CID), resulting in a collection that included various gene types. Through a process of elimination

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that removed duplicates and non-protein-coding genes, we arrived at a curated list of protein-coding genes for deeper analysis. This method mirrors previously established approaches to explore pharmacogenomics potentials within the ASD context, particularly regarding the GARS panel [54–60]. The core of our analysis encompassed evaluating Protein-Protein Interactions (PPIs), Gene Regulatory Networks (GRNs), and interactions between genes, diseases, drugs, and chemicals. We utilized STRING-MODEL for PPIs and Cytoscape for examining signaling pathways. The analysis of GRNs was facilitated by miRTarBase, while interactions with drugs and chemicals were explored using DrugBank and the Comparative Toxicogenomics Database (CTD), respectively.

Our comprehensive review included 17,184 associations across 881 studies. After synthesizing this data, we refined our gene list down to 990 unique entries. Further validation through GeneCards (http://www.genecards.org/) led to a distilled list of 667 genes after the removal of RNA genes and pseudogenes. The most significant genes were identified using STRING-MODEL based on their network centrality, with the top 20 genes being CTNNB1, ESR1, RHOA, CDC42, FOXO3, MAP3K7, PRKCD, ERBB4, UBE2D3, MAPT, CTNND1, HDAC4, BRAF, ESR2, PPP2R5C, ERBB3, TLR4, HTT, SRC, and GRIN2B. These, along with 10 genes from the GARS panel, formed the basis of our subsequent analyses.

We observed notable interactions, particularly highlighting extensive engagement of hsa-miR-16-5p with several genes. The examination of protein-drug and protein-chemical interactions unearthed significant links, notably between ESR1 and various chemicals, with Aflatoxin B1 standing out due to its importance. Further in silico analyses integrating results from Reactome 2022, KEGG 2021, and Panther 2016, revealed the dopaminergic synapse pathway as highly significant. Metabolomics Workbench Metabolites 2022 data pinpointed dopamine as a critical metabolite in ASD and related disorders. Gene Ontology (GO) analyses highlighted the dopamine metabolic process as notably significant. Clinical databases such as DisGeNET and others underscored Obsessive-Compulsive Disorder as a highly associated phenotype. Our multi-omics analysis, utilizing five different libraries, solidified the conceptualization of ASD as an endophenotype. A meta-analysis via Metascape, employing several databases, further refined our understanding, identifying the dopamine metabolic process as crucially involved in ASD. This research, by integrating comprehensive genetic data and multiple analytical frameworks, enhances our understanding of ASD's genetic basis and its potential implications for therapeutic strategies.

Clinical Relevance

The Pharmacogenomics Variant Annotation Assessment (PGx-VAA) aimed to explore the potential and limitations of a gene set proposed in our study for pioneering personalized treatment approaches for ASD patients, alongside identifying ASD risk alleles using a refined GARS test. This comprehensive analysis covered 30 genes, yielding 1,419 annotations, of which 571 were deemed significant and potentially useful for therapeutic targeting and precision in medication dosing based on risk allele genotyping.

Significantly, no pharmacogenomics annotations were identified for a subset of genes including CDC42, FOXO3, MAP3K7, PRKCD, ERBB4, UBE2D3, HDAC4, PPP2R5C, BRAF, HTT, GABRA3, and SRC. These findings point to critical pharmacogenomics gaps in ASD and related disorders that future research must address. For instance, PPP2R5C shows a strong interaction with DRD2 in Clustered Protein-Protein Interactions (PPIs), and HDAC4 links MAOA with ESR1 and CTNNB1, the latter being highly implicated in ASD as noted in our GWAS analysis. Two key interaction pathways emerged from our study: one involving DRD1, DRD3, DRD2, PPP2R5C, CTNNB1, and other genes, and a second pathway connecting DRD3, COMT, MAOA, HDAC4, MAP3K7, ESR1, and CTNNB1 with other genes. These pathways, particularly the second with its epigenetic significance involving COMT and HDAC4, highlight areas for future investigation. The interactions suggest a complex network of pharmacogenomic influences warranting further exploration.

The complexity of therapeutic strategies in ASD patients is on an equal footing with the intricate nature of the ASD syndrome *per se*, environmental impacts, patients' personalities, and their

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neurobiological characteristics with predispositions. Personalized medicine is the fitting setting for matching the innovative therapeutic strategies to eligible candidates [23]. For instance, our team's previous success with Personalized Transcranial Magnetic Stimulation (PrTMS) in treating another disorder from the RDS continuum [81,82] namely post-traumatic stress disorder) supports the PrTMS potential utility in addressing RDS in ASD guided by the pharmacogenomic framework anchored in our findings.

A harm reduction approach employed in opioid addicted patients is an illicit opioid' substitution with an opioid drug with the with lesser addiction potential e.g., buprenorphine [83]. It is notable that buprenorphine, which has been shown to stabilize reward function [84,85] has also been successfully tried in an ASD patient [86]. It would be of interest to explore whether genetic testing may identify ASD patients who are eligible for the opioid medication assisted therapy. While the reasoning behind administering an addictive substance to individuals prone to addiction may appear counterintuitive, this method could be beneficial for patients experiencing significant RDS symptoms. In that regard, there are reports on a subgroup of depressed patients who failed the treatment with conventional antidepressants but had a beneficial response to buprenorphine [87].

There are non-addictive alternatives to opioids that may restore pathological states of reward deficiency [24,88] including antidepressants [89]. anticonvulsants and neuroleptics [88] as well as dietary supplement, Acetyl-L-carnitine [90], a neurotrophic, neuroprotective and antidepressant agent and a nutraceutical combination of DA precursors with inhibitors of the DA degrading enzyme Catechol-O- methyl transferase [91]. Antagonism of the N-Methyl-D- aspartate glutamate receptors is another strategy specifically effective for reward deficiency [92]. Reward deficiency can be also targeted by behavioral techniques e.g., Positive Affect Treatment purported to improve anticipatory, consummatory and learning reward components [93] as well as by the Positive Affect Stimulation and Sustainment therapy providing the tools for enhancing the salience and learning how to sustain positive affects [94].

To enhance readership comprehension, we hereby provide Table 8 demonstrating known clinical relevance for each gene we found in our study.

Table 8. clinical relevance for Autism genes we found in the PGX study.

GENE	CLINICAL RELEVANCE	SOURCE	COMMENT
CTNNB1	*CTNNB1* neurodevelopmental disorder (*CTNNB1*-NDD) is characterized in all individuals by mild-to-profound cognitive impairment	PMID: 35593792.	Up to 39% of reported individuals by exudative vitreoretinopathy, an ophthalmologic finding consistent with familial exudative vitreoretinopathy (FEVR).
ESR1	There is evidence for the role of Esr1^{+} neurons in aversion and sexually dimorphic stress sensitivity.	PMC10322719	Excitatory projections from the lateral hypothalamic area (LHA) to the lateral habenula (LHb) drive aversive responses
RHOA	Knockdown of RhoA in the dHIP enhanced METH-induced CPP, whereas RhoA	PMID: 34313802.	Findings indicate that the miR-31-3p/RhoA

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	overexpression attenuated the effects of METH.		pathway in the dHIP modulates METH-induced CPP in mice. Results highlight the potential role of epigenetics represented by non-coding RNAs in the treatment of METH addiction.
CDC42	cdc42-activated, nonreceptor tyrosine kinase, Ack1, is a DAT endocytic brake that stabilizes DAT at the plasma membrane and is released in response to PKC activation.	PMID: 26621748	Findings reveal a unique endocytic control switch that is highly specific for DAT.
FOXO3	Forkhead transcription factor (FOXO) family to be a downstream mechanism through which SIRT1 regulates cocaine action.	PMID: 25698746	Overexpressing FOXO3 in NAc enhances cocaine place conditioning
MAP3K7	The multi-component and multi-target properties of *Acanthopanax senticosus* (AS) play an important role in the alleviation of anxiety and memory impairment caused by AD, and the mechanism is involved in the phosphorylation and activation of the MAPK signaling pathway.	PMID: 38545117	The levels of MAP3K7 and P38 phosphorylation increased, and there was also an increase in the expression of HSP27 proteins.
PRKCD	It was reported that social choice-induced voluntary abstinence prevents incubation of methamphetamine craving in rats. This inhibitory effect was associated with activation of protein kinase-Cδ (PKCδ)-expressing neurons in central amygdala lateral division (CeL).	PMID: 32205443	shPKCδ CeL injections decreased Fos in CeL PKCδ-expressing neurons, increased Fos in CeM output neurons, and reversed the inhibitory effect of social choice-induced abstinence on incubated drug seeking on day 15.
ERBB4	Natural genetic variants of Neuregulin1 (NRG1) and its cognate receptor ErbB4 are associated with a risk for schizophrenia.	PMID: 32354758	Findings indicate that ErbB4 signaling affects tonic DA levels and modulates a wide array of behavioral

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			deficits relevant to psychiatric disorders, including schizophrenia.
UBE2D3	It is well known that dysfunction of the ubiquitin-proteasome protein degradation system (UPPDS) is one of the major mechanisms of the pathogenesis of PD.	PMID: 24993970	Decreasing transcript levels of genes may indicate decrease in the efficiency of the UPPDS on the whole which in turn may lead to the accumulation of abnormal proteins and toxic protein aggregates and subsequent death of the neurons.
MAPT	*MAPT* involves myelination, neurite elongation and guidance in cytoskeletal organization and is involved in structural connectivity	PMID: 38438384	Structural connectivity measures are highly polygenic
CTNND1	Functional and positional QTL gene-based approaches identified 249 significant candidate risk genes for OCD, of which 25 were identified as putatively causal, highlighting *WDR6*, *DALRD3*, *CTNND1* & genes in the MHC region	doi: 10.1101/2024.03.13.24304161.	Obsessive-compulsive disorder (OCD) affects ~1% of the population and exhibits a high SNP-heritability
HDAC4	One family of epigenetic molecules that may regulate maladaptive behavioral changes produced by cocaine use are the histone deacetylases (HDACs)-key regulators of chromatin and gene expression. In particular, class IIa HDACs (HDAC4, HDAC5, HDAC7 and HDAC9) respond to changes in neuronal activity by modulating their distribution between the nucleus and cytoplasm-a process controlled in large part by changes in phosphorylation of conserved residues.	PMID: 28635037	Despite high sequence homology, HDAC4 and HDAC5 are oppositely regulated by cocaine-induced signaling in vivo and have distinct roles in regulating cocaine behaviors.
BRAF	Expressing BRAF K499E (KE) in neural stem cells under the control of a Nestin-Cre	PMID: 39964758	Results demonstrate that ERK hyperactivity contributes to

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	promoter (Nestin;BRAFKE/+) induced hippocampal memory deficits		astrocyte dysfunction associated with Ca2+ dysregulation, leading to memory deficits of BRAF-associated RASopathies.
ESR2	Studies indicates that the epigenetic dysregulation of ESR2 may govern the development of autism.	PMID: 28299627	Detailed analyses revealed that eight specific CpG sites were hypermethylated in autistic individuals and that four specific CpG sites were positively associated with the severity of autistic symptoms.
PPP2R5C	Pathogenic variants resulting in protein phosphatase 2A (PP2A) dysfunction resulting in mild to severe neurodevelopmental delay.	PMID: 39978342	PPP2R5C total loss-of-function variants could be inherited from a non-symptomatic parent. This implies that a dominant-negative mechanism on substrate dephosphorylation or general PP2A function is the most likely pathogenic mechanism.
ERBB3	Significant gene-gene interactions were found between (i) *NRG1*MBP* (*perm p*-value = 0.002) in the SSD trios sample, (ii) *ERBB3*AKT1* (*perm p*-value = 0.001) in the SSD case-control sample, and (iii) *ERBB3*QKI* (*perm p*-value = 0.0006) in the ASD trios sample.	PMID: 34338147	Schizophrenia-spectrum disorders (SSD) and Autism spectrum disorders (ASD) are neurodevelopmental disorders that share clinical, cognitive, and genetic characteristics, as well as particular white matter (WM) abnormalities.

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TLR4	After TLR4 activation, CD14+ monocytes from autistic children produced increased IL-6 compared to monocytes from children with typical development. IL-6 concentration also correlated with worsening restrictive and repetitive behaviors.	PMID: 35203983	Findings suggest dysfunctional activation of myeloid cells, and may indicate that other cells of this lineage, including macrophages, and microglia in the brain, might have a similar dysfunction.
HTT	Prenatal alcohol exposure (PAE) results in cerebral cortical dysgenesis. AE inhibited *Bcl11a*, ***Htt***, *Ctnnb1*, and other upstream regulators of differentially expressed genes and inhibited several autism-linked genes, suggesting that neurodevelopmental disorders share underlying mechanisms.	PMID: 33997709	Episodic PAE persistently alters the developing neural transcriptome, contributing to sex- and cell-type-specific teratology.
SRC	Src protein tyrosine kinase plays an important role Opioid addiction	PMID: 34243625	Src inhibitors have the potential to be used in combination with opioids to achieve synergy
GRIN2B.	The N-methyl-D-aspartate (NMDA) glutamate receptors play important roles in the pathophysiology of substance dependence (SD). Results from gene-based association tests showed that the association signal derived mostly from GRIN2B.	PMID: 23855403	Rare variants (RVs) with minor allele frequency <1% in the NMDAR-related genes influence the risk of OD
GARS GENES	The GARS panel can accurately predict preaddiction	PMID: 28930612	Genetically identifying risk for all RDS behaviors, especially in compromised populations, may be a frontline tool to assist municipalities to provide better resource allocation.

Limitations

One potential issue with the utilization of large databank retrieval data may induce some concern but is currently unavoidable lies in the robustness of the GWAS location collected from the database. While computational and meta-analyses have been a mainstream analytical acceptable tool,

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we must point out that these loci may not always reflect consistently reliable across studies, given the inherent bias introduced by heterogeneous sample recruitment and ascertainment criteria. Certainly, these could involve variability in population demographics, diagnostic definitions, and phenotypic measurements across studies and might impact the reproducibility of GWAS findings. While Genetic liability and clinical heterogeneity in ASD are heavily influenced by sex, in the current study we did not explore in any detail the possibility of gender differences [95,96]. While we provided the various genes showing significant load onto the ASD phenotype, and of course the data in these utilized data banks report many annotations and risk polymorphisms, with known ASD risk genes or exhibit consistent cell-type-specific expression patterns relevant to ASD, the actual identification of all these risk polymorphisms per se was not the focus of the current study but will be addressed in future analyses. It is noteworthy that several postmortem studies, utilizing both bulk and single-cell RNA sequencing from fetal to adult stages, have demonstrated a functional convergence of ASD risk genes in pathways related to early neurogenesis, particularly during mid-fetal development [97,98].

Conclusions

Through data mining of GWAS and *in silico* analyses, this study uncovers genetic and epigenetic markers related to ASD, proposing the disorder as an endophenotype of RDS. Innovative methodologies, including protein-protein interactions, gene regulatory networks, and systems biology analyses, are employed to identify significant pathways and gene ontologies associated with ASD. The multi-omics analysis integrates findings across genomics, proteomics, metabolomics, and phenomics, reinforcing the concept of ASD as an endophenotype of RDS. This comprehensive approach offers new insights into the genetic architecture and molecular mechanisms of ASD, paving the way for personalized medicine strategies and novel therapeutic interventions.

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Conflicts of Interest: Prof. Kenneth Blum holds patents both domestic and foreign related to pro-dopamine regulation complexes and genetic testing for addiction risk. Through his company Synaptamine inc., licensed patents on KB220Z to Victory nutrition international, LLC. There are no other conflicts to report.

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