Research Article

A systematic computational analysis of pharmacological options in neuroinflammatory-induced autism spectrum disorder in children: A potential for drug repositioning

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Abstract

Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in social communication and the presence of restricted or repetitive behaviors. Although its underlying pathophysiological mechanisms remain unclear, growing evidence indicates that neuroinflammation plays a significant role, especially in children. **Objective:** This study aims to explore neuroinflammatory pathways in children aged 12 and under, with a focus on potential therapeutic opportunities through drug repositioning. **Methods:** We conducted a systematic computational analysis using data from 27 studies and bioinformatics resources such as DrugBank and PubChem, identifying over 8,000 potential drug candidates from the initial 29 treatments retrieved from the literature. **Results:** Key compounds such as cannabidiol, fluoxetine, and risperidone were highlighted for their broad therapeutic potential. In addition, emerging treatments, including cell-based therapies and dietary interventions, were explored. **Conclusion:** Our findings support drug repositioning as an effective strategy for developing new ASD treatments during critical developmental periods, emphasizing the need for further research to validate these pathways and the efficacy of innovative therapies.

Keywords: Brain, Bioinformatics, Data science, Inflammation, Pharmacology, Knowledge graph, Youth

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition, identifiable in children through challenges in social communication and restricted, repetitive patterns of behavior and interests.[1] Due to a spectrum of manifestations and severity levels, ASD is acknowledged as a heterogeneous phenotype, posing substantial issues for diagnosis and treatment.[2] Over recent decades, the prevalence of ASD has risen markedly. In the United States alone, rates have escalated from 1 in 150 children in 2000 to 1 in 36 in 2023, reflecting in part changes in diagnostic criteria and reporting practices.[3]

ASD frequently co-occurs uniquely or in combination with other neurodevelopmental disorders such as attention deficit/hyperactivity disorder (ADHD), intellectual disabilities, or specific learning disorders, complicating its clinical presentation and management.[4] In addition, children with ASD often exhibit various comorbidities such as epilepsy, sleep

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disturbances, anxiety, depression, bipolar disorder, sensory processing disorders, and schizophrenia. The complexity of ASD is further highlighted by findings that autistic children are 67% more likely to develop inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis, than their peers.[5] These overlapping conditions, particularly in cases of ADHD co-occurrence with Crohn's disease and psoriasis, where the prevalence is higher among girls, underscore the multifaceted nature of ASD.[4] The presence of these comorbidities and associated conditions not only complicates the diagnosis and treatment of ASD but also emphasizes the need for a multifaceted therapeutic approach. Understanding the broader neurodevelopmental and immunological context of ASD is critical for developing targeted treatments that address not just the core symptoms of ASD but also the complex interplay of co-occurring disorders and inflammatory pathways. This complexity is compounded by the fact that, despite extensive research efforts, the underlying causes of ASD remain elusive. There is still no consensus on the exact interplay of genetic, environmental, and biological factors.[6] Important environmental exposures, such as xenobiotics during prenatal life, heavy metals, and environmental toxicants during critical developmental periods, have been associated with ASD[7]. These exposures are thought to interfere with metabolic and biochemical pathways, potentially leading to oxidative stress, mitochondrial anomalies, and immune dysregulation.[8]

In recent years, the focus has shifted toward understanding the role of neuroinflammatory pathways in ASD. Studies have explored how activating the brain's immune system may alter neurological development and function. Evidence of immunological alterations, including atypical microglial activity and elevated cytokine levels, suggests that these disturbances may play a critical role in disrupting the neural circuits integral to ASD.[9-12]

In light of these findings, the potential for therapeutic interventions targeting neuroinflammatory pathways becomes crucial, especially since current treatments for ASD, particularly in pediatric populations, are largely aimed at symptom management to improve daily functioning and quality of life.[13] However, despite the adoption of holistic frameworks, ASD is still predominantly treated as a mental disorder, with a strong emphasis on cognitive-behavioral therapies. While these therapies, such as applied behavior analysis, have demonstrated some effectiveness, their success is often contingent on intensive and prolonged intervention, sometimes requiring up to 40 h/week, which can be difficult to sustain and may yield only modest improvements.[14,15] This reality underscores the urgent need for additional therapeutic strategies, including pharmacological interventions, which are increasingly recognized as vital for managing core and co-occurring symptoms like irritability and anxiety.[14,16,17]

One promising strategy in ASD treatment is drug repositioning. This approach leverages existing knowledge of the safety and pharmacokinetic profiles of approved drugs, reducing both the cost and time required for development, while facilitating quicker access to effective treatments.[10,18] Given the complexity of ASD, repositioning existing drugs to target well-understood biological pathways, including those involved in neuroinflammation, holds significant promise.[11,19] For example, drugs initially developed for other conditions, such as anti-inflammatory or immunomodulatory agents, may offer therapeutic benefits for targeting neuroinflammatory pathways implicated in ASD.[20]

To explore this potential, this study aims to develop a systematic computational approach, investigating the role of neuroinflammation in ASD and the potential for drug repositioning in children with ASD. The focus is on three key elements: therapeutic categories, protein targets, and biological pathways.

2. Methodology

2.1. Study design and data sources

This study involved a comprehensive two-tiered selection process (Figure 1) of original research studies and systematic reviews, focusing on neuroinflammation in ASD among children aged ≤12 years. This approach was taken to reduce the clinical and biological heterogeneity of the analyzed data. ASD presents considerable variability depending on age, developmental stage, and the co-occurrence of comorbidities. Expanding the age range would have introduced an additional layer of complexity, making the systematic computational analysis less consistent. Thus, we opted to focus on the pre-adolescent period to better circumscribe our study population. By targeting a relatively homogeneous age group, we were able to structure the data around therapeutic categories, proteomic targets, and biological pathways that are specific to this developmental phase. Finally, this choice enabled the formulation of therapeutic hypotheses that were more relevant for early intervention strategies, which are currently a priority in the management of ASD.

The selection of studies was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to ensure rigorous methodology (Figure 2).

2.1.1. Primary data sources

Initially, we identified relevant original research studies using predefined inclusion criteria (i.e., children aged ≤12 years and studies published in English between 2008 and 2023) by targeting various study designs such as case studies, observational reports, and randomized controlled trials. Non-human experimental studies, research involving

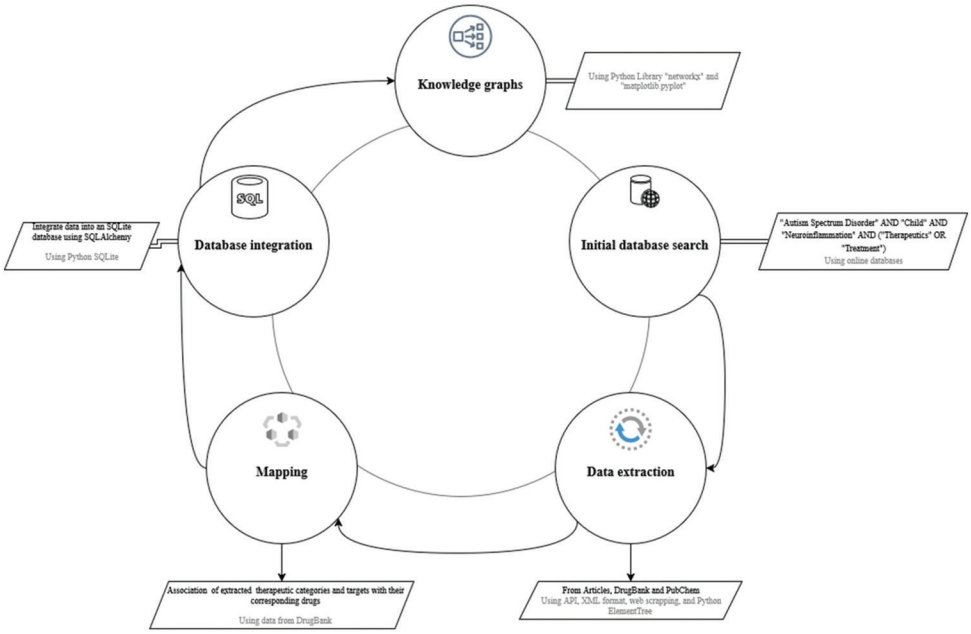

Figure 1. Overview of the methodological steps

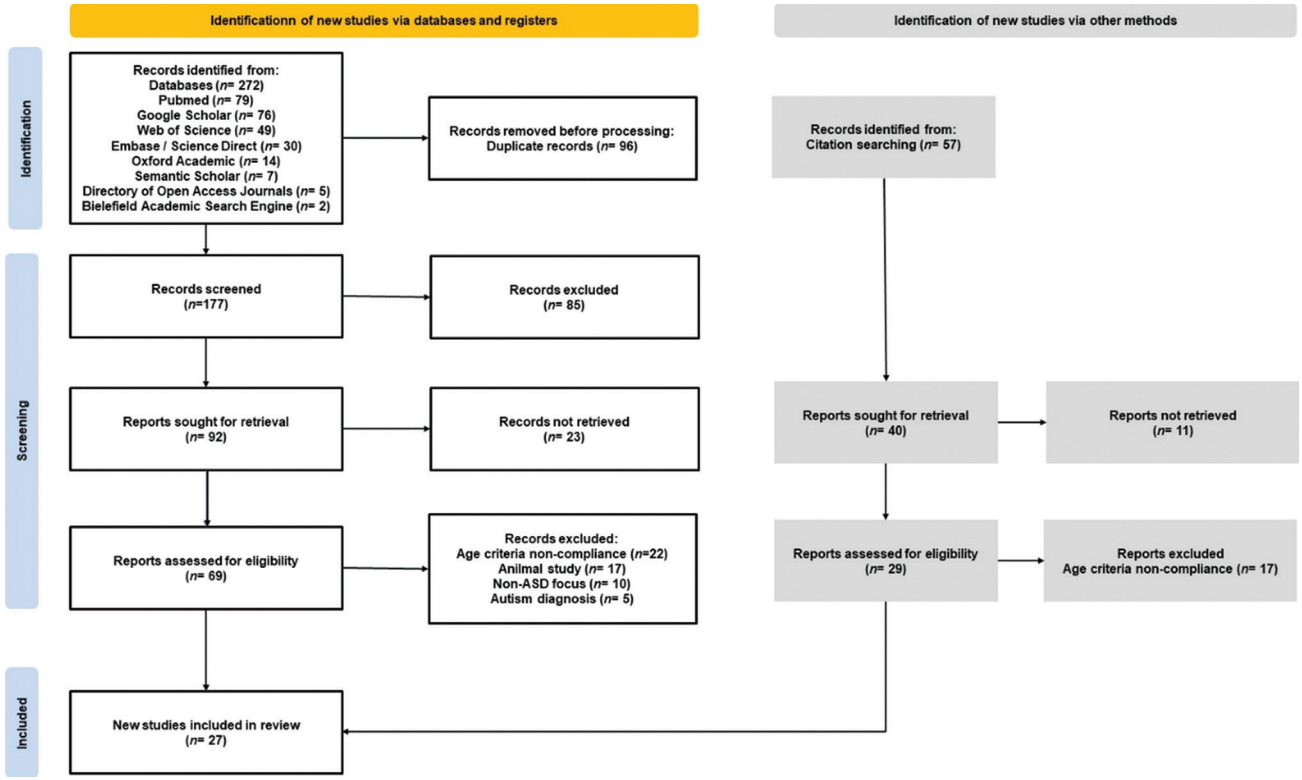

Figure 2. Flowchart for the selection of studies
Abbreviation: ASD: Autism spectrum disorder.

adolescents or adults with ASD, and studies focusing solely on behavioral therapies without addressing neuroinflammation or biomedical treatments were excluded.

The search for relevant studies employed the following search equation: "Autism Spectrum Disorder" AND "Child" AND "Neuroinflammation" AND ("Therapeutics" OR "Treatment"). Supplementary methods, including citation searching, were also used. Duplicate records were removed, and the remaining records underwent title and abstract screening. Full-text assessments were independently conducted by two authors to determine eligibility, with discrepancies resolved through discussion.

2.1.2. Data mapping process

To enhance the clarity and comprehensiveness of our data analysis, we developed a methodological approach involving a systematic data mapping process. This process included two main steps:

(i) Data extraction from articles: We began by extracting the treatments described in the selected articles, focusing on their specific components (e.g., drugs or nutritional supplements). For each component, we retrieved therapeutic categories, proteomic targets, and biological pathways using data from DrugBank, PubChem, and the Small Molecule Pathway Database (SMPDB), respectively.

(ii) Cross-referencing with external data sources: In parallel, we identified additional therapeutic categories, proteomic targets, and biological pathways that corresponded to the retrieved components. The additional data points helped identify new drugs linked to the same therapeutic categories, proteomic targets, or pathways, which were not initially part of the treatment components. These drugs were considered potential candidates for therapeutic repositioning. We then integrated this information by mapping drug categories, targets, and pathways to their respective drugs through XML scans and cross-referencing with DrugBank and SMPDB.

2.1.3. Database management

The extracted and processed data were integrated into an SQLite database (version 3.38.4) managed through SQLAlchemy (version 2.0.30), an object-relational mapping library for Python (version 3.11.0). This database was designed not just as a data repository but as a comprehensive knowledge base, facilitating the analysis of complex relationships between various treatments and their biological pathways. Custom scripts ensured efficient data insertion and updates.

2.1.4. Secondary data sources

The secondary data sources consisted of systematic reviews, meta-analyses, and other secondary sources used to elucidate the biological mechanisms underlying neuroinflammation in ASD. Specifically, 13 secondary articles were reviewed to identify and extract relevant biological pathways. From these articles, we predefined six general biological pathways: "inflammation," "immune system," "oxidative stress," "neurotransmitter regulation," "gene expression regulation," and the "gut–brain axis" based on the following criteria:

(i) Relevance to ASD: Pathways were considered relevant if they were directly linked to key aspects of ASD pathology or symptomatology, such as behavioral regulation, neurodevelopment, or immune responses

(ii) Frequency of mention: We prioritized pathways that were mentioned in multiple articles, suggesting a consensus or recurring focus in the literature

(iii) Impact on neuroinflammation: Pathways were selected based on their demonstrated or hypothesized role in modulating neuroinflammatory processes, particularly those known to affect neural circuits involved in ASD.

While these articles provided important context and background, they were not included in the final core study count. Instead, they served to inform our understanding of the broader biological mechanisms and guided the interpretation of findings from primary research.

2.2. Data collection and processing

Study metrics, protocols, and treatments were extracted from the selected articles. Two co-authors independently reviewed the full texts to ensure data accuracy and relevance. The names of the drugs or nutritional supplements used in the treatments were standardized using the DrugBank database.

2.2.1. Pathway classification

Following the identification of relevant compounds and building on the foundational knowledge provided by the 13 key articles, as well as additional information from scientific literature and the SMPDB, keywords and actions associated with each compound were extracted to align them with the predefined pathways.

This categorization process was conducted by two independent authors who identified specific terms describing each compound's action or mechanism. For treatments and compounds with data available in the SMPDB, relevant biological mechanisms were identified. When specific pathway data for a compound were not available in SMPDB, a literature search was conducted to find pertinent information using search terms like "treatment_name AND biological pathway" or "treatment_name AND mechanism of action." Each compound's alignment to a pathway was supported by bibliographic sources or SMPDB data.

2.2.2. Data retrieval

Based on the identification of relevant compounds, Python scripts were used to parse and retrieve detailed information from DrugBank academic resources and data from PubChem. Specifically, the "requests" library (version 2.31.0) was employed to interact with the PubChem application programming interface, fetching target data for specific PubChem Compound IDs to ensure clinical relevance. The Python ElementTree XML parser was utilized to extract drug information from DrugBank XML files, focusing on drug

categories. Predefined drug IDs were referenced to identify and extract relevant categories accurately.

2.2.3. Data analysis and visualization

To enhance data analysis and visualization, knowledge graphs were constructed using the networkx library (version 3.3) and visualized using matplotlib.pyplot (version 3.8.3). To explore potential drug repositioning for ASD, three types of knowledge graphs were created for each treatment: therapeutic categories, biological pathways, and proteomic targets.

The therapeutic categories graph was displayed according to its "components" (breaks down the treatment into main components); "categories" (link components to therapeutic categories); "associated drugs" (i.e., additional drugs in each category for repositioning); and "reported effects" (i.e., summary of therapeutic effects observed). The biological pathways graph was composed with its "components" (details about main treatment components); "biological pathways keywords" (link components to keywords describing their biological actions); "biological pathways box" (connection of predefined pathways); "SMPDB pathways and drugs" (i.e., additional drugs associated with the same pathways); and "reported effects." Finally, the proteomic targets graph included its "components" (outline of main treatment components); "targets" (links of components to proteomic targets); "associated drugs" (depicting additional drugs targeting the same proteomic entities); and "reported effects."

However, a comprehensive dashboard using Python and JavaScript (version 8.0 for Chrome 140) was developed to visualize the full spectrum of outcomes. The interactive dashboard is available at https://autism-research.univ-lille.fr/index.html, while certain advanced features can be provided on request. This interactive interface should allow users to progressively select and explore the full range of information of interest, enabling them to examine specific pathways, proteomic targets, and therapeutic categories. The dashboard could generate a list of drugs associated with these elements, providing a more detailed exploration and a user-friendly navigation. Additional development was conducted on common proteomic targets to assist in identifying treatments from the selected studies that share common proteomic targets.

3. Results

3.1. Data sources

We identified 27 studies focusing on ASD interventions (Figure 2), sourced from peer-reviewed journals. These studies were conducted across multiple continents, with the largest contributions from the United States ($n = 12$), followed by New Zealand ($n = 2$), Brazil ($n = 2$), Iran ($n = 2$), China ($n = 2$), and single studies from Jordan ($n = 1$), France ($n = 1$), Italy ($n = 1$), Chile ($n = 1$), Poland ($n = 1$), Slovakia ($n = 1$), and Vietnam ($n = 1$).

Our dataset included a mix of randomized controlled trials ($n = 17$), case reports ($n = 2$), *in vitro* studies ($n = 1$), and observational studies ($n = 8$). More detailed information about the study designs is available in Table S1.

3.2. Knowledge base

As shown in Table 1, the integrated analysis revealed over 8,000 potential drug candidates: 8,093 through therapeutic categories, 1,373 through proteomic targets, and 64 via pathways.

The resulting knowledge base consisted of 29 unique treatments, each corresponding to a distinct compound. Therapeutic categories were identified for 22 compounds, while seven (e.g., ketogenic diet, umbilical cord blood cells, and high-protease pancreatic enzyme) lacked categorization. Proteomic targets were available for 20 compounds; no targets were retrieved for nine, including valproic acid and Vitamin D3. Biological pathways remained undefined for six compounds due to limited data. Pathway information from SMPDB was available for 11 compounds (fluoxetine, risperidone, memantine, methylphenidate, atomoxetine, Vitamin D3, loxapine, oxytocin, bumetanide, prednisolone, and docosahexaenoic acid), allowing a comprehensive mapping of associated drugs.

The knowledge base also contained extensive, specific details for several compounds associated with numerous therapeutic categories, proteomic targets, and drug candidates for repurposing (Table 2).

3.3. Knowledge graphs

Using the available database information, several types of knowledge graphs were created at different levels of granularity. To ensure clarity and readability, the amount of information displayed on the graphs was intentionally limited. Comprehensive details are available in the interactive dashboard developed in this study. Examples of graphs related to the combined treatment of sulforaphane and risperidone for its "therapeutic categories" (Figure 3), "biological pathways" (Figure 4), and "proteomic targets" (Figure 5).

3.3.1. Therapeutic categories graph

The therapeutic categories graph (Figure 3) starts by decomposing the combined treatment of sulforaphane and risperidone into its main components. Sulforaphane is linked to antineoplastic and anticarcinogenic agents. Risperidone is associated with adrenergic alpha-1 receptor antagonists and

Table 1. Summary of potential drug candidates by therapeutic categories, proteomic targets, and biological pathways

Treatment	Compound	Therapeutic categories total	Proteomic targets total	Biological pathways total	Number of potential drugs through categories	Number of potential drugs through proteomic targets	Number of potential drugs through pathways
Methylphenidate	Methylphenidate	20	4	2	3,160	168	17
Memantine	Memantine	23	38	1	2,031	304	5
Atomoxetine	Atomoxetine	20	19	1	2,747	241	16
Fluoxetine+cyamemazine+valproic acid	Valproic acid	71	0	0	3,742	0	0
	Cyamemazine	11	4	0	3,062	157	0
	Fluoxetine	51	33	2	3,230	460	18
Fluoxetine+risperidone+loxapine	Loxapine	27	34	1	2,945	514	0
	Fluoxetine	51	33	2	3,230	460	18
	Risperidone	45	38	2	2,605	508	14
Fluoxetine+risperidone	Fluoxetine	51	33	2	3,230	460	18
	Risperidone	45	38	2	2,605	508	14
Fluoxetine+risperidone+melatonin	Melatonin	24	27	0	4,524	323	0
	Fluoxetine	51	33	2	3,230	460	18
	Risperidone	45	38	2	2,605	508	14
Controlled-release melatonin	Melatonin	24	27	0	4,524	323	0
N-acetylcysteine	Acetylcysteine	22	13	0	3,376	68	0
Buspirone	Buspirone	25	22	0	2,584	283	0
Intranasal oxytocin	Oxytocin	18	4	1	2,376	16	4
Vitamin D3	Vitamin D3	0	0	1	0	0	9
DHA; an omega-3 fatty acid	Doconexent	14	13	1	1,719	197	13
Vitamin D3+DHA	Vitamin D3	0	0	1	0	0	9
	Doconexent	14	13	1	1,719	197	13
Cannabidiol	Cannabidiol	59	66	0	2,456	565	0
Sulforaphane+risperidone	Sulforaphane	6	1	0	2,154	0	0
	Risperidone	45	38	2	2,605	508	14
Sulforaphane	Sulforaphane	6	1	0	2,154	0	0
Memantine+risperidone	Risperidone	45	38	2	2,605	508	14
	Memantine	23	38	1	2,031	304	5
Prednisolone	Prednisolone	45	7	2	3,053	93	18
Bumetanide	Bumetanide	30	24	1	3,087	112	8
Ketogenic diet+modified Atkins diet+low glycemic index treatments	Low glycemic index treatments	0	0	0	0	0	0
	Ketogenic diet	0	0	0	0	0	0
	Modified Atkins diet	0	0	0	0	0	0
Ubiquinol	Ubidecarenone	16	6	0	1,007	24	0
adrenal corticosteroid	Prednisolone	45	7	2	3,053	93	18
Luteolin	Luteolin	6	18	0	1,836	20	0
Umbilical cord blood infusion	Umbilical cord blood cells	0	0	0	0	0	0
Chondroitin sulfate+phosphatidylcholine+vitamin D3	Lecithin	13	1	0	1,273	0	0
	Vitamin D3	0	0	1	0	0	9
	Chondroitin sulfate	8	0	0	1,142	0	0
High protease pancreatic therapy	High-protease pancreatic enzyme	0	0	0	0	0	0
Single infusion of autologous umbilical cord blood	Autologous umbilical cord blood	0	0	0	0	0	0
Autologous bone marrow mononuclear cells	Autologous bone marrow mononuclear cells	0	0	0	0	0	0
Global	All				8,093	1,373	64

adrenergic antagonists. Each therapeutic category is linked to two additional drugs.

For example, under antineoplastic agents, 10-hydroxycamptothecin and 2-(4-chlorophenyl)-5-

quinoxalinecarboxamide are listed. For adrenergic alpha-1 receptor antagonists, alfuzosin and acepromazine were mentioned. The therapeutic categories then converge into a "reported effects" box, showing improvements in irritability and hyperactivity/noncompliance. This graph suggested new candidates for ASD treatment, such as 10-hydroxycamptothecin and alfuzosin, which share therapeutic categories with existing ASD treatments.

Table 2. Selection of identified compounds/treatments and samples of associated drug candidates for repurposing

Compound/Treatment	Examples of candidates
Valproic acid	• Tacedinaline • Fimepinostat
Cannabidiol	• Theobromine • Naxifylline • Theophylline
Fluoxetine	• Tetrahydrobiopterin • Pyridoxal 5'-phosphate
Risperidone	• Carbamoylcholine • Fesoterodine
Prednisolone	• Paromomycin • Troxipide
Bumetanide	• Lornoxicam • Salsalate

3.3.2. Biological pathways graph

Figure 4 illustrates an example of a biological pathways graph, which first links the components sulforaphane and risperidone to specific biological actions. Sulforaphane was associated with actions such as upregulating antioxidants, activating nuclear factor erythroid 2-related factor 2, inhibiting nuclear factor kappa B, enhancing extracellular signal-regulated kinase, increasing neuronal autophagy flux, and reducing interleukin 6/tumor necrosis factor alpha and interleukin 1 beta. These actions were connected to the predefined pathways, including oxidative stress, inflammation, neurotransmitter, and immune system pathways. Risperidone was linked to inhibiting D2 dopaminergic receptors and reducing dopaminergic neurotransmission. These actions were associated with the neurotransmitter pathway. The

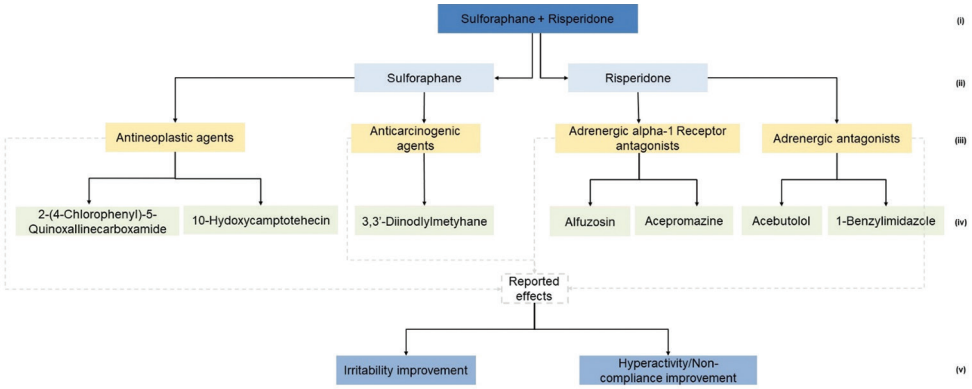

Figure 3. Therapeutic categories graph for sulforaphane + risperidone treatment. The numbers point to a given category level as follows: (i) Treatement; (ii) Compound; (iii) Therapeutic categories; (iv) Associated drug; (v) Observed effects.

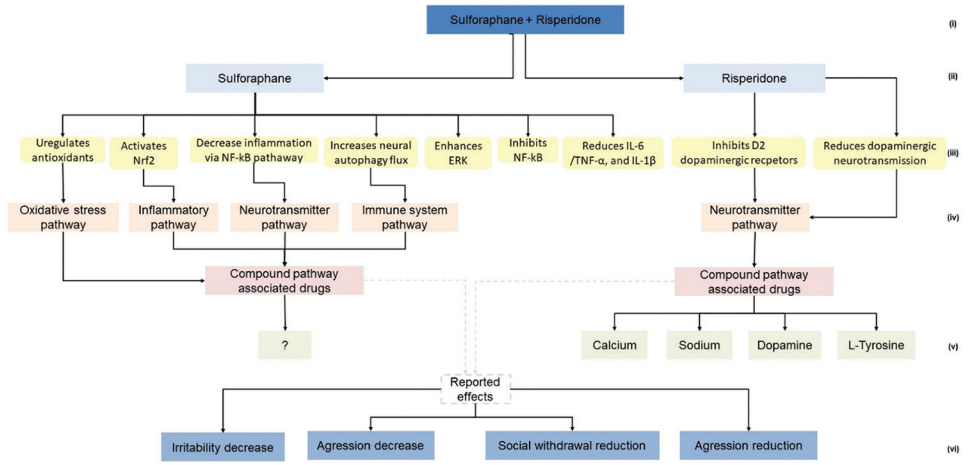

Figure 4. Biological pathways graph for sulforaphane + risperidone treatment. The numbers point to a given category level as follows: (i) Treatement; (ii) Compound; (iii) Pathway information; (iv) Compound global pathway; (v) Associated drugs; (vi) Observed effects.
Abbreviations: ERK: Extracellular signal-regulated kinase; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; NF-κB: Nuclear factor kappa B; Nrf2: Nuclear factor erythroid 2-related factor 2; TNF-α: Tumor necrosis factor-alpha.

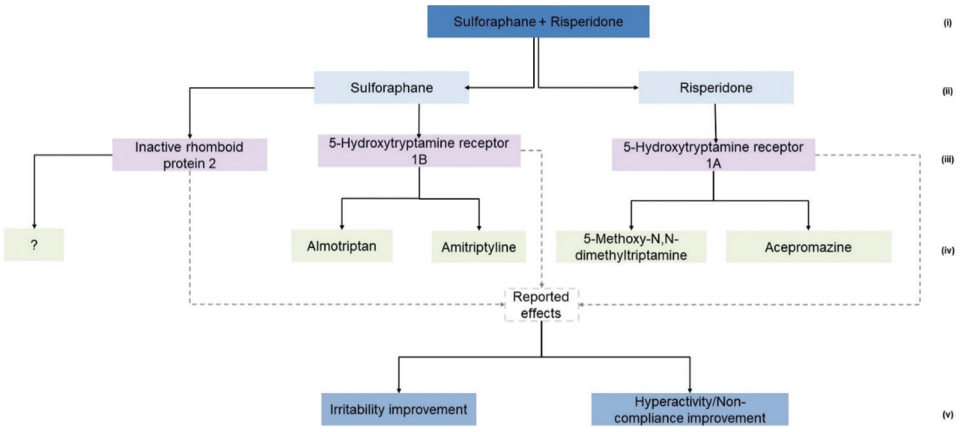

Figure 5. Proteomic targets graph for sulforaphane + risperidone treatment. The numbers point to a given category level as follows: (i) Treatement; (ii) Compound; (iii) Proteomic targets; (iv) Target-associated drugs; (v) Observed effects.

global biological pathways box combined these actions and pathways into a summarized view of the affected pathways. For risperidone, the graph included connections to drugs linked to the same SMPDB pathway. Where the SMPDB global compound pathway information for sulforaphane was missing, a question mark indicated the absence of data. The global pathways ultimately converged into a "reported effects" box, illustrating observed improvements in irritability and hyperactivity/non-compliance.

This graph identifies potential repositioning candidates such as calcium and carbon dioxide, which share biological pathways with risperidone but have not yet been tested for ASD.

3.3.3. Proteomic targets graph

In Figure 5, the specific proteins targeted by sulforaphane and risperidone were identified. Sulforaphane targets included inactive rhomboid protein 2, while Risperidone targets 5-hydroxytryptamine receptors 1A and 1B. Each target was linked to additional drugs that interacted with the same proteins. For inactive rhomboid protein 2, a question mark indicated the absence of available data on drugs targeting this proteomic target, while for the 5-hydroxytryptamine receptors, linked drugs include 5-methoxy-N, N-dimethyltryptamine and acepromazine.

This graph suggests repositioning candidates like 5-methoxy-N, N-dimethyltryptamine, which targets proteins similar to risperidone but has not been tested for ASD. Additional examples of different graphs are available on the study's dashboard.

3.4. Common proteomic targets

A detailed analysis of common proteomic targets among various treatments was conducted to assist in identifying treatments that share these targets. The findings for cannabidiol treatment, compared to the 28 other treatments, are summarized in Table 3.

Cannabidiol and memantine shared eight common targets, including neuronal acetylcholine receptor subunit alpha-7 and cytochrome P450 2D6. Both treatments have shown improvements in social interaction. Cannabidiol was also involved in reducing anxiety and psychomotor agitation, while memantine was linked to improvements in communication.

Cannabidiol and the combination of fluoxetine, cyamemazine, and valproic acid shared 10 common targets, including cytochrome P450 2C9 and adenosine triphosphate-dependent translocase ABCB1. Both treatments reduced anxiety and improved social interaction. Cannabidiol was also associated with an increased number of daily meals and enhanced concentration, while the combination treatment demonstrated decreases in self-injurious behavior.

Cannabidiol and the combination of fluoxetine, risperidone, and loxapine shared 11 common targets, including cytochrome P450 2C9 and adenosine triphosphate-dependent translocase ABCB1. Both treatments improved social interaction and reduced aggressive behaviors. In addition, cannabidiol demonstrated reductions in anxiety and psychomotor agitation, while the combination treatment led to decreases in risperidone dosages.

Moreover, cannabidiol and the combination of fluoxetine, risperidone, and melatonin shared 13 common targets, including cytochrome P450 2C9 and adenosine triphosphate-dependent translocase ABCB1. Both treatments demonstrated improvements in social interaction and reductions in anxiety. Cannabidiol also showed an increased number of daily meals and enhanced concentration, while the combination treatment exhibited decreases in ADHD-like symptoms.

Cannabidiol, memantine, and risperidone shared 13 common targets, including neuronal acetylcholine receptor subunit alpha-7 and adenosine triphosphate-dependent

Table 3. Comparison of shared proteomic targets between cannabidiol and other treatments

Main treatment	Comparator	Total common targets	Names of the targets
Cannabidiol	Methylphenidate	1	5-hydroxytryptamine receptor 1A
Cannabidiol	Memantine	8	Neuronal acetylcholine receptor subunit alpha-7; Cytochrome P450 2A6;Glycine receptor subunit alpha-1; Glycine receptor subunit beta; Cytochrome P450 2C19;Cytochrome P450 2B6;Glycine receptor subunit alpha-3; 5-hydroxytryptamine receptor 3A
Cannabidiol	Atomoxetine	2	Cytochrome P450 2D6; Cytochrome P450 2C19
Cannabidiol	Fluoxetine+cyamemazine+valproic acid	10	Cytochrome P450 2C9; ATP-dependent translocase ABCB1; Cytochrome P450 1A2; Cytochrome P450 3A4; 5-hydroxytryptamine receptor 2A; Cytochrome P450 3A5; Cytochrome P450 2D6; Cytochrome P450 2C19; Cytochrome P450 2B6; 5-hydroxytryptamine receptor 1A
Cannabidiol	Fluoxetine+risperidone+loxapine	11	Cytochrome P450 2C9; ATP-dependent translocase ABCB1; Cytochrome P450 1A2; Cytochrome P450 3A4; 5-hydroxytryptamine receptor 2A; Cytochrome P450 3A5; Cytochrome P450 2D6; Cytochrome P450 2C19; Cytochrome P450 2B6; 5-hydroxytryptamine receptor 3A; 5-hydroxytryptamine receptor 1A
Cannabidiol	Fluoxetine+risperidone	10	Cytochrome P450 2C9; ATP-dependent translocase ABCB1; Cytochrome P450 1A2; Cytochrome P450 3A4; 5-hydroxytryptamine receptor 2A; Cytochrome P450 3A5; Cytochrome P450 2D6; Cytochrome P450 2C19; Cytochrome P450 2B6; 5-hydroxytryptamine receptor 1A
Cannabidiol	Fluoxetine+risperidone+melatonin	13	Cytochrome P450 2C9; ATP-dependent translocase ABCB1; Indoleamine 2,3-dioxygenase 1; Cytochrome P450 1A2; Cytochrome P450 3A4; Cytochrome P450 1A1; 5-hydroxytryptamine receptor 2A; Cytochrome P450 3A5; Cytochrome P450 2D6; Cytochrome P450 2C19; Cytochrome P450 2B6; Cytochrome P450 1B1; 5-hydroxytryptamine receptor 1A
Cannabidiol	Controlled-release melatonin	7	Cytochrome P450 2C9; Indoleamine 2,3-dioxygenase 1; Cytochrome P450 1A2; Cytochrome P450 1A1; 5-hydroxytryptamine receptor 2A; Cytochrome P450 2C19; Cytochrome P450 1B1
Cannabidiol	Buspirone	8	ATP-dependent translocase ABCB1; Cytochrome P450 3A7; Cytochrome P450 3A4; 5-hydroxytryptamine receptor 2A; Cytochrome P450 3A5; Cytochrome P450 2D6; 5-hydroxytryptamine receptor 3A; 5-hydroxytryptamine receptor 1A
Cannabidiol	DHA (an omega-3 fatty acid)	4	Prostaglandin G/H synthase 1; Cytochrome P450 2C9; Peroxisome proliferator-activated receptor gamma; Prostaglandin G/H synthase 2
Cannabidiol	Vitamin D3+DHA (an omega-3 fatty acids)	4	Prostaglandin G/H synthase; Cytochrome P450 2C9; Peroxisome proliferator-activated receptor gamma; Prostaglandin G/H synthase 2
Cannabidiol	Sulforaphane+Risperidone	5	ATP-dependent translocase ABCB1; Cytochrome P450 3A4; 5-hydroxytryptamine receptor 2A; Cytochrome P450 2D6; 5-hydroxytryptamine receptor 1A
Cannabidiol	Memantine+Risperidone	13	ATP-dependent translocase ABCB1; Neuronal acetylcholine receptor subunit alpha-7; Cytochrome P450 3A4; 5-hydroxytryptamine receptor 2A; Glycine receptor subunit alpha-1; Cytochrome P450 2D6; Glycine receptor subunit beta; Cytochrome P450 2C19; Cytochrome P450 2B6; Cytochrome P450 2A6; Glycine receptor subunit alpha-3; 5-hydroxytryptamine receptor 3A; 5-hydroxytryptamine receptor 1A
Cannabidiol	Prednisolone	2	ATP-dependent translocase ABCB1; Cytochrome P450 3A4
Cannabidiol	Bumetanide	1	Prostaglandin G/H synthase 2
Cannabidiol	Ubiquinol	2	3-hydroxy-3-methylglutaryl-coenzyme A reductase; ATP-dependent translocase ABCB1
Cannabidiol	Adrenal corticosteroid	2	ATP-dependent translocase ABCB1; Cytochrome P450 3A4

Abbreviations: ATP: Adenosine triphosphate; DHA: Docosahexaenoic acid.

translocase ABCB1. Both treatments showed reductions in irritability and stereotypic behavior. In addition, cannabidiol demonstrated improvements in social interaction, while the combination treatment was linked to reductions in hyperactivity.

4. Discussion

This study provides a comprehensive analysis of neuroinflammation's role in ASD and explores the potential for drug repositioning in pediatric populations. By focusing on children aged 12 years or younger, we targeted a critical period of brain development, where interventions could have the most profound impact on long-term outcomes. This age group was chosen specifically because early childhood is a period of significant neurodevelopmental plasticity, during which therapeutic interventions might alter developmental trajectories more effectively than in older populations[21]. This approach, while narrowing the generalizability to older individuals, ensures that the findings are particularly relevant to the most vulnerable and developmentally significant phase of life.

Our results underscore the complexity of ASD and its associated neuroinflammatory pathways, reinforcing the multifaceted nature of the disorder. We identified more than 8,000 potential drug candidates through therapeutic categories, proteomic targets, and biological pathways, highlighting the

vast potential for drug repositioning. This approach leverages existing drugs with known safety profiles, reducing the time and cost associated with developing new therapies. Notably, drugs such as cannabidiol, fluoxetine, and risperidone were mapped to multiple therapeutic categories and proteomic targets, indicating their broad potential utility in treating ASD symptoms in this population. For example, cannabidiol interacts with the adenosine receptor A1 and the serotonin receptor 5-HT1A, which may contribute to its anxiolytic and neuroprotective effects. Fluoxetine, a selective serotonin reuptake inhibitor, modulates serotonin levels, potentially improving mood and social behavior by enhancing synaptic plasticity. Risperidone, an atypical antipsychotic, antagonizes dopamine D2 and serotonin 5-HT2A receptors, reducing irritability and aggression in ASD. The common proteomic targets shared between these drugs, such as the serotonin receptor pathways, suggest potential synergistic effects that could stabilize neurotransmitter imbalances and modulate neuroinflammatory responses in ASD. Exploring these shared targets may lead to new combination therapies that maximize efficacy while minimizing side effects. Further clinical studies should investigate how these mechanisms interact in the context of neurodevelopment to refine and optimize treatment strategies for ASD. This aligns with the growing body of evidence supporting the role of neurotransmitter regulation and neuroinflammatory pathways in ASD.[22] Another critical next step is the experimental validation of the identified drug candidates through *in vivo/in vitro* assays, as well as robust clinical trials.

One of the primary limitations of this study is the age range restriction, which, while deliberate, limits the applicability of the findings to older populations. This focus on early childhood, however, is justified by the critical nature of this developmental period, where the brain is most malleable and responsive to interventions.[23] Future studies could expand the age range to assess whether the identified treatments maintain their efficacy in older children and adolescents, providing a more comprehensive understanding of the developmental trajectory of ASD and the long-term effects of these interventions.

Another limitation is the inherent bias introduced by excluding studies that did not report significant effects. While this exclusion criterion is necessary for identifying effective treatments, it potentially overlooks important data on less successful or neutral outcomes. Including these studies in future analyses could offer a more balanced view of the efficacy and limitations of various treatments, contributing to a more nuanced understanding of ASD therapies.

Furthermore, the selection of biological pathways in our study, while based on current scientific understanding, also presents a limitation. The pathways, such as inflammation, immune system, oxidative stress, neurotransmitter regulation, gene expression regulation, and the gut–brain axis, were chosen due to their frequent association with ASD in existing literature.[13,24] However, the empirical validity of these pathways as central mechanisms in ASD remains an area of ongoing research and debate. While these pathways are supported by some studies, they may not fully encapsulate the complexity of ASD's pathophysiology. Future research should validate these pathways through more extensive empirical studies to ensure they represent the most relevant biological processes in ASD.[25]

In addition, to provide a clear and concise visual representation of the results, we initially presented the graphs with a limited amount of information. This paper-based approach was intended to help readers quickly grasp the key findings without being overwhelmed by data. However, this limitation in the visualization of data could potentially obscure some valuable insights. To address this, we developed an interactive dashboard that allows users to explore the full range of data, including more detailed information on therapeutic categories, proteomic targets, and biological pathways. This tool helps overcome the limitations of static graphs by providing a more comprehensive and user-friendly exploration of the study's findings.

The creation of an interactive dashboard to visualize and explore the extensive data collected in this study represents a significant advancement in making complex data more accessible and actionable for clinicians and researchers.[26] This tool enables the dynamic exploration of therapeutic categories, biological pathways, and proteomic targets, facilitating the identification of potential drug candidates for further investigation. Future enhancements could include integrating more advanced bioinformatics tools and machine learning algorithms to predict treatment outcomes based on the data collected, thus personalizing treatment plans for individual patients.

Moreover, promising but poorly understood treatments, such as those involving cell-based therapies and specific dietary interventions, warrant further investigation. The lack of detailed data on the mechanisms of these treatments complicates the assessment of their efficacy. Future research should focus on elucidating these mechanisms, particularly in the context of neuroinflammatory processes and gut–brain interactions, which have shown potential in managing ASD symptoms.[27]

5. Conclusion

This study highlights the potential of drug repositioning as a promising strategy for addressing the complex neuroinflammatory pathways involved in ASD in children. By focusing on early childhood, a critical period for brain

development, we have identified numerous potential therapeutic candidates that could be repurposed to target ASD's multifaceted symptoms. For instance, cannabidiol, known for modulating serotonin and adenosine receptors, and fluoxetine, a selective serotonin reuptake inhibitor that enhances neuroplasticity, emerged as strong candidates for repurposing based on their interactions with key proteomic targets relevant to ASD. The results underscore the need for further research into the underlying mechanisms of cell-based therapy and dietary supplementation, particularly within the context of neuroinflammation and gut-brain interactions. Overall, this work lays a strong foundation for future studies aimed at developing more effective and personalized treatments for ASD, with the ultimate goal of improving the quality of life for children.

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Conflict of interest

The authors declare no conflict of interest.

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Not applicable.

Consent for publication

Not applicable.

Data availability statement

A dashboard from this work is available through https://autism-research.univ-lille.fr/index.html.

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