Supplementary Data

Autism spectrum disorder: pathogenesis, biomarker and intervention therapy

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# Hongbin Zhuang and Zhiyuan Liang have contributed equally to this study.

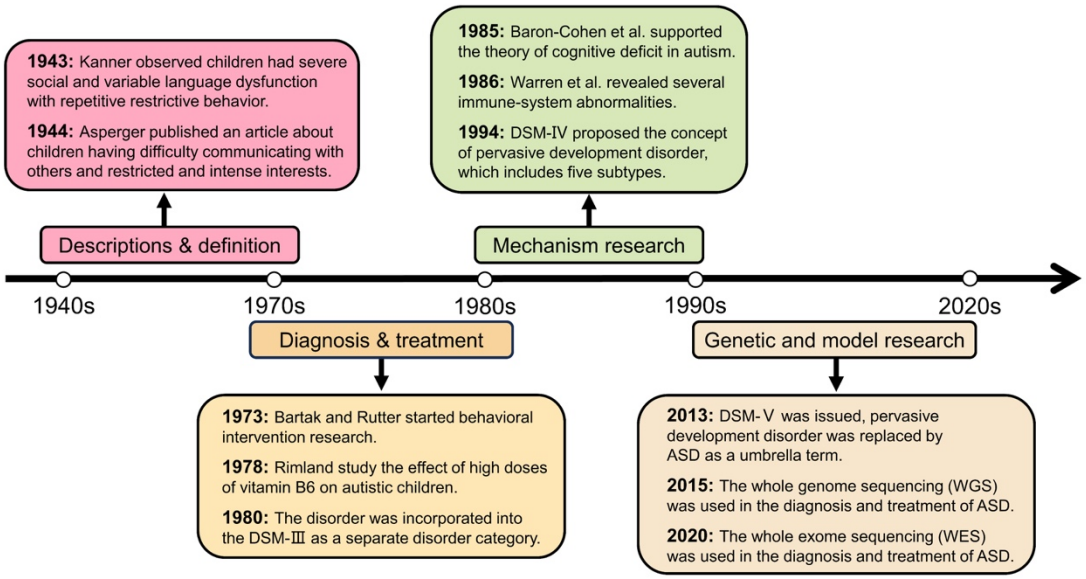

Figure S1. The milestone events associated with autism spectrum disorder.

Original description of autism was in 1940s, subsequently leading to a series of studies on the definition, diagnosis and treatment of autism in 1960s and 1970s. From the behavioral intervention research in 1973, people began to explore ways to effectively intervene in the disorder. Up to now, advances in whole genome sequencing (WGS) and whole exome sequencing (WES) have revealed patterns of inheritance and the types of genetic variation that result in ASD and studies in models have identified a mountain of evidence for molecular mechanisms for ASD.

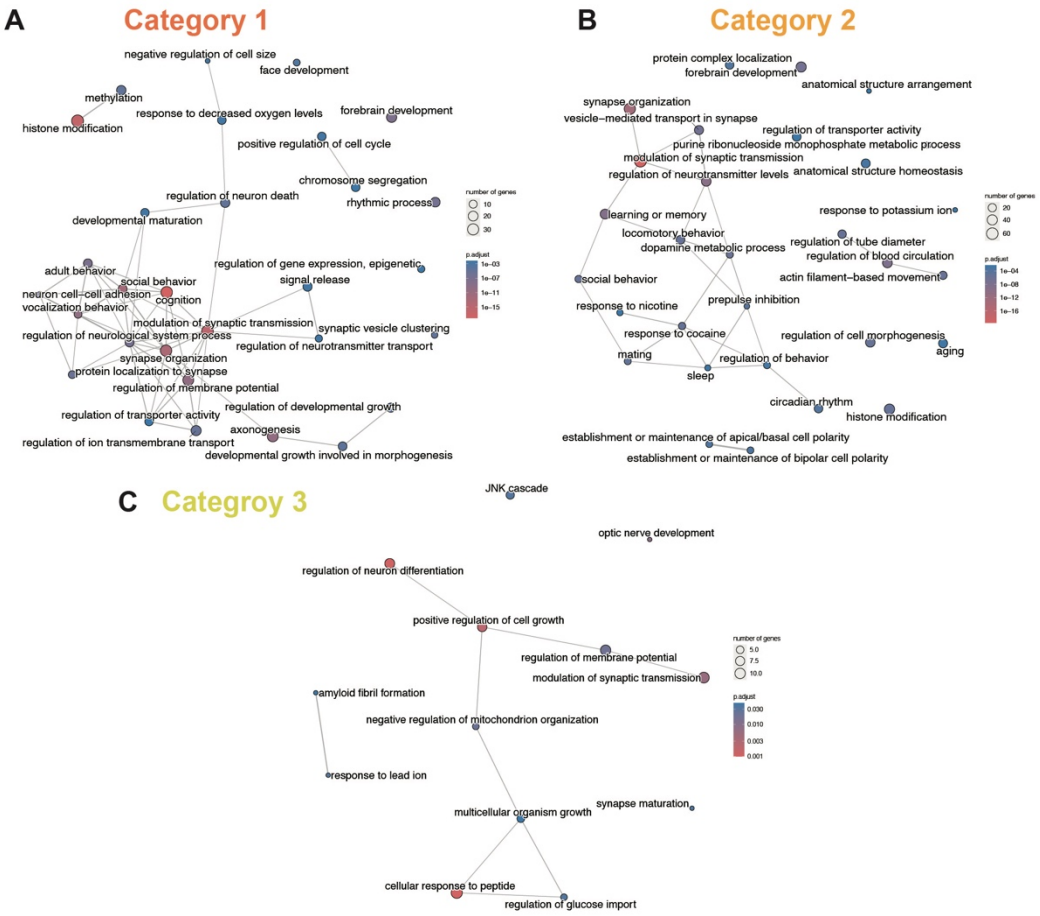

Figure S2. The network analysis of ASD risk genes from SFARI database.

The risk genes ASD are classified into three grades based on SFARI database and analyzed respectively. The significant pathways are involved in interaction network. The size of the circle represented the number of risk genes contained in the pathway and the line between circle indicated that two pathways have an interaction based on Gene Ontology (GO) database. The pathway which placed in core location showed a wide interaction. The enriched analysis was performed on R packages Clusterprofiler (v4.2.1).

Table S1. Convergent pathways related to ASD pathology by omics methods.

No.	Author	Method	Converged pathway	Sample	Gene type	Major finding
1	Niere, F., et al.	Proteome	PI3K/Akt/mTOR	Rat brain	-	1. Both ASD and epilepsy phenotypes and has overactive mTORC1 signaling. 2. A brief repression of mTORC1 activity leads to a significant remodeling of synaptic proteins.
2	Lombardo, M.V., et al.	Transcriptome	PI3K/Akt/mTOR	Mouse brain	-	1. MIA induced transcriptional dysregulation of mTOR and EIF4E-dependent signaling.
3	Mencer, S., et al.	SNO-proteome	PI3K/Akt/mTOR	Mouse brain	InsG3680(+/+)	1. The mammalian target of mTORC1 signaling pathway as one of the shared molecular mechanisms.
4	Wesseling, H., et al.	Proteome	PI3K/Akt/mTOR	Mouse brain	Tsc1+/−	1. proteomic alterations in the hippocampus validated the pathways “myelination”, “dendrite,” and “oxidative stress”, an upregulation of ribosomal proteins and the mTOR kinase.
5	Wan, H., et al.	Proteome	PI3K/Akt/mTOR	Mouse brain	*wdr45* KO	1. Proteomics anlyais reveals the accumulation of ER proteins in *wdr*45 KO mice 2. Suppression of ER stress or activation of autophagy through MTOR inhibition alleviated cell death.
6	Gazestani, V. H., et al.	Transcriptome	PI3K/Akt/mTOR	Human leukocyte	-	1. Whie the majority of rASD genes are not fully penetrant to ASD, the regulatory consequence of many rASD genes on the DE-ASD network are channeled through the PI3K/AKT, RAS/ERK, WNT/β-catenin signaling pathways.
7	Matic, K., et al.	Phosphoproteome	ERK/MAPK signal	Mouse brain	Fmr1 KO	1. Downregulation of the MEK/ERK pathway are obeserved in the absence of FMRP, with decreased phosphorylation on ERK1/2. 2. Several key proteins from the p53 pathway were detected, pointing to the involvement of p53 signaling in dysregulated cell cycle control in Fragile X syndrome.
8	Yang, J., et al.	Proteome	ERK/MAPK signal	Human plasma	-	1. The study showed increased levels of MAPKAPK3 and MRPL33 in ASD. 2. MAPK/ERK signaling pathway and mitochondrial dysfunction are involved in the pathogenesis of ASD
9	Berg, J. M., et al.	Proteomic interactome	JAKMIP1 pathway	Mouse brain	-	1. JAKMIP1 interacted with proteins related to signaling and interaction, nervous system development and function, and protein synthesis.
10	Nishimura, Y., et al.	Transcriptome	JAKMIP1 pathway	Human lymphoblastoid cells	-	1. This study identified common regulation of two other dysregulated genes, JAKMIP1 and GPR155, downstream of FMR1 or CYFIP1
11	Reilly, J., et al.	Proteome / Transcriptome	Calcium signaling	Human, mouse and rat datasets	-	1. Calcium signalling and the glutamatergic synapse were found to be highly interconnected among pathways in the combined geneset. 2. Converging ASD candidate genes on E/I balance and calcium signalling pathway.
12	Wen, Y., et al.	Network anlaysis	ERK/MAPK signal / Calcium signaling	Human databases	-	1. Calcium signaling pathways and MAPK signaling pathway are interactive hubs with other pathways. 2.The analysis showed convergent indications that the process "calcium-PRC (protein kinase C)-Ras-Raf-MAPK/ERK" is likely a major contributor to ASD pathophysiology.
13	Baucum, A. J., et al.	Proteome / Interactome	Calcium signaling	Mouse brain	-	1. Significantly more CaMKAPs co-precipitated with WT CaMKII holoenzymes in the synaptic fraction compared to the membrane fraction, with functions including scaffolding, microtubule organization, actin organization, ribosomal function, vesicle trafficking, and others.

Abbreviation: ASD, autism spectrum disorder; E/I, excitation/inhibition; ER, endoplasmic reticulum; KO: kncok out; MIA, maternal immune activation; SNO, S-nitrosylation; WT, wild type.

Table S2. Non-targeted metabolomics study of intestinal microbial metabolites as biomarkers of ASD

No.	Author	Sample	Method	Related Metabolites (# : ASD compared to Ctrl, * : ASD compared to TD)	Metabolic Process Involved
1	Wang et al. (2012)[222]	Fecal	HPLC-GC	**Increased#:** Acetic, Butyric, Isobutyric, Propionic acid, Valeric, Isovaleric, Total chain fatty acid, SCFA.	Intestinal permeability, Total SCFA metabolism, Gut microbiota metabolism.
2	Angelis et al. (2013)[215]	Fecal	GC-MS/ SPME	**Decreased#:** GABA, Ethanol, SCFA1-Pentanol2-propyl-1-pentanol. **Increased#:** Pentanoic, 3,7-Dimethyl-2,6-Octadien-1-ol, P-cresol, Acetic acid methyleseter, Indole, 3Methylindole, Acetic, Propionic acid.	Metabolism of gut microbiota, FAA metabolism
3	Kang et al. (2018)[223]	Fecal	1NMR spectroscopy	ASD compared to Neurotypical: **Decreased:** Lactate, Formate, Acetate. **Increased:** P-cresol, Caprate, Isopropanol.	Respiratory depression, the homeostasis of colonic epithelial cells
4	Wang et al. (2019)[224]	Fecal	LC-MS/MS	**Decreased*:** 2-Keto-glutaramic acid, Palmitic amide, Glutamate, Fumaric acid, Epinephrine, Cinnamicacid, Benzaladehyde, Cortisol, L-Aspartic acid **Increased*:** Chenodeoxycholic acid 3-sulfate, Taurocholic acid.	Composition of gut microbiota, Intestinal hormone metabolism
5	Wang et al. (2020)[225]	Fecal	GC-MS; UHPLC-MS /MS	**Decreased#:** Acetic acid, Propionic acid, Butyric acid, Homovanillic acid. **Increased#:** Serotonin, SCFAs.	Ecological imbalance of gut microbiota, Disorder of dopamine metabolism
6	Dan et al. (2020)[226]	Fecal	LC/MS	**Decreased*:** Tetracosahexaenoic acid, 2'-lndoleacetaldehyde, Acetaldehyde, Deoxyguanosine, Adenine, Xanthine, lmidazo4e-4-acetaidhyde. **Increased*:** Desaminotyrosine, Chloroneb, Hexanoic acid, Cytosine, Deoxyinosine, Glutamylproline, DL-2 aminooctanoic-acid, 2,5-Dioxopentanoate, 2'Deoxyuridine.	Phenylalanine and Tyrosine metabolism, Arginine and proline metabolism, Histidine and Aspartate metabolism, Glutamate metabolism
7	Xiao et al. (2021)[183]	Fecal	LC-MS/MS	**Decreased*:** 6-Hydroxymelatonin, 5-Hydroxyindole-3-acetic acid **Increased*:** Tryptamine, 5-Hydroxytryptophan, Serotonin, 5-Hydroxy-N-formylkynurenine,	Intestinal microbiota, Tryptophan metabolism, Serotonin metabolism.
8	Zhu et al. (2022)[187]	Fecal	LC-MS/MS	**Decreased*:** TPP, DHF, 5MTHF, Retinol, Vitamin C, L-Ascorbic acid, Dihydrofolic acid, Lumichrome, Pyridoxamine. **Increased*:** All-trans-retinal, B,e-carotene-3,3-diol, Tocopherol, 5-Hydroxytryptophan, Serotonin, Homocysteine, Xanthurenic acid, 4'-apo-beta-	Tryptophan metabolism, Retinol metabolism, Vitamin digestion and absorption.

				carotenal.	
9	Kuwabara et al. (2013)[227]	Blood	CE-TOF-MS	**Decreased#:** 5-Oxoproline, Lactic acid. **Increased#:** Taurine, Arginine.	Oxidative stress, Mitochondrial disorder
10	West et al. (2014)[216]	Blood	GC-MS, LC-HRMS	**Decreased*:** Citric acid, Fatty acids, Creatinine, the branched chain amino acid isoleucine, Homocitrulline. **Increased*:** Succinic acid, 3-Aminoisobutyric acid, Aspartic acid, Glutamate, DHEAS, Glutaric acid.	Mitochondrial disorder, Oxidative phosphorylation, Changes in gut microbiome, Transport and accumulation of metabolites in urine.
11	Wang et al. (2016)[228]	Blood	UPLC/Q-TOF MS/MS	**Decreased#:** Adrenic acid, L-acetylcarnitine, Acetylcarnitine, Uric acid, Arachidonic acid, Docosahexaenoic acid, Docosapentaenoic acid. **Increased#:** LPA, LysoPE, Phytosphingosine, Pregnanetriol, 9,10-epoxyoctadecenoic acid, Sphingosine 1 phosphate.	Fatty acids β Oxidation, Mitochondrial dysfunction.
12	Anwar et al. (2016)[229]	Blood	HPLC, LC-MS/MS	**Decreased#:** Thiamine Pyrophosphate.	Interorganizational, Exchange of metabolites, Mucosal immunity, Host microorganisms.
13	Jory et al. (2016)[230]	Blood	GLC	**Decreased#:** Docosahexaenoic acid, Arachidonic acid, Linoleic acid, Eicosapentaenoic acid, ω-3/ω-6 ratios.	Abnormal Fatty acid Metabolism, Cobalamin metabolism, Intestinal microbiota, Propionic acid production
14	Grayaa et al. (2018)[231]	Blood	GC–MS	**Decreased#:** 7α-Hydroxycholesterol, 25-Hydroxycholesterol. **Increased:** 24-Hydroxycholesterol.	Cholesterol metabolism, Cytotoxicity, Oxidative stress, Cell apoptosis, Synaptic dysfunction
15	Rangel et al. (2019)[232]	Blood	UPLC–MS/MS	**Decreased#:** Glutamate, Nicotinamide. **Increased#:** Isovalerylcarnitine, Isobutyrylcarnitine, Tryptophan, kynurenine, N-methyl-2-pyridone 5-carboxamide, 3-Indole sulfate, 1-methyl Nicotinamide, 5-Bromophosphatidylcholine Indolelactate.	Amino acid, Lipid, Nicotinamide metabolism, Oxidative stress
16	Wang et al. (2020)[225]	Blood	UHPLC-MS /MS	**Decreased#:** Homovanillic acid, Kynurenine. **Increased#:** 5-HT, L-glutamine, L-arginine, L-histidine, 5-hy- droxyindoleacetic acid	Intestinalmicrobiota, Dopaminemetabolism, Tryptophan metabolism.
17	Kang et al. (2020)[219]	Blood	LC-MS	**Decreased*:** IMP, Iminodiacetate, Sarcosine, Methylsuccinate, Valylglycine, Leucylglycine,	Mucosal homeostasis, Energy production,

				Galactonate, Nicotinamide riboside. **Increased*:** Caprylate, Heptanoate, p-cresol sulfate	Folate metabolism.
18	Needham et al. (2021)[233]	Blood	LC-MS/MS	**Decreased*:** saturated fatty acid levels, long-chain acyl-carnitines. **Increased*:** Cresol derivatives, 4EPS, 4-Methylbenzene sulfonate, 2-Ethylphenyl sulfate, 4-allylphenyl sulfate.	Oxidative stress, Mitochondrial dysfunction, Hormone level elevations, Lipid profile changes, Phenolic microbial metabolites.
19	Yap et al. (2010)[234]	Urine	1H NMR Spectroscopy	**Decreased#:** Glutamate, Hippurate, PAG. **Increased#:** Taurine, Acetate, DMA, NAG, Succinate, NMNA, NMND, 2PY.	Gastrointestinal Dysfunction, Oxidative stress, Energy metabolism, Inflammation, Sleep disorder.
20	Ming et al. (2012)[235]	Urine	UPLC/MS/MS	**Decreased#:** Glycine, Serine, Threonine, Alanine, Histidine, glutamyl amino acids, The organic acid, Taurine, Antioxidants, 3-(3-Hydroxyphenyl) propionate, 5-Aminovalerate. **Increased#:** 2-(4-hydroxyphenyl) propiona, Taurocholenate sulfate.	Abnormal amino acid metabolism, Increased oxidative stress, Changes in gut microbiome.
21	Diémé et al. (2015)[236]	Urine	UPLC–HRMS, NMR	**Decreased#:** Dihydrouracil, Desaminotyrosine, Guanidinosuccinic acid, Methylguanidine. **Increased#:** Valine, Dihydroxy-1H-indole glucuronide I, N-α-acetyl-l-arginine, lndoxyl, Norvaline, 5-Aminopentanoic acid, α-N-phenylacetyl-l-glutamine, P-cresol sulfate, Glucuronic acid Indoxyl sulfate, N-acetylasparagine.	Fatty acids β Oxidation, Mitochondrial dysfunction, Oxidative stress, Amino acid metabolism, Intestinal microbiota dysfunction
22	Gevi et al. (2016)[237]	Urine	HILIC-UHPLC	**Decreased#:** N-acetyl-5-methoxy tryptamine, Melatinin, Kynurenic acid. **Increased#:** indolyl-3-acetic acid indolyl lactate, Inosine, Hypoxanthine, Xanthine, Xanthurenic acid, Quinolinic acid.	Vitamin B6, Reduced synthesis of melatonin, Intestinal ecological imbalance, Tryptophan and Purine metabolism pathways
23	Xiong et al. (2016)[238]	Urine	GC/MS	**Increased#:** 3-Hydroxyphenylacetic acid, 3-Hydroxyhippuric acid, 3-(3-Hydroxyphenyl)-3 hydroxypropionic acid.	Intestinal microbiota composition, Gastrointestinal symptoms, Urinary excretion
24	Lussu et al. (2017)[239]	Urine	1H NMR Spectroscopy	**Decreased#:** Lactate, Valine, Betaine, Taurine, Glutamate, Creatinine. **Increased#:** Glycine, D-threitol, Hippurate, Tryptophan.	Oxidative stress conditions, Gut microbiota modification

25	Bitar et al. (2018)[240]	Urine	LC-MS, NMR	**Decreased#:** N-methylglutamate, Creatine, Glucose-1-phosphate, Tyrosine, Urocanic acid, Cysteic acid, Citric acid, Guanine, Acetylcarnitine, Nacetylalanine, Serine, Threonine, Hydroxybenzoic, Hydroxyproline. **Increased#:** Riboflavin, Cholic acid, Phosphoserine, Glutamic acid, Trigonelline, 5-Aminioimidazole-4-carboxamide.	Intestinal microbiota, Energy metabolism, Oxidative stress, Purine and creatine metabolism
26	Chen et al. (2019)[218]	Urine	GC-MS, XGBoost	**Decreased*:** 3-Oxoglutarate, 3-Oxoglutaric, Aconitic, Phosphoric acid, Carboxycitric acid, Phosphoric acid. **Increased*:** Phenylactic acid.	Amino acid metabolism, Gut microbiota, Energy metabolism, Bone salt metabolism.
27	Gevi et al. (2020)[241]	Urine	UHPLC-MS	**Decreased#:** GABA, MHPG, P5P, VMA, Noradrenaline, Adrenaline, Vanillylmandelic acid. **Increased#:** Dopamine, Vitamin C, 4-Cresol, HVA.	Intestinal microbiota composition, Neurotransmission, Biological composition, DNA hypomethylation
28	Liang et al. (2020)[242]	Urine	1H NMR Spectroscopy	**Decreased#:** Trigonelline, Melatonin, Pantothenate, Serotonin, Taurine. **Increased#:** Tryptophan, Hippurate, Glycine, Creatine	Changes of cysteine metabolism, Oxidative stress, Thiometabolism, Melatonin production
29	Anna et al. (2022)[243]	Urine	LC-MS/MS	**Decreased#:** Phenylalanine, Quinolinate, Asparagine, Piridoxyne, Methyl-histidine, Xanthosine, Uridine,Ornithine, D-glucarate, Tyrosine. **Increased#:** Hypoxantine, Guanine, Cystine, Acetylysine, Thiamine-phosphate, Deoxyribose-phosphate, Phenylpyruvic, Phenylacetic acid.	Intestinal microbiota derived compounds, Reduction and oxidative, metabolism of gut bacteria, Vitamin B6 and B12
30	Sharon et al. (2019)[184]	Intestinal	GC-MS	**Decreased*:** 5-AV, GABA, Taurine. **Increased*:** 3AIBA, Soy-derived isoflavones, Genistein, Daidzein	Metabolites of intestinal bacteria, E/I balance, Neuronal development
31	Lu et al. (2021)[183]	Intestinal	LC-MS/MS	**Decreased*:** 6-Hydroxymelatonin, Indole-3-lactic acid, 5-Methoxy-Indoleacetate. **Increased*:** Trp-Trp, Kynurenic acid, Indole-3-acetic acid	Changes in gut microbiota, Tryptophan and Serotonin Metabolism

Abbreviations: DMA: dimethylamine, DEHA: dehydroepiandrosterone sulfate, DHF: dihydrofolic acid, GABA: γ- aminobutyric acid, HC: healthy children, HVA: homovanillic acid, LPA: lysophosphatidic acid; LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry; TPP: thiamine pyrophosphate, TD: typical development, Trp-Trp: a dipeptide of tryptophan, SCFA：short-chain fatty acid, NAG: N-acetyl glycoprotein fragments, NMNA: N-methyl nicotinic acid, MHPG: 3-methoxy-4-hydroxyphenethyleneglycol, NMND: N-methyl-nicotinamide, PAG: phenylacetylglutamine, P5P: pyridoxal phosphate, VMA: vanillylmandelic acid, 2PY: N-methyl-2-pyridone-5-carboxamide, 5-AV: 5-aminovaleric acid, 3AIBA: 3-aminoisobutyric acid, 4EPS: 4-ethylphenyl sulfate, 5MTHF: 5-methyltetrahydrofolate, 5-HT: 5-hydroxytryptamine.