CHAPTER THREE

Genomics, transcriptomics, proteomics and big data analysis in the discovery of new diagnostic markers and targets for therapy development

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Contents

Abstract

Highly complex endophenotypes and underlying molecular mechanisms have prevented effective diagnosis and treatment of autism spectrum disorder. Despite extensive studies to identify relevant biosignatures, no biomarker and therapeutic targets are available in the current clinical practice. While our current knowledge is still largely incomplete, -omics technology and machine learning-based big data analysis have provided novel insights on the etiology of autism spectrum disorders, elucidating systemic impairments that can be translated into biomarker and therapy target

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candidates. However, more integrated and sophisticated approaches are vital to realize molecular stratification and individualized treatment strategy. Ultimately, systemic approaches based on -omics and big data analysis will significantly contribute to more effective biomarker and therapy development for autism spectrum disorder.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with a wide range of conditions characterized by repetitive behaviors, intellectual disability, and deficits in social interaction, communication and language skills. ASD has a high rate of comorbidity with other psychiatric conditions including depression and anxiety.[1,2] Despite its low life prevalence, the disease is highly heritable and estimated to have a median prevalence of 62 in 10,000 worldwide.[3]

While human genetic studies found that a large number of genes are associated with ASD, no major gene which accounts for more than 1% of the cases was found[4] indicating that ASD is not a single disorder with single cause.[5,6] In fact, ASD includes several diseases previously categorized as separate disorders including autistic disorder, Asperger syndrome (AS) and pervasive developmental disorder not otherwise specified (PDD-NOS). In this regard, identifying molecular mechanisms and diagnosis/treatment targets for ASD has been challenging due to the highly heterogenous genetic and symptomatic architecture.

Omics technology has been a powerful tool to identify biomarkers and pathways which could explain the etiology of the psychiatric disorders. With the help of -omics technology, risk genes and proteins involved in neuronal calcium signaling (CACNA1E, CACNA2D1 and CAMK2 alpha), dopaminergic neurotransmission (DRD2), glutamatergic neurotransmission (GRIA1, GRIA2, GRIK5, GRIN2B and GRM5), presynaptic vesicle trafficking (PCLO and STXBP1) and proinflammatory cytokine response (IFN gamma, CCL4 and CXCL8) have been implicated for MDD.[7–10] Diverse pathways such as angiogenesis and vascular system development (ANGPT2 and STAB1), insulin secretion (INS, IRS1 and IRS2) and endocannabinoid signaling (CNR1, DAGLA and DAGLB), as well as ion channel and transporters (SCN2A and SLC4A1), and synaptic component (RIMS1 and ANK3) were associated with BD.[11,12] In SCZ, susceptible pathways involved in glutamatergic neurotransmission and synaptic plasticity (AMPAR, DPYSL2, DRD2, GNB1, GRIA1,

GRIN2A, GRM3, GLUL, NEFL, NEFM, SRR and VDAC), neuronal development (CTNNA1, GPM6A, SOX2OT), calcium channel (CACNA1C, CACNB2, CACNA11, CALM1, CALM2, CAMK2B, CAMK2D, CAMK2G, PMCA4, S100A6, S100A12), chromatin regulation (PU.1 and RPB2), and immune system and inflammation (alpha-defensins, interleukins and COX2) were identified.[13–16] In addition, several biological pathways including calcium signaling (CAMKMT) and mitochondria-related functions (PREPL, ATP5H, MT-CO1, MT-ND2, MT-ND6, NDUFA2, NDUFS6, COX7A2 and COX7C) were found to be related to anxiety disorders.[17,18]

Despite limitations by polygenicity, consequent variable disease symptoms and comorbidity with other psychiatric conditions, -omics studies have identified valuable ASD biomarker and therapy candidates with the help of data resources such as the Simon Foundation Autism Research Initiative (SFARI) (https://gene.sfari.org/), the autDB (http://autism.mindspec.org/autdb/Welcome.do) and the Autism Sequencing Consortium (ASC) (https://genome.emory.edu/ASC/). Recent advances in big data processing with computational algorithms further accelerate ASD research by helping data integration and interpretation. Here, we present the state-of-the-art progress made in -omics and big data studies, and discuss the future directions that will provide a breakthrough in the ASD biomarker and therapy discovery.

2. Omics and big data studies of autism spectrum disorder

2.1 Immune and inflammation pathways

Dysregulated immune system has been shown to highly contribute to the autism-related neurodevelopmental and behavioral abnormalities. Particularly, in utero immune system dysfunctions including maternal production of autoantibodies reactive to fetal brain, altered immunoglobulin levels and innate/adaptive immune cell response have been suggested to play a critical role in the etiology and pathology of ASD.[19] To support this, numerous omics studies have provided evidence of immune dysfunction and autoimmunity in ASD.

Gandal et al. integrated genomics and transcriptomics data of brain tissues acquired from ASD and healthy individuals.[20] They found that gene, isoform and non-coding RNA expression were significantly dysregulated in several brain regions of ASD patients. The differential expression profiles

enriched diverse immune-related pathways including inflammatory response, response to cytokine, innate immune response and cytokine production.

Gupta et al. analyzed transcriptome from postmortem human cortical tissues corresponding to Brodmann Area 10, Brodmann Area 19 (BA19) and Brodmann Area 44.[21] The authors identified 12 co-expression modules using weight gene correlation network analysis. The module upregulated in autism patients significantly enriched activated microglial cells and multiple immune pathways.

Voineagu et al. used transcriptomics to analyze postmortem frontal and temporal cortex tissues from ASD and control cases.[22] Genes involved in immune response, inflammation, astrocyte and activated microglia were upregulated in ASD cortices. These genes enriched a network module highly related to autism disease status. Since further genome wide association study (GWAS) enrichment analysis using published data did not enrich the immune and glial module, the authors suggested the non-genetic etiology for immune dysregulation in ASD.

Abnormal immune system in ASD may be also supported by comorbidity with cancer. By analyzing electronic health record, young ASD adults without intervention was shown to be at high risk for cancer development by midlife.[23] Genome-wide exome sequencing analysis showed that risk genes associated with immune system including ERBB2IP (involved in NF-kB signaling, proinflammatory cytokine secretion and TGF beta signaling) and PAX5 (involved in B cell development, leukemia, acute lymphoblastic leukemia and Wnt signaling pathway) are shared between ASD and cancer.[24] Fores-Martos et al. performed transcriptomics meta-analysis and found that a number of genes related to immune system (CDK2, CDKN1A, COL4A1, COL4A2, DDIT4, F2R, IL4R, ITGA5, MYC, NFKB1 and VEGFA) and oxidative stress (ATP5F1, ATP5J, ATP50, COX7B, CYC1, DLD, OGDHL, PFKM, NDUFAF1, NDUFB2, NDUFB6, NDUFV1 and UQCRFS1) were commonly regulated between ASD and several types of cancer cases.[25]

Redox proteomics analysis identified altered protein oxidation profiles in blood plasma of autistic children.[26] The study found that two carbonylated plasma proteins, Ig kappa chain C and complement component C8 alpha chain, were significantly higher in autistic patients than healthy controls. Protein carbonylation is an irreversible post-translational modification induced by reactive oxygen species. Elevated levels of carbonylated immunoglobulin and complement in the study indicated activated oxidative stress and autoimmune response in ASD.

Momeni et al. performed blood plasma proteome profiling in children with ASD.[27] They found that children with ASD had significantly higher levels of complement factor I, a protease for complement protein C3b. In line with this, C3f, a cleaved C3b, and its fragment C3f-des-arginine levels were found to be elevated in ASD children compared to healthy controls.[28]

Urinary proteomics analysis revealed the differential excretion of kininogen-1 (KNG1), IgG1 heavy chain variable region and mannan-binding lectin serine protease-2 (MASP2) isoform-2 precursor between ASD and typically developing children.[29] KNG1 is a precursor protein for high-molecular-weight kininogen that is suggested to play a role in the inflammatory disorders by stimulating cytokine and chemokine secretion.[30] MASP2 activates complement system by cleavaging the components such as C2 and C4.[31] Thus, the findings supported a crucial role of complement system in ASD.

Junaid et al. used two dimensional gel electrophoresis followed by proteomics identification and direct DNA sequencing of frontal lobe gray matter obtained from ASD patients and controls.[32] The authors identified a single nucleotide polymorphism (SNP) in glyoxalase I (GLO1) gene which causes Ala111Glu substitution in the protein sequence. GLO1 is mainly involved in methylglyoxal detoxification and oxidative stress. It was also shown to be associated with other psychiatric disorders such as anxiety disorders and depression.[33–35] The study revealed that the GLO1 polymorphism results in reduced protein activity and methylglyoxal-derived advanced glycation end product accumulation which may affect ASD-susceptible pathways such as cell growth and differentiation.[36,37]

Vogel Ciernia et al. used a mouse model of maternal allergic asthma (MAA) which induces ASD-like behaviors in offspring.[38] Using microglial fraction isolated from the offspring, they performed whole genome bisulfite sequencing and RNA sequencing to investigate DNA methylation and gene expression, respectively. Differentially methylated regions were associated with a number of cytokine and chemokine-mediated immune pathways and transcription factor binding motifs for early microglial development and immune activation including runt-related transcription factor 1 (RUNX1), PU.1, interferon regulatory factor 8 (IRF8), nuclear factor kappa-B (NF-kB) and MAF BZIP transcription factor B (MAFB). In addition, voltage-gated ion channel genes involved in neuronal connection regulation and microglia sensitivity to environmental signals were differentially expressed between MAA and control mice. The study suggested that maternal immune activation during pregnancy can contribute to ASD susceptibility by affecting fetal synaptic function and development.

2.2 Synapse and neurodevelopment

The ultimate culprit of ASD is more likely to be dysregulated neurodevelopmental process during gestation and early postnatal stage. The resultant functional and structural impairment at the synapse can cause cognitive and psychological dissonance.

Multiple synaptic features such as synaptic vesicle trafficking, neurotransmitter release, GABAergic transmission, and balance between excitatory and inhibitory synaptic transmission were found to be impaired in ASD.[39] Also, abnormalities in dendritic spine density/length, branching and morphology have been extensively associated with ASD.[40]

De Rubeis et al. performed whole exome sequencing and integrated several datasets for de novo, inherited and case-control variations, and de novo missense variants.[41] The study showed that genes affected in ASD are highly enriched for synaptic networks and chromatin remodeling. Several chromatin regulators have been shown to be essential for neural development processes including neural progenitor proliferation, migration, differentiation, synaptogenesis and synaptic pruning.[42] Thus, the findings supported synaptic connectivity and plasticity alterations in ASD.

Callaghan et al. performed whole genome sequencing using whole blood of ASD probands.[43] The authors identified five de novo subject-specific damaging variants using computational prioritization based on predicted damage, population frequency, literature evidence and phenotype concordance. The variants included two novel de novo variants of SCN2A, a well-documented ASD gene.[44] SCN2A encodes voltage-gated sodium channel Nav1.2 which is expressed in the axon initial segment. SCN2A was shown to be important for action potential initiation and propagation.[45]

Integrated genomics and transcriptomics analysis by Pain et al. identified 14 differentially expressed genes between ASD and control cases.[46] The authors found that the protein disulfide-isomerase A6 precursor (PDIA6) involved in protein folding was significantly downregulated in blood of ASD individuals. Gene sets including synaptic vesicle, presynapse, abnormal axon guidance and early cortical development represented nominal significance.

Sanders et al. analyzed de novo CNVs using the Simons Simplex Collection (SSC) cohort, the resource of the SFARI.[47] They integrated published de novo CNV data from the Autism Genome Project, and exome sequencing data from the SSC and the ASC. The integrated analysis confirmed previously reported de novo CNV loci, and identified 65 ASD genes.

Protein-protein interaction (PPI) analysis further revealed that the ASD genes enrich interconnected networks for synapse and chromatin regulation.

Grunwald et al. performed transcriptomics analysis using human induced pluripotent stem cells (iPSCs) and -derived neurons generated from dermal fibroblasts of ASD and SCZ patients.[48] While iPSCs-derived neurons from three ASD patients shared a small number of dysregulated genes, transcriptome profiles were discriminative between the ASD and SCZ patients. The authors found that several genes linked to neural development such as SHH, PTCH1, GREM1, FEZF1 and FEZ-AS1 were significantly altered in the ASD iPSCs-derived neurons compared to those from healthy controls.

Single cell transcriptomics was used to analyze gene expression profiles from doublecortin expressing immature neurons in the dentate gyrus of mouse hippocampus.[49] While the study identified several subpopulations with distinct developmental stages, genes positively associated with neuronal maturation progression from stem-like to neuron-like cells were enriched for autism-related gene sets. This suggested that autism development may be attributed to gene and network dysregulation involved in neuronal maturation process during neurogenesis.

DeRosa et al. examined temporal transcriptome changes during cortical neuron differentiation of iPSCs derived from peripheral blood mononuclear cells (PBMCs) of individuals with idiopathic ASD.[50] The authors compared transcriptome differences between two time points, early (DIV 35) and later (DIV 135) developmental stages. Differentially regulated genes in early stage exhibited expression changes in opposite direction in later stage, indicating dramatic gene expression changes during development. Several biological processes including synaptic activity, calcium signaling and neuronal cell migration were found to be dysregulated in the early stage of ASD iPSCs. Consistent with transcriptomics data, neuronal process migration, spontaneous neural spiking activity and calcium transients were found to be impaired.

Pinto et al. identified 36 pathogenic CNVs in ASD-affected individuals.[51] The CNVs implicated genes linked to ASD-related neurodevelopmental disorders such as CHD2, HDAC4 and GDI1. Several synaptic processes including cell projection, neural development, axonogenesis and neuronal synapse were enriched by rare exonic gene deletions. Integrative analysis with de novo loss-of-function SNVs from four ASD exome sequencing studies further revealed the convergent pathway enrichment in neuronal development and axon guidance as well as microtubule associated protein kinase (MAPK) signaling and chromatin modification/transcriptional regulation.

Hu et al. performed quantitative trait analysis using ASD patient subgroups based on the Autism Diagnostic Interview-Revised (ADI-R) scores from five symptom categories including spoken language skills, non-verbal communication, play skills, social development and insistence on sameness.[52] Quantitative trait locus associated with distinct ASD subgroups enriched several cellular pathways including neurogenesis, axogenesis and long-term synaptic potentiation. Network analysis using genes associated with intronic SNPs identified hubs such as HTR4 and GCH1, indicating a role of serotonergic system in the ASD etiology.

Bralten et al. performed GWAS to identify SNPs associated with autistic traits.[53] The authors measured autistic trait scores from Dutch general population using self-report questionnaire. They used GWAS data from Psychiatric Genomics Consortium autism group to find overlaps between genetic variants for ASD susceptibility and three autistic traits (childhood behavior, rigidity and attention to detail). A number of neurite outgrowth-related genes including MET showed a significant association with rigidity trait.

Using exome sequencing, O'Roak et al. identified recurrent de novo mutations of chromodomain helicase DNA binding protein 8 (CHD8) from ASD probands.[54] CHD8 is a chromatin remodeling factor which regulates Wnt signaling pathways.[55,56] CHD8 deficiency was shown to impair axon and dendrite growth, and delay cortical neuron migration.[57] In addition, CHD8 mutations have been strongly associated with clinical features including macrocephaly, speech delay and psychopathology.[58]

Sugathan et al. performed RNA-seq and ChIP sequencing using iPSC-derived human neural progenitor cells with CHD8 reduction.[59] Several critical pathways including synapse, axon guidance and neuron differentiation were enriched by downregulated genes. Furthermore, most CHD8 binding sites were found to be active transcription start sites, supporting a crucial role of CHD8 in chromatin and transcription regulation.

Genomic DNA methylation study using blood specimens examined genetically defined ASD patient subgroups with 16p11.2 deletion or with $CHD8^{+/-}$ variant.[60] Both genotypes have been strongly associated with behavioral and neurodevelopmental deficits of ASD patients.[58,61] Methylated gene profiles obtained from the subgroups were able to distinguish the subgroups from undefined individuals, showing that ASD subgroup stratification could provide more sensitive and specific diagnostics.

Neurexin (NRXN) is a neuronal presynaptic cell adhesion molecule which is crucial for synaptic function and development.[62,63] One study

identified CNV losses of chromosome 2p16 which results in NRXN1 coding exon deletion in families with ASD individuals.[64] Zahir et al. identified de novo NRXN1-alpha deletion from a ASD patient.[65] Feng et al. identified two putative missense structural variants in NRXN1 beta gene from several ASD patients.[66] Lam et al. performed single cell RNA-seq analysis using human iPSC-derived neural stem cells and differentiated cells generated from autism patients carrying bi-alleic NRXN1-alpha deletion.[67] The authors found that NRXN1-alpha deletion resulted in astroglia generation, depressed calcium signaling and impaired excitatory neuron maturation.

Single nucleus RNA sequencing using ASD patient cortical tissues revealed that genes important for synaptic function and brain development such as NRXN1, STX1A, SYN2, TCF25, SOX5 and RBFOX3 are differentially regulated in upper-layer excitatory neurons, microglia and protoplasmic astrocytes.[68] The authors examined correlation of cell type-specific gene expression changes with the clinical ASD symptom severity. Transcriptomic changes in L2/3 neurons and microglia were found to be the most predictive of symptom severity. By comparing single cell profiles, they further revealed that cortico-cortical projection neurons across layers are commonly affected in multiple patients, suggesting that upper-layer cortical circuit dysfunction may be relevant to ASD pathology.

Provenzano et al. used microarray to analyze hippocampal transcriptome of two ASD mouse models, BTBR T^{+} $Itpr3^{tf}$/J (BTBR) and Engrailed-2 knockout mice, to identify conserved ASD molecular signatures.[69] While more than 150 genes were commonly expressed between the two groups, differentially expressed gene profiles common to both mouse models enriched biological processes such as regulation of ion transmembrane transport and synaptic transmission.

Parikshak et al. performed ribosomal RNA-depleted RNA sequencing using postmortem human frontal cortex, temporal cortex and cerebellum of ASD and control subjects.[70] Significant alterations in long non-coding RNAs (lncRNAs) expression and neuron-specific alternative splicing were found in ASD cortices. In addition, multiple lncRNAs were predicted to interact with microRNA-protein complexes that are differentially regulated between control and ASD cortices, and with fragile X mental retardation protein (FMRP) that is strongly associated with intellectual disability and ASD.[71] The study revealed that cortical patterning between the frontal and temporal cortices were attenuated in ASD. In addition, transcription factor binding site enrichment analysis further found that SOX5, a mammalian corticogenesis regulator, might be involved in cortical patterning

dysregulation in ASD. The co-expression network analysis showed that age-related microglial and synaptic function were changed, suggesting that ASD genetic risks may affect cortical gene expression.

Lee et al. performed transcriptomics analysis using zebrafish embryos and larvae treated with valproic acid as a model of ASD.[72] They found that ASD-associated genes such as ADSL, MBD5, SHANK3 and TSC1b were differentially regulated between the control and drug-treated larvae. Several synapse-related processes including generation of a signal involved in cell-cell signaling, synaptic transmission and transmission of nerve impulse were enriched for genes altered in a concentration-dependent manner. Therefore, the authors suggested that valproic acid-treated zebrafish can be a promising alternative animal model for ASD research.

Multiple clustering algorithms based on scores from the ADI-R were used to identify ASD proband subgroups.[73] While the clustering analysis identified four phenotype clusters with distinct behavioral deficits, the authors further performed DNA microarray using lymphoblastoid cell lines from three of the subgroups (groups with severely impaired language, mild phenotype and savant skills).[74] Differentially expressed genes in severely language-impaired subgroup were involved in biological processes including circadian rhythm and apoptosis/cell death of neuroglia, astrocytes and neurons. Interestingly, differentially expressed genes common to all three ASD subgroups were found to be in uncharacterized intronic or intergenic regions associated with cellular response to androgen. Seven of the transcripts showed significant expression changes in response to dihydroxytestosterone, suggesting that the transcripts responsive to androgen may be related to a higher rate of ASD prevalence in males.

Transcriptomics and proteomics profiling of the BTBR mouse hippocampus identified several differentially regulated genes and proteins such as blood-brain-derived neurotrophic factor (BDNF) and SH3 and multiple ankyrin repeat domains 3 (SHANK3).[75] The altered genes and proteins represented several pathways including axon guidance, endocytosis, and regulation of actin cytoskeleton, indicating synaptic dysfunction in the BTBR mouse hippocampus.

Broek et al. performed targeted proteomics analysis using postmortem brain tissues collected from ASD patients and typical controls.[76] The authors found that proteins involved in axon myelination and synaptic regulation were oppositely regulated between prefrontal cortex and cerebellum. The study indicated that distinct brain regions can be differentially affected in ASD.

Meta-analysis of 14 different studies including more than 2700 participants found that peripheral BDNF levels are significantly higher in ASD patients compared to controls.[77] Despite inconsistent reports on BDNF levels, the results concluded that peripheral BDNF alteration can be significantly implicated in ASD.

Synaptic interactome studies using co-immunoprecipitation (Co-IP) coupled mass spectrometry-based proteomics identified key PPIs. One interactome study with developing mouse telencephalon synaptosome revealed that p140Cap protein interacts with more than 300 proteins highly associated with multiple psychiatric disorders including ASD, SCZ, and BD.[78] Another study using developing mouse neocortical synaptosome found that MET receptor tyrosine kinase interaction networks include several key synaptic proteins such as SHANK3, synaptic Ras GTPase-activating protein 1 (SYNGAP1) and glutamate ionotropic receptor NMDA type subunit 2B (GRIN2B).[79] Mejia et al. immunoprecipitated tuberous sclerosis 1 (TSC1) interactome in mouse neuro2a cells and primary rat cortical neurons. The authors found a novel interaction between huntingtin associated protein 1 (HAP1) and TSC1. Furthermore, HAP1 was found to regulate mammalian target of rapamycin complex 1 (mTORC1) signaling and axon-dendrite morphogenesis/positioning in brain.[80]

Baucum et al. performed Co-IP followed by quantitative proteomics to identify CaMKII interactome from mouse forebrains.[81] The study employed the CaMKIIα T286A knock-in mutation which results in reduced CaMKIIα and β phosphorylation. The mutation was found to alter CaMKII interaction with ASD-related synaptic scaffolding proteins including SHANK3, disk large-associated protein 2 (DLGAP2) and SYNGAP1. Since dysregulated CaMKII signaling has been linked to Angelman syndrome which exhibits features overlapping with ASD, the authors concluded that altered PPIs in CaMKII networks may contribute to the ASD etiology.

2.3 Mitochondrial energy metabolism

Mitochondria are key organelles for cellular energy generation. Most energy produced in the brain is consumed on synaptic mechanisms mediating synaptic transmission.[82] Mitochondria were also shown to mediate critical neurodevelopmental processes with a high energy demand such as genetic reprogramming during neuronal differentiation.[83] It has been shown that mitochondria are transported to the active synapse and support dendritic

spine density and synaptogenesis[84] and synaptic transmission.[85] Thus, mitochondrial impairment is strongly implicated in dysregulation of synaptic vesicle trafficking, neurotransmitter release and concomitant synaptic transmission in ASD.

Gordon et al. analyzed transcriptome from cortices and hippocampi of three mouse lines, Df(h15q13)/+, Df(h22q11)/+ and Df (h1q21)/+, carrying ASD and SCZ-associated mutations.[86] While several cortical and hippocampal co-expression networks were found to be shared among the three mouse models, one cortical module associated with neuronal mitochondria and firing rate showed gene expression patterns overlapping with transcriptomic changes in ASD and SCZ postmortem human brains. The common gene expression patterns in mice and humans suggested that neuronal bioenergetics can be a crucial mechanism for ASD.

He et al. analyzed 11 transcriptomics datasets of different human tissues from ASD and control individuals.[87] Downregulated genes in ASD patients' brain and blood specimens significantly enriched several pathways including mitochondria-related functions and oxidative phosphorylation. The authors further analyzed eight transcriptomics datasets from ASD rodent models to validate the findings from the human subjects. While all differentially expressed genes in the animal models were found to be upregulated, similar biological processes were enriched between the ASD animal and humans.

Transcriptomic meta-analysis mentioned in Section 2.1 also found that a number of genes in mitochondrial processes including oxidative phosphorylation, mitochondrial electron transport and ATP synthesis were downregulated in ASD.[25]

While Broek et al. identified differential synaptic protein expression patterns between prefrontal cortex and cerebellum as mentioned in Section 2.2, the authors also found that creatine kinase B-type (CKB) levels were altered in ASD.[76] Creatine kinases play a central role in energy storage and distribution by catalyzing phosphate transfer between ATP and creatine phosphate. CKB was shown to affect formation and maintenance of mossy fiber connections in the hippocampus.[88] Glial CKB was associated with high energy demands related to ion homeostasis and TCA cycle metabolite/neurotransmitter trafficking with neurons.[89] Thus, altered CKB levels in ASD brains suggest that disturbed CKB-mediated energy metabolism may be involved in synaptic dysfunction.

Shen et al. performed proteomics analysis using PBMCs of autistic and healthy children.[90] The authors reported that 17 proteins involved in carbon metabolism, pyruvate metabolism, TCA cycle and respiratory electron transport were significantly altered in the ASD PBMCs.

West et al. interrogated blood plasma metabolome of ASD and healthy children.[91] The authors revealed that mitochondrial metabolites including isoleucine, glutaric acid, aspartate, glutamate and TCA cycle-associated molecules (citric acid and succinic acid) are significantly altered in children with ASD. Levels of dehydroepiandrosterone sulfate which affects oxidative energy metabolism by altering mitochondrial respiratory chain complex contents and activities[92,93] were found to be different between autistic and healthy children.

Metabolomics analysis of the prefrontal cortex gray matter obtained from ASD individuals showed that significantly altered metabolites enrich glutathione metabolism and TCA cycle.[94] Transcriptomics data analysis in the human prefrontal and temporal cortices further found that genes for ASD-related metabolites were elevated in autistic individuals.

Blood plasma metabolome was analyzed in healthy controls and children with ASD, idiopathic-developmental delay and Down syndrome.[95] The study revealed that metabolites involved in one-carbon metabolism (serine and glycine) and TCA cycle (alanine and ornithine) were significantly elevated only in children affected by ASD. Since serine and glycine are agonists for NMDA and glycine receptors, respectively, the results suggest the close relationship between energy metabolism and synaptic activity.

2.4 Lipid transport and metabolism

Lipids are essential components of cellular membranes and vesicles. Most cholesterol in the brain are required in myelin sheath, neurons and astrocytes. Particularly, more than 70% of rodent brain cholesterol is in myelin,[96] indicating its major role in synaptic transmission regulation. Lipid rafts which contains high concentration of cholesterol and glycosphingolipids were shown to play a major role in maintenance of synapses and dendritic spines.[97] Lipids are also crucial for brain development. Mammalian brain lipids including cholesterol, phospholipids, cerebrosides, sulfatides and gangliosides highly increase during development. Several studies have demonstrated a role of lipid transport and metabolism in CNS development.[98,99] Therefore, perturbed lipid homeostasis can directly hamper critical synaptic functions which can lead to cognitive, behavioral and emotional impairment in ASD.

Gudenas et al. re-analyzed previously acquired RNA-seq data to identify differentially expressed lncRNAs in ASD brain cortices.[100] More than 200 lncRNAs were found to be altered in ASD cortices. Five among the identified lncRNAs were neighboring and antisense to ASD risk genes including

RAPGEF4, DLX6, STXBP5, KLC2 and DMXL2, suggesting that the lncRNAs may be cis-regulatory elements. Furthermore, differentially expressed lncRNAs enriched functional expression modules related to lipid transport along with synaptic transmission, immune response, drug response and nucleic acid metabolism, indicating a role of lipids at the nexus of other essential pathways.

Corbett et al. performed proteomics analysis using sera obtained from autistic and healthy children.[101] The authors separated the autism children into low-functioning autism (LFA) and high-functioning autism (HFA) subgroups based on intelligence quotient. While apolipoprotein B100 precursor (APOB100), complement factors and fibronectin 1 isoform 1 preproprotein (FN1) levels were altered in general autistic children, comparison between the autism subgroups revealed that apolipoprotein A-IV (APOA4) and APOB100 levels were significantly lower in LFA than HFA sera. Since most brain cholesterols are locally produced by glial cells,[102] apolipoprotein-mediated lipid transport can play a crucial role in diverse neuronal pathways.

Yang et al. analyzed blood sera of Han Chinese children with ASD to identify peptide biomarkers.[103] The authors found that eight peptide peaks were higher in ASD than control sera. The peptides were found to be derived from proteins such as fatty acid binding protein 1 (FABP1) and apolipoprotein C-I precursor (APOC1) involved in lipid metabolism regulation.

Steeb et al. used multiplex immunoassay profiling and proteomics to analyze sera of adults with AS.[104] The authors found that several lipid transport and metabolism-related proteins including apolipoprotein A1 (APOA1), apolipoprotein E (APOE), apolipoprotein C2 (APOC2), adiponectin (ADIPO), fetuin-B precursor (FETUB) and D-glucuronyl C5-epimerase (GLCE) were altered specifically in female AS patients. In addition, sex hormone binding globulin (SHBG) expression patterns between men and women were found to be opposite. SHBG is a major transporter of sex steroids such as testosterone and other androgens which were suggested to contribute to the ASD development.[105] Based on their results, the authors demonstrated that gender subgroup stratification may be required in the studies of AS biomarkers and drug targets.

Wang et al. found the serum metabolomic profile differences between autistic and healthy individuals.[106] The authors revealed that lipids including sphingosine 1-phosphate (S1P), docosahexaenoic acid (DHA) and docosapentaenoic acid were significantly altered in ASD cohorts. S1P and

DHA were significantly correlated with the autism behavior checklist (ABC) score, representing biomarker candidates for ASD diagnosis.

2.5 Gut microbiome

The microbiota-gut-brain axis coordinates communication and interaction between enteric and central nervous system, therefore affecting emotional and cognitive functions in the host. Gut microbes provide neuroactive compounds and immune modulators including serotonin, dopamine, γ-aminobutyric acid (GABA), acetylcholine, histamine and short-chain fatty acids (SCFAs). In addition, gut microbiome was shown to regulate blood-brain barrier formation and hypothalamic-pituitary-adrenal stress response.[107] increasing evidence strongly suggests that abnormal gut microbiome could serve as one of major contributors to ASD development.[108,109] Various gastrointestinal symptoms in autistic individuals also support a significant link between gut microbiome and ASD.[110]

de Theije et al. analyzed microbiota composition of caeca samples from mouse pups exposed to in utero valproic acid.[111] Pyrosequencing analysis revealed that prenatal valproic acid exposure resulted in transgenerational gut microbiota changes in the offspring. Microbiota differences of drug-exposed male offspring were significantly correlated with increased caecal butyrate and decreased ileal serotonin levels.

Ming et al. performed metabolomics analysis to identify urinary biosignatures for ASD. They found that microbial metabolites including 2-(4-hydroxyphenyl)propionate, taurocholenate sulfate, 3-(3-hydroxyphenyl)propionate and 5-aminovalerate (5-AV) were significantly altered in children with ASD.[112]

Yap et al. analyzed urinary metabolite profiles of ASD patients using ^{1}H NMR spectroscopy.[113] The authors found that gut microbial and mammalian cometabolites including dimethylamine, hippurate and phenyacetylglutamine were significantly different between ASD and control subjects. The metabolites were suggested to be involved in interconnected pathways among gut microbiome, energy metabolism and methylamine, suggesting the systemic effects of gut microbiome.[114]

Kaluzna-Czaplinska interrogated urinary metabolome profiles to find organic acid level differences between autistic and control children.[115] The author suggested that gut bacterial metabolism might affect several metabolites including hydroxyphenylacetate and hippurate.

Emond et al. examined urinary metabolome to investigate biomarker candidates for ASD.[116] Nineteen metabolites including succinate, glycolate, hippurate, 3-hydroxyphenylacetate, hydroxyacetate, 1H-indole-3-acetate, phosphate, palmitate, stearate and 3-methyladipate were found to be discriminative between autistic and healthy children, thus representing potential biomarkers for ASD diagnosis. Altered levels of microbe-derived metabolites such as hippurate, 3-hydroxyphenylacetate, 3-hydroxy-hippurate and 1H-indole-3-acetate implicated significant effects of gut microbiome on the host metabolome.

De Angelis et al. analyzed fecal metabolome of children with PPD-NOS and ASD.[117] The authors found that beneficial metabolites such as free amino acids (FAAs) and SCFAs were significantly decreased in feces of PDD-NOS and ASD compared to healthy individual. They also revealed significant correlations between metabolically active bacteria abundance and metabolites levels. *Faecalibacterium*, *Ruminococcus* and *Bifidobacterium* genera were positively correlated with total SCFA levels. Total FAA levels were correlated with *Bacteroides* genus.

Sharon et al. performed metabolomics using colon contents from mice harboring ASD human gut microbiome.[108] Adult offspring from mice transplanted with gut microbiome of ASD patient donors showed significantly altered autistic behaviors, and microbiome and metabolome profiles. Weak $GABA_A$ receptor agonists including 5-AV and taurine were found to be lower in mice with ASD microbiome. 5-AV treatment significantly decreased cortical neuron excitability in BTBR mice. Taurine treatment was found to delay inhibitory neurotransmission switch in response to GABA exposure in primary rat cortical neurons. Therefore, the study suggested that neuroactive metabolite alterations by ASD gut microbiome may affect the host neuronal activity. Transcriptomics analysis using mouse prefrontal cortex and striatum further revealed differential expression of diacylglycerol lipase beta which is required for axonal growth and guidance.[118] In addition, alternative splicing events relevant for several ribosome binding protein targets were significantly altered in ASD mouse brains.

Kang et al. analyzed fecal microbial metabolites in children with ASD using ^{1}H NMR spectroscopy.[119] The authors found that isopropanol, *p*-cresol and GABA concentrations were significantly altered in ASD compared to healthy individuals. In addition, they showed that Fischer discriminant analysis model using caprate, nicotinate, glutamine, thymine and aspartate can separate ASD from control cases, therefore suggesting that the metabolite group may be used as biomarkers.

2.6 Ubiquitin-proteasome system

The ubiquitin-proteasome system was shown to control synaptic structure and activity by regulating synaptic protein composition that is important for synaptic formation and maintenance such as SNARE complex proteins, AMPAR and PSD95.[120,121] Thus, perturbed proteolysis processes including ubiquitin mis-conjugation to the protein of target and subsequent failure of proteasomal degradation can lead to synaptic dysfunction, implicating a crucial role of the defective machinery in ASD.

Yi et al. found the link between ubiquitin and Wnt signaling pathways in ASD. The authors compared published databases for ubiquitin protein ligase E3A (UBE3A) interactome and Wnt signaling regulators.[122] While multiple UBE3A interacting proteins were found to be negative Wnt signaling regulators, they quantitated ubiquitinated peptide profiles to identify UBE3A substrates. They revealed that ASD-associated de novo $UBE3A^{T485S}$ mutation depleted the original UBE3A interactors and substrates including proteasome 19S regulatory subunits (PSMD1, PSMD2, PSMD11, PSMC2, and PSMC5) and 20S core subunits (PSMA1, PSMA2, and PSMB1).

Glessner et al. interrogated whole genome CNVs which confer an ASD risk.[123] The authors found that neuronal development-associated genes such as NRXN1, CNTN4, ASTN2 and NLGN1 had a higher CNV frequency in ASD compared to control cases. Interestingly, CNVs associated with ubiquitin ligase genes including UBE3A, PARK2, RFWD2 and FBXO40 were found only in ASD patients, suggesting a role of ubiquitin-proteasome system for ASD susceptibility.

2.7 Epigenetic regulation

Epigenetic regulation including DNA CpG methylation and histone modifications determines chromatin architecture, transcription factor accessibility and gene expression levels. The important role of epigenetic regulation has been highlighted in ASD. Multiple ASD-associated epigenetic changes in PRRT1, FAM181A, CHFR, AURKA, MAP8KIP3, NALP1L5, TET, MECP2, DNMT1 and MTHFR were suggested to affect crucial synaptic and neurodevelopmental processes.[124]

Whole exome sequencing using whole blood obtained from 10-year-old autistic male identified a de novo mutation in HIST1H1E which encodes histone H1.4 or H1E proteins involved in heterochromatin formation.[125] The authors performed a systemic review using autism genetics databases

including SFARI and autDB. The analysis revealed that 42 genes related to ASD are directly associated with epigenetic machinery.

Ladd-Acosta et al. examined global DNA methylation profiles using postmortem brain tissues including dorsolateral prefrontal cortex, temporal cortex and cerebellum from autism and control cases.[126] Four differentially methylated regions near PRRT1, 11orf21, ZFP57 and SDHAP3 genes were found commonly across three brain regions. Ellis et al. analyzed DNA methylation using postmortem BA19 brain tissues from autistic and control subjects.[127] The authors found that several CpH sites were hypermethylated in autism brains. Interestingly, 10 histone methylations were only enriched in brains, but not in lymphoblastoid cell lines from the corresponding patients, indicating that epigenetic dysregulation can be tissue-specific.

2.8 Big data analysis with machine learning approach

Big data analysis empowered by machine learning methods has achieved promising advances in ASD research. Machine learning based on large-scale omics and clinical diagnosis data has assisted studies aimed at more precise diagnosis.

Gong et al. applied the KF algorithm to predict new ASD susceptibility genes.[128] The algorithm used datasets generated from biomedical text mining. The authors identified a number of ASD candidate genes for several members of orthodenticle homeobox, 5-hydroxytryptamine receptor (HTR), GABA receptor (GABAR) and protocadherin (PCDH).

Jiao et al. applied machine learning techniques to assess whether SNPs can predict ASD symptom severity.[129] They analyzed 29 SNPs of nine ASD-associated genes in ASD children subgroups with different symptom severity. SNP-based diagnostic models were generated using three different methods including decision stumps, alternating decision trees and FlexTrees algorithms. The study found the SNP rs878960 in GABRB3, a gene encoding GABAR subunit beta-3, to be predictive for symptom severity. Thus, the authors suggested that SNPs in ASD genes can be used as accurate classifiers for ASD symptom severity.

Oh et al. used the published microarray data obtained from peripheral leukocytes of ASD patients.[130] The authors established a predictive diagnosis model based on 19 differentially expressed genes to distinguish autistic and typically developing individuals. While the 19-probe set showed a high accuracy to discriminate the two groups, pyruvate kinase muscle isozyme involved in glycolysis was found to be the best discriminant.

Xiong et al. used deep learning algorithms to compute a score for RNA splicing by DNA variants in ASD.[131] The authors analyzed whole genome sequence of brain tissues from the Autism Tissue Program to identify causal SNPs for splicing misregulation. They found that genes with predicted mis-splicing were enriched in several ASD-relevant pathways including synaptic transmission, neuron projection, mitotic cell cycle, embryonic development and CNS development.

To predict functional and pathogenic de novo mutations in ASD, Zhou et al. analyzed whole genome of ASD simplex families using deep convolutional-network-based framework.[132] Non-coding mutations in ASD probands were predicted to affect brain-specific genes. In addition, genes in synapse and chromatin-related clusters showed similar expression patterns with those with high-impact proband mutations. Thus, the study discovered the causal contribution of non-coding mutations in the ASD etiology.

3. Conclusions and future directions in the biomarker and therapy discovery based on -omics and big data analysis

While -omics and big data analysis have profoundly advanced ASD research by providing information on affected biosignatures and pathways (Fig. 1), none has been validated as reliable clinical biomarkers and therapy targets. This may be the result of the current clinical diagnosis strategy based on standard tests including the Diagnostic and Standard Manual of Mental Disorders, the Childhood Autism Rating Scale, the ABC, the Gilliam Autism Rating Scale, the ADI-R and the Checklist for Autism in Toddlers. Since the standard tests are only based on phenotypic and behavioral traits, underlying biological mechanisms need to be considered for more precise diagnosis and treatment. Therefore, Research Domain Criteria (RDoC) has been suggested to be an alternative framework for biological classification system. The RDoC can address more biological and functional domains related to ASD. For instance, Hennessey et al. attempted to classify amygdala dysfunction in ASD using five RDoC domains including Negative Valence Systems, Positive Valence Systems, Cognitive Systems, Social Processes and Arousal and Regulatory Systems.[133] Systemic molecular profiling using -omics and big data analysis will strongly assist RDoC-based diagnosis strategy.

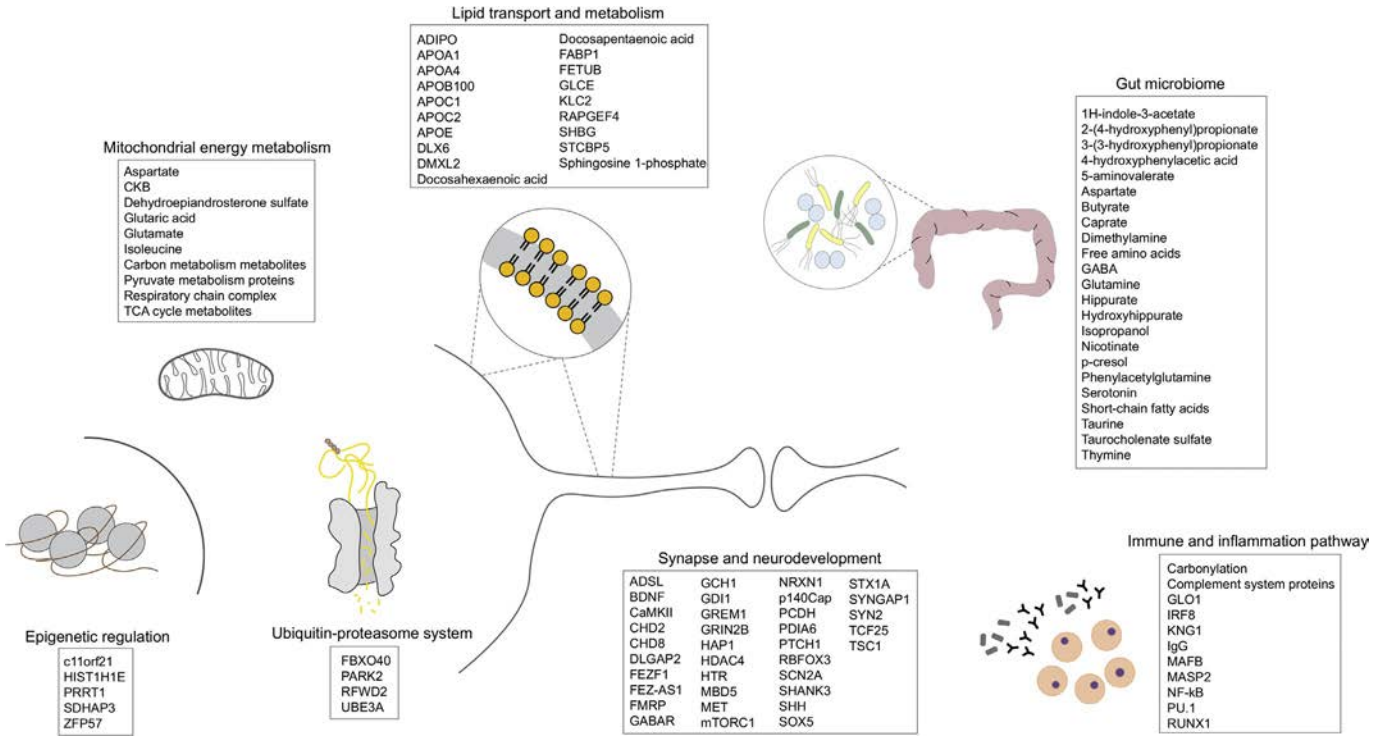

Fig. 1 The overview of biosignatures and pathways identified from -omics and big data analysis for autism spectrum disorder.

More research on ASD subgroup stratification based on molecular profiles is strongly required to overcome the current diagnostic and therapeutic limitations. The current studies have used clinical phenotypes and characterized single genotypes to stratify the subgroups. However, stratification efforts based on -omics and other big data are largely missing. The molecular subgrouping will not only provide information on underlying mechanisms specific to relevant subgroups, but also assist customized pharmacological interventions. While effective treatment for ASD core symptoms has been very challenging, several classes of drugs including atypical antipsychotics, selective serotonin reuptake inhibitors, tricyclic antidepressants, anticonvulsants, NMDA receptor antagonists, acetylcholinesterase inhibitors, psychostimulants, adrenergic alpha-2 receptor agonists and opiate antagonists have been shown to attenuate associated behavioral symptoms such as hyperactivity, anxiety, repetitive behavior, aggression and self-injury.[134] With the help of advanced machine learning methods, integrated multi-omics data may further help to improve pharmacological treatment efficiency.

Ultimately, there is an absolute necessity to establish the integrated molecular guideline based on databases acquired from -omics and other biological measurements. The molecular guideline databases will significantly advance clinical diagnosis and treatment of ASD.

Conflict of interest

The author declares no conflict of interest.

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