Review

Autism spectrum disorder pathogenesis – a cross-sectional literature review

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Abstract: The etiology of autism spectrum disorder (ASD) has not yet been completely elucidated. Through time, multiple attempts have been made to uncover the causes of ASD. Different theories have been proposed, such as that it is caused by "Gods Wrath," alternations in the gut–brain axis with an emphasis on gut dysbiosis, post vaccine complications, and genetic or even autoimmune causes. In this review we present data covering over 170 000 participants at age ranging from 2 to 14 years focusing on human cell changes rather than using animal models found in previous studies. The male/female ratio was 4:1. Data collection occurred in many countries covering ethnically diversified subjects. Moreover, we aim to show how the progress in genetic techniques influence the explanation found in medical White Papers based on the human genetic samples we have observed from our studies

Keywords: keyword ASD; autism; Asperger syndrome; molecular sequencing

1. Introduction

Autism spectrum disorder (ASD) mechanism still remains unclear. Currently it is suspected to have multi-lineage etiology. The increasing incidence of these disorders represent a significant medical problem associated with an a surge of both medical and social care costs. Current progress of regarding our understanding of the genetic basis of ASD made an invisible progress of comprehension, and screening of ASD risk. ASD prevalence has been measured by special education and administrative records [1-4]. Generally the frequency of the occurrence of ASD in children is approximately 1.5%– 2%. A cross-sectional study by Christiansen et al. covering the years 2010, 2012, and 2014 showed that the estimated prevalence of ASD (per 1,000) among children aged 4 years was 13.4 in 2010, 15.3 in 2012, and 17.0 in 2014 [2]. However, the results of a screening study by Saito et al. (2020) covering 2013–2016 noted a higher ASD incidence with an adjusted ASD prevalence of 3.22%. The estimated male-to-female ratio of the prevalence was estimated at 2.2 [5].

2. Materials and Methods

This narrative review focuses on peer-reviewed literature. All reviewed sixty eight studies, reviews and experimental studies, ranging from 1998 to 2020, were obtained by means of systematic browsing of Pubmed and the Web of Science. Conducted searches included the following keywords: autism, ASD, Asperger syndrome, genetic profile, gut-brain axis, food intolerance, microbiota, cell migration, brain development. Reviewed studies contained demographically diverse samples originating from a wide range of

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Western cultural contexts. The research papers covered 171,345 participants. Four researches studied 135 post-mortem cases and samples from the Tissue Bank of Neurodevelopment Disorders, and two experimental researches were based on an animal model. Other cases were based on humans using laboratory tests, microbiologiacal tests and finally molecular analyses.

3. Results

The collected materials allowed us to present a multilineage approach to explain of ASD pathogenesis. All that we know to date about ASD has been provided by research conducted in Western high-income countries. It is believed that the majority of ASD patients live in low- and middle-income regions where access to modern medicine is still heavily restricted. There are multicultural differences in behavior norms, culture-specific approaches, beliefs, mental health literacy, or even stigmas [6]. To the present day the etiology of ASD is still enigmatic. Many reaserches tried to explain the driving abnormality but there is still no hard evidence. Looking from a time perspective there is a evident change of ASD etiology perception. The first highlighted idea was the gut-brain axis theory with emphasis on gut microbiota and especially the fungal colonization of the colon mucosa. The parallel theory centered around the influence of environmental changes, especially environmental pollution. Subsequently the advancements made in laboratory technology allowed for studies with their focus on stem cell pathways and molecular abnormalities.

The problem overview - from past to present day

3.1. Gut–Brain Axis

The discussion about the ASD pathway has been focused on the gut-brain axis for many years. A recent review by Fattorosso et al. (2019) presented a dependance of ASD symptoms with the gut dysbiota. Generally, intestinal dysbiosis and frequent gastrointestinal (GI) symptoms would partially explain the role of probiotics and other para-pharmaceuticals in the care on children. The microbiota composition built by collonies of Bacteroidetes, Firmicutes, and Actinobacteria, is much more concentrated in children with ASD than in controls. The ASD patients showed a abnormaly high ratio of Faecalibacterium and Phascolarctobacterium and a decreased number of Coproccocus and Bifidobacterium species. The authors concluded that the administration of probiotics would be the most promising treatment for neurobehavioral symptoms, however there is still a need of well-designed trials in this field [7-8]. Other recent systematic review by Ho et al. (2020) discredits gut-dysbiosis as a leading cause of ASD symptoms. According to the authors there are ambiguous conclusions in reports and consequently its causality cannot be confirmed [9]. Another proposed aspect in gut dysfunction coused by diet intolerance. It is believed that a gluten-free diet or other food-intolerance restriction that could reduce severity of ASD symptoms [10]. A controversial thesis studying the mother's glucose abnormalities during pregnancy was reported recently. The authors hypothesized that a glucose-rich diet by the mother a day before delivery would be a couse of fetal ischemic brain injury. Within the published study (Hoirisch-Clapauch and Nardi, 2019), meta-analysis and Swedish research were included as evidence of linkage of maternal hyperglycemia to ASD. However, initial findings were observed in animal models the authors found a deep need for future controlled studies to confirm a close correlation on maternal hyperglycemia and the risk of ASD [11].

3.2. Role of the Environment

A few studies investigating other ASD causes in diet or environment associations focus on mercury and biphenyl poisoning as a potential ASD cause. A review by McCaulley (2019) suggests the neurotoxicity of a common heavy metal, via genetic and epigenetic alterations [12]. The fact that the industrial progress has led to severe environmentla pollution is not negotiable. In the list of the most harmful chemicals there are

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polychlorinated biphenyls (PCBs) and heavy metals with their proven neurotoxicity. The reported reaserch highlighted the evidence of PCBs alternating dopamine (DA) neurotransmission. In addition, in vitro studies using the pheochromocytoma cells lines being exposed to the PCBs presented a significant reduction of dopamine concentrations (Pessah et al, 2019). Furthermore, the molecular mechanism underlying reduced cognition, attention, behavior, attention deficit, hyperactivity disorder, or ASD has been also highlighted. All these have been found to impact dopaminergic neurotransmission, hypothyreosis, calcium dyshomeostasis, or oxidative stress especially in early childhood presenting ASD specific symptoms [13]. Recently, it has been reported that high levels of reactive oxygen species (ROS) leading to oxidative stress, could also be a possible mechanism resulting in ASD. This pro-inflammatory state caused by the ROS results in dysregulation of the central nervous system and immune system which has been acknowledged as contributing factors of ASD development. Moreover, it is thought that reduced resources of free-radical scavengers as decreased glutathione level makes individuals more vulnerable to neurons injury. The contribution of the ROS toxicity has been highlighted in the autism animal model by alleviating the brain neuroinflammatory via overactivation of microglia [14]. Another metabolic route concerning L-carnitine deficiency was considered as a unique subgroup of ASD patients on the basis of suggesting mitochondrial dysfunction leading to first autisctic symptoms. Recently publisched two randomized clinical trials suggest that carnitine administration could extinguish severe symptoms in non-syndromic ASD. Unfortunatelly, in both trials, dose-dependent adverse reactions have been observed, but the same beneficial effect has also been reported for other comorbid disorders, such as intellectual disability and increased muscle tense. Authors concluded that following stydies are necessary, however, the beneficial features of carnitine treatment on the basis of mitochondrial dysfunction may alleviate the symptoms in non-syndromic ASD [15].

3.3. Stem Cells

A fresh, innovative thesis presented by Bankaitis et al. concerning L-carnitine impact was discussed last year. The authors coined the neural stem cell (NSC)/carnitine malnutrition hypothesis. According to the authors the ASD risk factor would be a diminished capacity for carnitine-dependent long-chain fatty acid β-oxidation in the neural stem cells of the developing central nervous system. The authors concluded that fetal carnitine status is a significant metabolic component in determining NSC vulnerability and further could contribute in abnormal cell maturation and dysfunction [16].

With obvious reason the studing of glial cells in ASD affected patients in impossible. The hope are cellular models providing us a ways to uncover disease mechanisms and develop novel therapeutic strategies. The ability of induced pluripotent stem cells (iPSCs) to generate diverse brain cell types offers great opportunity to study neurodevelopmental disorders.

This iPSC-based model was used to understand the neuronal and glial contributions to neurodevelopmental disorders, including ASD, Rett syndrome, bipolar disorder (BP), and schizophrenia. For example, many molecular hot-points have been shown to influence cellular phenotypes in three-dimensional iPSC-based models in patients with ASD. Delays in the differentiation of astrocytes and morphological changes of neurons are associated with Rett syndrome. In bipolar disease and schizophrenia, patient-derived models helped identify cellular phenotypes associated with neuronal deficits and mutation-specific abnormalities in oligodendrocytes [17].

3.4. Genetic Puzzle

The last tree decades is a time of a significant progress in genetic studies concerning neurobehavioral syndromes including ASD. First results provided a huge amount of molecular data that would be used to verify many hypotheses, models, and finally genetic pathway of ASD. To present day, several lines of evidence support the view that structural and genomic variation play a pivotal roles in ASD. All sophisticated molecular techniques

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for genome sequencioning, including array comparative genome hybridization and single nucleotide polymorphism analysis, has allowed for the detection of a large number of autism-related loci. Copy number variants (CNVs) are the most common form of molecular abnormality and are seen as very important contributors to the pathogenesis of neurodevelopmental disorders. Because of the complex synaptic architecture, deciphering the functional impact of ASD associated variants is an extremely arduous task. Currently, it is believed that at least hundreds of loci are associated with ASD. That complexity makes it difficult to identify singular potential causative pathway and then therapeutic approach. Analysing the databases we can find the huge number of reserches based on divesified genetic material and used molecular assay. That is why we are still far behind in interpreting the main causative molecular sequences in ASD. The system biology/network analysis approaches would provide new insights into the molecular driving pathway. To help with these analyses the animal models, in vitro studies, and expreimental appoaches would contribute as well. It is suggested that comparison of these data on a multilevel plane would allow to create a ASD template model and then to explain both psychiatric disfunction and other somatic problems [18]. Previously mentioned CNVs are particularly important in the cases of complex syndromes, such as when ASD symptoms occur in association with intellectual disability and/or congenital malformations (e.g., Angelman syndrome, Phelan–McDermid syndrome). The model proposed by Bourgeron to explain the complex genetic landscape in ASD appears to be very reasonable. Because of massive molecular heterogeneity in affected subjects the authors try to focus on to the interplay between a genetic background with a low- to high-risk predisposition to ASD onset. Moreover, there are pointed new rare or ultra-rare variants with low to high-risk potential wchich can contribute in ASD as well. The combination of these different categories of variants in the population results in the massive phenotypic heterogeneity of ASD. Reported data provided an evidence concomitance of different models of inheritance in the heterogeneous ASD population but there is still lack of driving core. Here we can find monogenic in subjects carrying ultra rare or de novo variants, extremely deleterious and highly penetrating mutations; oligogenic with the concomitance of medium/high-risk predisposing variants; and, finally, a polygenic presence of multiple low-risk genetic variants [18-19]. The sybtyping would explain clinical ASD types and symptoms diversity and severity. During the last two decades, the launching of high throughput sequencing revolutionized genetic research and allowed studing ASD on significaty wider molecular landscape. The early documentation on karyotype chromosomal abnormalities shed a light on susceptible loci screening on those highly involved genomic regions such as chromosome 7q, 1p, 3q, 16p, and 15q [20]. Sequencing technology undoubtedly confirmed that the etiology of ASD is multigenic and highly heterogeneous. At this moment in know that any ASD patient bears various CNVs variations raising ASD susceptibility and provides an additional proof of the multigenic etiology. Although there is no clearly defined biomarker nor the driving molecular route identified, the fresh research studied DNA methylation present othergenes involvement in ASD. Only a little handful of ASD-related diseases have monogenic cause, such as Rett syndrome, tuberous sclerosis, fragile X syndrome, and Schuurs–Hoeijmakers syndrome [20-23]. All of that very well sign in Bourgeron hypothesis.

The complexity of neurodevelopment and signal transduction makes for the possible multilevel neurocyte dysfuntion. Genes output as a functional proteins, contribute in synapse formation, transcription regulation, and even chromatin remodeling. In the group, there were included of synapse-related risk genes were included those coding especially cell-adhesion proteins and ion channel. The synapsin family proteins contribute to synaptogenesis and realese of neurotransmiters. Mutations in synapsin-1 (SYN1) and synapsin-2 100 (SYN2) gene are common in neuropsychiatric disorders including ASD. Another problem obserwed in ASD is the changed signal transduction and influence on neutrotransmiter secretion. Reported data highlighted a high ratio of mutations in genes encoding ion channel protein as sodium voltage-gated channel alpha subunit 2 (SCN2A), potassium voltage-gated channel subfamily D member 2 (KCND2) calcium voltage-gated

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channel subunit alpha1 E (CACNA1E), Ankyrin a protein encoded bymultiple ankyrin repeat domains 3 (SHANK3) play important role in synaptogesis, maintenance of membrane channels and clearing of synaptic cleft. [24-30]. Clinical observation and key-ASD symptoms confirm an implication of synapse disfunction and abnormal dendritic etwork in ASD. A wide gene panel sequencioning allowed to find an increased number of de novo mutation (DNM) in regulatory genes However, correlation of DNM elements within the targeted genes on neuropsychiatric disorders was not identified yet, the DNMs were found to be specifically upregulated in early prenatal brain development [31-33]. Another no less meaning aspect in ASD genesis is chromatin-remodeling pathways. Into this gene group were incuded methyl CpG binding protein 2 (MeCP2) working as activator or inhibitor other genes. Ubiquitin Protein Ligase E3A (UBE3A), chromodomain helicase DNA binding protein 8 (CHD8) is an enzym contributing in proteasome degradation of proteins via connection their with ubiquitine. Mechanically, the UBE3A silencing could be activated by metahylation as imprinting what was proofed in Angelman and Willi-Prader syndrome. Tran and co-workers recently showed that fragile X mental retardation protein (FMRP) and fragile X related protein 1 (FXRP1) mutations could lead to abnormal RNA editing enzyme activity leading to hypoedition of adenosine--inosine transformation in neurons [34-36].

It is thought that disease-causing gene mutation are germinal and are present in almost all somatic cell. Obviously, post zygotic aquired mutation coud lead to is a somatic mosaicism, wchich is common in neurodevelopmental diseases, including autism. Neurogenesis is a crucial period in tissue development and maturation where aquired SNVs could lead to gene polymorhism. It was especially observed on example of the sodium channel alpha-1 subunit, SCN1A [37-39]. Generally, accordind to many reported data the aquired mutation prevalence is calculated about 5%–7%. However the frequency of post zygotic mutation in autism is unknown, some reaserches highlihgt its role as important pathway in ASD genesis [40-41]. Most reported mutations are not pathogenic or are classified as unkwown meaning, but some exons polymorhism could be extremely detrimental. All of that have been associated with ASD, Rett syndrome, tuberous sclerosis, and intellectual disability. Until next-generation sequencining, our understanding of somatic mosaicism in ASD was created only by simple molecular tests and were reported as case reports. The whole-exome sequencing (WES) data from large cohorts have been milestone in understanding of the somatic mosaicism role. According to Krupp et al. and Lim et.al. studis the mosaicysm prevalence is estimated on 3%–5%. Other reaserch based on larde cohort covering 5,947 families affected by ASD studied the meaning of critical exons variations wchich were much more common in ASD children than in unaffected siblings. In addition, the authors pointed that these exons variants showed higher expression in the amygdala—an area critical for emotional response and social awareness [42]. Similar report showed that aquired cerebellum located genes could be a cause of coordination difficulty that could be related to gait disorders typica for ASD patients [43]. A similar large cohort WES study be Freed and colleagues reported similar conculsion concerning somatic mosaicism as a significant factor in ASD etiology [44].

Currently, CNV is now seem as a critical driving factor in ASD susceptibility. Additionally, there is a thesis that these variations directly cause about 10% of all ASD cases. The molecular tests pointed 16p11.2 duplications as a very important region of DNA chain In this region are located at least 25 genes involved in neurodevelopment and maturation. Golzio et al. hypothesized that only one gene in thisregion—potassium channel tetramerization domain containing 13 (KCTD13) —is a pivotal driver for neuropsychiatric disease [45]. That strand undergo further deeper investigation. There was observed that CNVs in 16p11.2 region makes wrong synaptic transmission through altered regulation of Ras homolog family member A (RhoA) [46]. In addition, Escamilla et al. concluded that KCTD13 deletions is crucial in ASD genesis but in contrary to Golzio concluded that this mutation alone are not likely to be sufficient to cause the disease. Likely, the real driver of disease in 16p11.2 duplications or deletions is not from just one gene but from an interaction of all 25 genes contributing to susceptibility. Iyer et al. systematically investigated

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the interaction between genes in the 16p11.2 region, using RNAi in Drosophila to test 565 pairwise knockdowns. Moreover, they presented 24 interactions between pairs of genes within the 16p11.2 region, and Additionally 46 interactions between 16p11.2 genes and others ASD related genes. This information would be crucial in searching for a leading pathway in ASD genesis. Data suggest that interactions within CNVs result in the ASD [47]. The other CNVs loci are less frequently studied. The most common ASD-related region, such as 15q11-13 as well as 16p11.2, are present in only approximately 1% of cases [48].

Working with the heterogeneity, we can see that paying attention to common affected functional networks proves to be a useful method of study. It is a prevalent outcome in numerous studies, that autistic people have deletions in synaptic genes, namely SHANK3, dipeptidyl peptidase-like 10 (DPP10), neuroligins, and neurexins [49-51]. Among gene sets with rare CNVs, it is usual to see those related to cell development and proliferation, chromatin regulation, and ubiquitin pathways. In cases of some CNVs, copy number dosage seems to have an influence on disease phenotype. Stefansson et al. looked at 15q11.2 CNV region of autistic people and discovered that there were two areas of the brain with dose dependent structural and functional effects [52]. It is a curious thing to see, that non ASD/schizophrenic, dyslexic and dystaxic controls manifested the very same structural changes. Girirajan et al. (2010) found a dose dependent effect based on the microarray analysis with identified CNVs in genes associated with ASD. The reported correlation was one between duplication size increase and autism severity. However, there was not found any relation between duplication size and non verbal IQ [52-54]. The question to ask here is that how come non-causative modifiers play a part in modulating CNV pathogenicity? Epigenetic gene modulating functions have a great deal of involvement in ASD. There was a study which proposed that highly penetrative ASD risk related genes were usually located in the nucleus and that they have an involvement in modulation of either expression or silencing of the protein-protein network, key for CNS development [55]. Different studies show how the deep epigenetic changes could modify disease phenotype As an example, there was a study of 50 pairs of monozygotic twins discordant of ASD, who were reported to have many autism associated differentially methylated regions, some patterns of CpG sites in line with symptom groups. Even though there still remains a great amount of knowledge to be grasped about the epigenetic modulation od ASD, the large scale epigenomic studies have already provided the scientific and medical communities with valuable patterns. It is believed that methylation of KMT2C, lysine methyltransferase 5-6B, MeCP2, CHD8, and POGZ, FMRP and the RBFOX family, UBE3A and E3 ubiquitin-protein ligase 1 would seriously improoved the ASD susceptibility [24]. These proteins are varied function wise and often inlude pathways seen in autism, for example the synaptic formation. In order to see how single epigenetic regulator mutation could account for the modification of numerous other risk genes, we could focus on the two leading susceptibility genes, namely MeCP2 and UBE3A. MeCP2 is a chromatin modifier which is with no doubt involved in ASD). It is seen in a case of a healthy individual that the binding action of MeCP2 regulates numerous synaptic funtion genes, GABRB3, brain-derived neurotrophic factor (BDNF), distal-less homeobox 5 (DLX5), insulin-like growth factor-binding protein 3 (IGFBP3), cyclin dependent kinase-like 1 (CDKL1), protocadherin beta 1 (PCDHB1), protocadherin 7 (PCDH7), and lin-7 homolog A (LIN7A) [57]. The E3 ubiquitin protein ligase UBE3A is the second crucial epigenetic regulator closely related to ASD. It is modulated by MeCP2 but it can also be causative itself.

UBE3A's location is 15q11-13, regularly duplicated in autism cases. Dose dependent effects correlated positively with decreased excitatory synaptic transmission speech delay and psychomotor regression [60-61]. Lee et al. (2014) identified four proteosome-related UBE3A direct subtrates proteins. UBE3A and subtrate proteasome 26S subunit, non-ATPase 4 (Rpn10) caused the growth in accumulation of ubiquitinated proteins, hinting at a proteostatic imbalance. Protesome health is strongly implicated in dendritic spine outgrowth, making for a connection between UBE3A and abnormal neurocytes as seen in autism cases [63]. Additionally, its involvement in Wnt signalling could also cause for a

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serious perturbation during development period. [64]. MeCP2 and UBE3A are two fine examples of a gene mutation causing very extensive effects. It was the wide ranging epigenetic studies that have provided us with a broad view of epigenetic disregulation seen in ASD. Ladd-Acosta et al. (2014) measured above 485,000 CpG loci in 40 individuals' post mortem brain tissue finding four differentially methylated regions. Three methylated regions were found to be located in the cortical tissue: the proline-rich tranmembrane protein 1 (PRRT1) 3′ UTR, promoter regions of tetraspanin 32 (TSPAN32), and C11orf21. The other site was identified in the cerebellar tissue, an alternative promoter for succinate dehydrogenase complex flavoprotein subunit A pseudogene 3 (SDHAP3) [65]. The evidence of abnormal DNA methylation in ASD cases exists in numerous aspects, ranging from genetic mutations in epigenetic machinery to loci specific and genome wide changes. Epimutations in DNA methylation are possible to be aquired, meyhylation reprogramming and imprinting are active during early embrogenesis and postnatal peak synaptogenesis. [66]. Mor et al. approached the matter using small RNA sequencing data, relating the results to genome wide DNA methylation in order to find disregulated miRNAs. The result proved to be in line with many other studies, the significantly expressed miRNAs in ASD patients' brains were related to synaptic activity. There was also a mention of a connection to the oxytocin receptor OXTR gene, which hints at attenuated OXTR expression in the autistic brain. A study of preterm birth fetal membranes supports this conclusion, showing hypermethylated OXTR and suggesting a potential environmental factor to be linked to this pathological process [67]. Homeobox 2 (EN2) is another epigenetic function risk gene, its patterns of methylation in ASD cases are found to be unusual, the gene is speculated to cause abnormal cerebellar Purkinje growth and maturation. The list of epigenetic function ASD risk genes is a vast one, the mechanism of broad gene expression disregulation could rely on just a few mutations [68].

In Table 1 we collected studied genes and their main function.

Table 1. A presentation of studied mutations in ASD cases according to its function.

Function	Gene
nucleus	KDM5B, MAGEL2, SMARCB1, KMT2A, MYT1L, KMT2C, CHD8, CTNND2, CHD7, CHD2, AAAS, PHF8, SYNE1, RAI1, NIPBL, NSD1, DMD, HOXA1, PCDHA2, MBD5, RBFOX1, CDKL5, CRADD, ATRX, DYRK1A, HUWE1, EN2, ASH1L, ARID1A, SMARCA2, ASPM, KANSL1, SETBP1, TBL1XR1, ASXL3, BCOR, ADNP, CC2D1A, ZNF674
plasma membrane	NRXN1, CACNA1C, CACNA1F, GRIP1, RAB40A, RELN, PCDHA1, PCDHA4, SCN7A, PCDHA3, DMD, PCDHA9, ATP7A, PCDHA8, MICA, PCDHAC1, SCN1A, KIRREL3, KCNJ10, ABCC8, ATP6AP2, CACNA2D3, ANK3, GABRG3, PRSS12, GRIN1, DLG3, SLC9A9, CNTN3, CNTN4, CDH15, SHANK3, SHANK2
transcription, DNA-templated	KDM5B, MAGEL2, SMARCB1, MYT1L, KMT2C, CHD8, CTNND2, ATRX, CHD7, ARID1A, CHD2, SMARCA2, MED13L, PHF8, TBL1XR1, NSD1, ASXL3, KMT5B, HOXA1, BCOR, ADNP, CC2D1A, ZNF674
nucleoplasm	KDM5B, SMARCB1, CDKL5, KMT2A, KMT2C, CHD8, DYRK1A, HUWE1, FANCB, ASH1L, ARID1A, CHD2, AAAS, SMARCA2, SYNE1, PHF8, RAI1, NIPBL, KANSL1, TBL1XR1, NSD1, MCPH1

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nervous system development	MBD5, RBFOX1, SMARCB1, MYT1L, DYRK1A, SMARCA2, PCDHA10, DLG3, PCDHA1, OPHN1, PCDHA5, PCDHA4, CNTN3, PCDHA3, CNTN4, PCDHA2, NDP, PCDHA8, PCDHAC1
DNA binding	KDM5B, MBD5, SMARCB1, KMT2A, MYT1L, KMT2C, CHD8, ATRX, HUWE1, ASH1L, ARID1A, CHD2, SETBP1, ASXL3, ADNP, ZNF674
cell adhesion	LAMC3, CTNND2, PCDHA10, RELN, PCDHA1, PCDHA5, PCDHA4, CNTN3, PCDHA3, CNTN4, PCDHA2, CDH15, PCDHA8, PCDHAC1
calcium ion binding	NRXN1, PCDHA10, GRIN1, PCDHA1, PCDHA5, OTOF, FAM20C, PCDHA4, PCDHA3, PCDHA2, CDH15, PCDHA9, PCDHA8, PCDHAC1
integral component of plasma membrane	KCNJ10, NRXN1, PCDHA10, GRIN1, PCDHA1, PCDHA5, PCDHA4, PCDHA3, PCDHA2, SLC16A2, ATP7A, PCDHA8, MICA, PCDHAC1
positive regulation of transcription from RNA polymerase II promoter	IGBP1, SMARCB1, KMT2A, CHD8, ATRX, CHD7, EN2, ASH1L, SMARCA2, GRIN1, RAI1, NIPBL, TBL1XR1
homophilic cell adhesion via plasma membrane adhesion molecules	KIRREL3, PCDHA1, PCDHA5, PCDHA4, PCDHA3, PCDHA2, CDH15, PCDHA9, PCDHA8, PCDHA10, PCDHAC1
chromatin binding	MBD5, NIPBL, KMT2A, CHD8, NSD1, ATRX, CHD7, ADNP, ASH1L, SMARCA2, PHF8
positive regulation of transcription, DNA-templated	GRIP1, RAI1, KMT2A, TBL1XR1, CHD8, NSD1, NDP, ARID1A, SMARCA2, CDK5RAP2, PHF8
postsynaptic membrane	GRIP1, DLG3, DMD, ANK3, GABRG3, DLGAP2, SHANK3, SHANK2, SYNE1, GRIN1
cell junction	GRIP1, OPHN1, NRXN1, OTOF, GABRG3, DLGAP2, SHANK3, SHANK2, GRIN1, CDK5RAP2
negative regulation of transcription from RNA polymerase II promoter	IGBP1, NIPBL, TBL1XR1, WFS1, CHD8, NSD1, BCOR, ARID1A, SMARCA2, CC2D1A
endoplasmic reticulum	GRIP1, PCDHA1, RPL10, WFS1, NRXN1, ANK3, PCDHA2, DHCR7, ATP7A, GRIN1
dendrite	KIRREL3, GRIP1, RELN, WFS1, CTNND2, ANK3, ADNP, PRSS12, GRIN1
brain development	NIPBL, RELN, CHD8, CNTN4, CDK5RAP2, PHF8, AFF2
neuron projection	GRIP1, TPH2, ANK3, ATP7A, SHANK3, SHANK2, GRIN1
cell surface	NRXN1, DMD, ANK3, NDP, PPFIA4, MICA, GRIN1
regulation of ion transmembrane transport	KCNJ10, CACNA2D3, SCN7A, CACNA1F, CACNA1H, SCN1A
covalent chromatin modification	SMARCB1, CHD8, ATRX, CHD7, ARID1A, CHD2
sensory perception of sound	NIPBL, WFS1, CHD7, OTOF, HOXA1, NDP
postsynaptic density	DLG3, CACNA1C, DLGAP2, SHANK3, SHANK2, GRIN1
neuronal cell body	DLG3, NRXN1, ADNP, ATP7A, SHANK2, SCN1A
chromatin remodeling	SMARCB1, ATRX, CHD7, ARID1A, SMARCA2

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histone binding	TBL1XR1, CHD8, ATRX, CHD2, SMARCA2
calcium ion transmembrane transport	CACNA2D3, CACNA1C, CACNA1F, CACNA1H, GRIN1
axon guidance	RELN, OPHN1, NRXN1, ANK3, CNTN4
basolateral plasma membrane	KCNJ10, DLG3, OTOF, ANK3, ATP7A
protein C-terminus binding	GRIP1, RBFOX1, NIPBL, DLG3, SHANK3
calmodulin binding	ASPM, WFS1, CACNA1C, GRIN1, CDK5RAP2
visual perception	KCNJ10, WFS1, LAMC3, NDP, CACNA1F
transcription regulatory region DNA binding	KMT2A, TBL1XR1, BCOR, SMARCA2, CDK5RAP2
actin binding	OPHN1, DMD, TNS4, SHANK3, SYNE1
positive regulation of synaptic transmission, glutamatergic	RELN, NRXN1, SHANK3, SHANK2
positive regulation of excitatory postsynaptic potential	RELN, NRXN1, SHANK3, GRIN1
ionotropic glutamate receptor binding	OPHN1, DLG3, SHANK3, SHANK2
voltage-gated calcium channel complex	CACNA2D3, CACNA1C, CACNA1F, CACNA1H
membrane depolarization during action potential	SCN7A, CACNA1F, CACNA1H, SCN1A
histone-lysine N-methyltransferase activity	KMT2A, NSD1, KMT2C, KMT5B
social behavior	NRXN1, SHANK3, SHANK2, GRIN1
cerebral cortex development	ASPM, ATIC, MCPH1, GRIN1
learning	NRXN1, AAAS, SHANK3, SHANK2
synapse assembly	KIRREL3, NRXN1, SHANK3, SHANK2
post-embryonic development	KDM5B, ALDH5A1, KMT2A, DHCR7
beta-catenin binding	GRIP1, TBL1XR1, CHD8, CTNND2
multicellular organism growth	STIL, TBL1XR1, ATRX, DHCR7
helicase activity	ATRX, CHD7, CHD2, SMARCA2
dendritic spine	OPHN1, SHANK3, SHANK2, GRIN1
neuron migration	KIRREL3, ASPM, RELN, CDKL5
Z disc	DMD, ANK3, CACNA1C, SCN1A
negative regulation of neuron apoptotic process	WFS1, EN2, ADNP, GRIN1
NMDA glutamate receptor clustering	RELN, NRXN1, SHANK3
receptor localization to synapse	RELN, DLG3, NRXN1
npBAF complex	SMARCB1, ARID1A, SMARCA2
nBAF complex	SMARCB1, ARID1A, SMARCA2
SWI/SNF complex	SMARCB1, ARID1A, SMARCA2
vocalization behavior	NRXN1, SHANK3, SHANK2
histone methyltransferase activity (H3-K4 specific)	KMT2A, KMT2C, ASH1L
histone H3-K4 methylation	KMT2A, KMT2C, ASH1L

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ATP-dependent chromatin remodeling	SMARCB1, CHD8, ARID1A
adult behavior	NRXN1, SHANK3, SHANK2
MLL1 complex	KMT2A, KANSL1, CHD8
neuronal action potential	SCN7A, ANK3, SCN1A
adult walking behavior	KCNJ10, CHD7, SCN1A
long-term synaptic potentiation	RELN, SHANK3, SHANK2
cognition	NIPBL, CHD7, HOXA1
DNA duplex unwinding	CHD8, ATRX, CHD2
cardiac conduction	CACNA2D3, CACNA1C, CACNA1F
neuromuscular process controlling balance	RBFOX1, NRXN1, SHANK3
forebrain development	STIL, ATRX, ARID1A
neuron projection morphogenesis	KIRREL3, DMD, ATP7A
methylated histone binding	CHD8, ATRX, PHF8
negative regulation of neuron differentiation	ASPM, CNTN4, CDK5RAP2
terminal bouton	OPHN1, PRSS12, GRIN1
presynapse	KCNJ10, NRXN1, OTOF
canonical Wnt signaling pathway	TBL1XR1, CHD8, NDP
guanylate kinase-associated protein clustering	NRXN1, SHANK3
GKAP/Homer scaffold activity	SHANK3, SHANK2
axon initial segment	ANK3, SCN1A

5. Conclusions

This review increases our knowledge concerning ASD, but there are further challenges ahead. There is no evidence-based single pathway that results in symptoms of ASD. Patient management is constantly only symptomatic, and there is no ASD screening apart from the symptom-based diagnosis and parent-mediated interventions. Multi-gene sequencing or epigenetic alternations hold promise in solving the disjointed molecular puzzle. Further research is needed, especially in the field of biogenetics, because young children constitute the patient group most affected by ASD.

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