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The Genetic and Epigenetic Basis Involved in the Pathophysiology of ASD: Therapeutic Implications

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Abstract

The prevalence of autism has increased in an exponential way in the past few years. Many monogenetic mutations as well as copy number variants and single nucleotide polymorphisms have been associated with autism spectrum disorders (ASD), a large proportion of which occur in genes associated with synaptogenesis and synaptic function. However, the increase in appearance of genetic alterations does not explain the etiology of an elevated number of ASD cases. Recent research is now focusing on the role of environmental/epigenetic factors, which by themselves and/or in combination with classical genetic factors, may be the root cause of a large number of ASDs. In this chapter we review the current literature regarding the epigenetic changes involved in ASD, including their possible mechanisms of action such as oxidative stress, altered fatty acid metabolism, mitochondrial dysfunction, DNA methylation and histone methylation (via the one-carbon metabolism cycle), histone variants, and ATP-dependent chromatin remodeling. We discuss possible new biochemical markers related to autism as well as new lines of research for therapeutic targets.

Keywords: ASD, autism, children, betaine, choline, genetics, epigenetics, fatty acid, lipid, one-carbon metabolism cycle, PUFAs, peroxidation, methylation, mitochondrial dysfunction, oxidative stress, phospholipases, research

1. Introduction

Autism spectrum disorder (ASD) etiopathogenesis occurs result of a complex, engaging multiple environmental factors and epigenetic modifications, interacting with the genetic basis of the developmental processes. Although the ADS-risk genes are numerous, and many of them have a dual function involved in different neural networks, they seem to converge in a

limited number of molecular pathways. It has been proposed that genes implicated in “mono-genic” variants of syndromic and nonsyndromic ASD converge on molecular pathways and mechanisms related to synaptic dysfunction also known as “developmental synaptopathy.” The interplay between mutations in different genes can produce “idiopathic” autism, but the exposure to environmental modifiers as well as epigenetic factors may add up to a varying expression of autistic features, allowing to define autism as “synaptic and chromatin-remodeling disorders.”

Congenital epigenetic diseases are a newly delineated group of multiple congenital anomalies, intellectual disabilities, and ASD syndromes resulting from “Classical imprinting disorders” and “Mendelian disorders of the epigenetic machinery.” The advances in the identification of the DNA-methylation level of specific genes, histones modifications (highlighted dysregulation of histone methylation as a major contributing factor), histone variants, and ATP-dependent chromatin remodeling complexes throw light about the epigenetic effects of nongenetic biological risk factors and environmental factors in ASD.

Currently, new biochemical markers have been implicated in the etiology of autism, such as increased oxidative stress and lipid peroxidation, phospholipase release of fatty acid, and the formation of toxic metabolites, mitochondrial dysfunction, or alteration of DNA-methylation. In addition, other relevant epigenetic mechanisms such as impaired methylation via the “one-carbon metabolism cycle” including the folate metabolism, the methionine-homocysteine remethylation cycle (involving choline and betaine), and the transsulfuration pathway also help to complete the difficult puzzle of autism.

Therefore, epigenetic mechanisms constitute new lines of research and may open the door for new targets in the treatment of autism. The epigenetic changes are potentially reversible, and, therefore, a thorough understanding of these modifications may identify new therapeutic targets for the disease. So finally several questions arise: Is autism also an acquired and therefore preventable, treatable, and potentially curable epigenetic disease? Could alteration in diet and fat supplementation result in neuronal repair and reverse the deficit in autism? How could this knowledge be applied to clinical practice?

In this chapter, we conducted an updated review of the role of genetic and epigenetic changes involved in the pathophysiology of ASD including their possible mechanisms of action. We also included the latest lines, perspectives, and future directions of therapeutic approaches.

2. The etiopathogenesis of ASD

The autism, a debilitating neurological handicap in children, is a highly heterogeneous set of disorders with wide variations in symptom severity, intellectual level, and functional disability. Classically in DSM IV-TR [1] “*Pervasive developmental disorders*” (PDD) encompass a heterogeneous group of children characterized by severe and pervasive impairment in several areas of development: (1) reciprocal social interaction skills, (2) communication skills, and (3) the presence of stereotyped behavior, interests, and activities. The qualitative impairments that define these conditions are distinctly deviant relative to the individual's developmental

level or mental age. The specific disorders included five subtypes in this section: autistic disorder (AD), Rett's disorder (RD), childhood disintegrative disorder (CDD), Asperger's disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS). These disorders are usually evident in the first years of life and are often associated with some degree of *"intellectual disability."*

At present, a new edition of the DSM ("Diagnostic and Statistical Manual of Mental Disorders," from the American Psychiatric Association) has been published: DSM-5® [2]. In the new DSM-5 there is a single category of ASD (autism spectrum disorder) instead of five subtypes, and has deleted the term "pervasive developmental disorders." Scientific evidence and clinical practice show that a single spectrum better reflects the symptom presentation, time course, and response to treatment. ASDs is a new DSM-5® name that reflects a scientific consensus of four previously separate five subtypes disorders, and are actually a single condition. The three domains of DSM IV are combined into two core domains: (a) Deficits in social communication and social interaction. The rationale is that deficits in communication and social behaviors are inseparable. Social communication domain will be created by merger of key symptoms from the DSM-IV social and communication domains. This de-emphasizes language skills not employed in the context of social communication. (b) The second major criterion remains restricted repetitive behaviors, interests, and activities (RRBs).

In multiple communities of the United States (CDC surveillance data, 2010) [3], the overall prevalence of ASD was 14.7 per 1000 (1 in 68) children aged 8 years. Overall ASD prevalence estimates varied among sites from at least 5.7 to 21.9 per 1000 children aged 8 years. Consistent with previous reports, findings were marked by significant variations in ASD prevalence by geographic area, sex, race/ethnicity, and level of intellectual ability. The extent to which this variation might be attributable to diagnostic practices, underrecognition of ASD symptoms in some racial/ethnic groups, socioeconomic disparities in access to services, and regional differences in clinical or school-based practices that might influence the findings in this report is unclear.

The importance of accurately identifying individuals with autism has never been greater, particularly given the growing prevalence [4], considerable family and societal costs [5], and recognized importance of early diagnosis and intervention. The ASD frequency has been increasing for decades, with an astonishing 556% reported increase in pediatric prevalence between 1991 and 1997 (a prevalence higher than that of spina bifida, cancer, or Down syndrome). Even though researchers cannot agree on whether the trend is a result of increased awareness, improved detection and changing diagnostic criteria with expanding definition and reduced stringency of diagnostic criteria, rather than to new environmental influences and other factors of unknown nature [6, 7]. The classification systems used strongly affect prevalence studies, and is important to consider the changes that have occurred at this level when analyzing the possible causes of the increase in previously known as *pervasive developmental disorders* [8]. But this increased ASD prevalence cannot be attributed entirely to "nonetiological factors" such as changes in reporting practices, diagnostic ascertainment, and "diagnostic substitution" (i.e., ID diagnoses decreased at the same time ASD diagnoses increased) [9–11].

Because people with autism can have very different features and symptoms with very high degree of phenotypic heterogeneity, autism is a multifactorial condition and is thought of as a spectrum disorder [12, 13]. Autism is one of the most complex heritable disorders, and a paradigmatic complex genetic disorder, with convincing evidence for genetic factors and little or no support for the influence of the environment factors [14]. However, it is essential that the ASD not be reflected alone in overly restrictive genetic approaches if we want to better understand its pathogenesis. Today, different studies support that the etiopathogenesis of ADS involves an interaction between both susceptible genetic loci and a wide range of environmental risk factors on the developmental trajectory. So the pathogenesis of ASD is considered an authentic “puzzle,” for a multifactorial threshold model underlying ASD with all types of genetic variation (SNV/indel/CNV in coding and noncoding DNA, germline, somatic, epigenetic) and environmental factors (the most significant: toxic, immune dysregulation, and nutritional status) involved in clinical, neuropsychological, and neurobiological perspectives [13, 15–18].

Since there are no definitive biological markers of autism, for a majority of cases, diagnosis depends on a range of behavioral signs. But advances in system biology are becoming strategic for carrying out knowledge on the ASD etiology and for early diagnosis. The most relevant metabolic pathways and discriminant metabolites in ASD belong among other, amino acid metabolism, antioxidant status, mitochondrial metabolism, nicotinic acid metabolism, and altered fatty acid metabolic pathways. So, for the study of this “autism epidemic” it is of great interest to identify risk factors (genetic and nongenetic) as well as metabolic routes that can contribute to ASD; but not separately if not collectively and simultaneously through common pathways, allowing to insert the pieces of the ASD puzzle together. That is, from the approach to the different disciplines: genomics, epigenomics, metabolomics, and environmental metabolomics.

3. Genetic in ASD

Children with dysmorphic features, congenital anomalies, intellectual disability, or family members with developmental disorders are those most likely to benefit from extensive medical testing and genetic consultation. Increased diagnosis of ASD associated with genetic abnormalities has allowed its new specification in the DSM5: “associated with known medical or genetic condition.” However, for children with “nonsyndromic or idiopathic” autism the studies of genetic risk factors have been less conclusive

3.1. ASD associated with “known medical or genetic condition”

It is known that several monogenic disorders (Mendelian disorders) are associated with autism. The most common of these single-gene defects, including CGG trinucleotide repeats within the FMR1 gene as the cause of Fragile X syndrome, mutations in the *MECP2* gene in Rett syndrome and related disorders, tuberous sclerosis, and PTEN mutation account for but a small minority of cases (for up to 5% of ASDs) [19]. Less often and among other genetic diseases consistently associated with autism include: rare genetic developmental disorder

(*inter alia* examples the Cohen syndrome caused by a mutation in the vacuolar protein sorting 13B (*VPS13B*) gene on locus 8q22-8q23, or the Smith-Lemli-Opitz syndrome caused by mutations in the *DHCR7* gene on locus 11q13.4 leading to deficiency of the enzyme 3 beta-hydroxysterol-delta 7-reductase); and metabolic diseases like phenylketonuria, adenylosuccinate lyase deficiency, or mucopolysaccharidosis III (Sanfilippo syndrome). (For more comprehensive listing see [20].)

There are also many anecdotal reports of autism with visible chromosomopathies [21, 22], and Down syndrome have been found with high rates of ASD of up to 42% [23]; and well-known microdeletion syndromes like 22q11 deletion syndrome divided into distinct syndromes (e.g., DiGeorge syndrome, velocardiofacial syndrome, and cardiofacial syndrome) in which more than 40% meet the criteria for either ASD, ADHD, or both [24]; as well as Angelman syndrome, or Smith-Magenis syndrome, are well known autism. Although the latter mentioned can be produced by different genetic mechanisms, Angelman syndrome is caused by deletion of the 15q11.2-q13 critical region (60–75%), paternal uniparental disomy (2–5%), imprinting defect (2–5%), and mutation in the *UBE3A* gene (10%); and Smith-Magenis syndrome is caused either by a 17p11.2 deletion encompassing the retinoic acid-induced 1 (*RAI1*) gene (90%) or a mutation of the gene (10%) [25].

Currently, these diagnosable medical conditions, cytogenetic abnormalities, and single-gene defects together account for <10% of cases of ASD. The latest advances made in genetics and technology, mainly the widespread use of molecular techniques by chromosomal microarray studies have increased the diagnostic cost-effectiveness of conventional techniques (karyotype, subtelomeric analyses, etc.) from 3–5% to 30–40% in patients with developmental delay/intellectual disability (DD/ID) or ASD, describing numerous genetic alterations. Overall, identified genetic causes of autism can be classified as single-gene disorders (5%), cytogenetically visible chromosomal abnormalities (5%), and copy number variants (CNV) (10–20%) [26].

Chromosomal microarray analysis (CMA) is increasingly utilized for genetic testing of individuals with unexplained DD/ID, multiple congenital anomalies (MCA), and has become increasingly important in the assessment of patients' ASD. CMA was initially restricted to ASD patients with specific additional phenotypic characteristics or comorbidities (DD/ID or MCA); at present, it has been established as the general recommendation and consensus statement that CMA should be offered as a first-tier diagnostic test in the assessment of patients with ASD in place of G-banded karyotyping [27]. Since CMA is higher (8.9%) than conventional karyotyping (1.6%) and other genetic analyses (3.8%) they are changing the frequency of the causes of ASD [28].

CNV is the most prevalent type of structural variation in the human genome [29] and they have been described as having strong association of "*de novo*" CNV with autism [30]. So, at least 8% of sporadic ASD cases carried a *de novo* CNV and they are clarifying the etiology for many cases of autism previously considered idiopathic [31, 32]. The *de novo* nature of these CNVs, together with their absence in the general population, suggests they represent a class of highly deleterious and highly penetrant mutations. Smaller microdeletions or microduplications may occur within this highly dynamic with frequent rearrangements

using alternative low-copy repeats (LCRs), also known as segmental duplications (SDs), as recombination substrates during nonallelic homologous recombination (NAHR).

The presence of these sequences in LCRs concentrated on “hotspots,” in recurring break points, it confers great instability, with frequent deletions, duplications, and the formation of small chromosome markers, since these repeated sequences can lead to incorrect meiotic recombination by NAHR and can be interchromosomal (between homologous chromosomes) or intrachromosomal (in the same chromosome); and every day are described new loci for recurrent NAHR-mediated CNV [33]. Specially “recurrent microdeletions” in ASD include: 22q13 deletion or Phelan-McDermid syndrome, 15q13.2-q13.3 (BP4–BP5) deletion, 16p11.2 deletion syndrome, 17q12 deletion. And “recurrent microduplications” like: duplication of 15q11-q13 or Dup15q syndrome in Prader Willi/Angelman region; duplication 7q11.23 in Williams's syndrome region; duplication of 22q11.2 in DiGeorge syndrome region (for review see [34]). Some of the genes contained within the *de novo* CNVs we identified are good candidates for autism [35]. Although specific “rare *de novo*” CNVs are individually infrequent, but combined they account for a significant fraction of patients with autism and their study could contribute to a better understanding of the phenotypic manifestations in ASD [36, 37].

We want to highlight as Phelan-McDermid syndrome (22q13.3 deletion syndrome) with a disruption of the *SHANK3* gene, it is believed to be underdiagnosed due to lack of specific clinical features, and because until recently it has inadequate genetic testing [38]. So when ASD is associated with intellectual disability, *SHANK3* mutations or deletions have been found in up to 2.3% of cases of ASD individuals [39].

The region 15q11 contains a number of LCRs (regions of repetitive DNA which are susceptible to rearrangements) known as breakpoint (bp) bp1, bp2, bp3, bp4, and bp5; and is especially linked to autism. The 15q11.2 BP1-BP2 microdeletion is emerging as a recognized new syndrome, its appearance ranging from 0.57 to 1.27% of patients sent for microarray analysis. Its expression can be variable: patients may present with developmental and language delay, neurobehavioral disturbances, and ASD in 27% of patients. The BP1-BP2 region include four genes (*TUBGCP5*, *CYFIP1*, *NIPA1*, and *NIPA2*) which are not subject to imprinting (a differential expression depending on maternal or paternal origin gene) and therefore with expression biallelicis [40]. Larger deletions involving BP3 (BP1-BP3 and BP2-BP3) cause either Prader-Willi or Angelman syndrome (PWS/AS) depending on which parent the deleted chromosome is inherited from (paternal or maternal, respectively). And also worth noting is duplications of the chromosome 15q11-q13.1 region (Dup15q syndrome) typically of maternal origin, which are associated with an estimated 1–3% of all autism cases, making this CNV one of the most frequent chromosome abnormalities associated with ASD. The region including ubiquitin protein ligase E3A (*UBE3A*)—the gene disrupted in Angelman syndrome—is involved in neural function and may play important roles in the neurobehavioral phenotypes associated with Dup15q syndrome. Genome-wide analyses suggest that common neuronal pathways may be disrupted in both the AS and Dup15q syndromes [31].

3.2. Genetic factors in ASD “nonsyndromic or idiopathic”

The concordance remarkably from 60 to 92% in monozygotic twins and from 0 to 10% in dizygotic pairs suggests a strong genetic component [41, 42]. Nevertheless, the recurrence rate of ADS in siblings of affected children is approximately 2% [43], which is 16 times higher than in the general population but much lower than in single-gene diseases, so the underlying genetic determinants are still largely unknown [41]. Recent advances in genetic screening (as next-generation sequencing, NGS, and whole-exome sequencing, WES) and systems biology approaches have extended our knowledge of the genetic etiology of ASD, and may lead to the discovery of underlying genetic factors for ASD, and may thereby identify novel therapeutic targets for this disorder [35].

3.2.1. Linkage and candidate gene association studies

During the past decade, research about the genetic variations that underlie susceptibility to ASD has been focusing on linkage and candidate gene studies. Even though genetics is considered to play a significant role in ASD, until recently solely 16–17% of autistics are carriers of a known genetic variant. A large number of linkage studies have been conducted and have identified possible susceptibility loci on multiple chromosomes. Although there is no full agreement among different studies, certain regions, such as those on chromosomes 2q, 3, 7q, 11, 16, 17q, and 19, have been involved in multiple occasions (for review see [44]), but only few common alterations were recognized as candidate genes in association studies including support from meta-analysis [45, 46]. So, only a few loci show recurrent mutations (particularly the 7q22-q37 and the 11p12-p13 locus) [47, 48], and these “recurrent mutations” account for only about 1–2% of patients [49]. The studies have identified several ASD candidates including genes encoding neuroligins [50], neurexins [47], or *SHANK3* [51].

This is now changing, linkage data with WES data has achieved great success for identifying many Mendelian disease genes [52–55] and the number of such studies is increasing gradually (for review see [35]). Studies carried out in twins, in which one sibling had a diagnosis of autism and the other was not affected, bioinformatics and gene ontological analyses implicate genes which are involved in nervous system development, inflammation, and cytoskeletal organization, in addition to genes which may be relevant to gastrointestinal or other physiological symptoms often associated with autism; and these processes may be modulated by cholesterol/steroid metabolism, especially at the level of androgenic hormones (higher levels of testosterone in the autistic sibling) [56].

3.2.2. Genome-wide association studies (GWAS)

The genetic architecture of ASD comprises a diversity of rare single nucleotide variants (SNVs), copy number variations (CNVs), chromosomal abnormalities, and common polymorphic variations [57–59]. Genome-wide association study (GWAS) is an examination of a genome-wide set of genetics variants in different individuals to see if any variant is associated with a trait. Advances in genomic technology, including GWAS of single nucleotide

polymorphisms (SNPs), and CNV studies, allow the detection of *de novo* or inherited mutations in the coding regions and new candidate genes for ASD. Principally, CNVs implicate many ASD-associated genes like *CHD2*, *HDAC4*, *GDI1*, *SETD5*, *MIR137*, *HDAC9*, *SHANK2*, *SYNGAP1*, *DLGAP2*, and the X-linked *DDX53-PTCHD1* locus [60, 61]. But this only represents a selected sample of examples of the numerous studies in this field; inasmuch as these CNVs are widely distributed across the genome, at more than 100 different loci. The statistical distribution of influences across the genome suggests that hundreds of different human genes can mutate to influence autism risk [53]. Also, there is recent interest in rare highly penetrant SNV analogous to these traditional genetic models that may influence risk for “idiopathic autism.” SNVs most commonly identified in ASD cohort including among others: *NRXN1*, *SHANK3*, *CTNNA3*, *CHD8*, *SCN1A* and *2A*, *ADNP*, *PTEN*, *DYRK1A*, and *SYNGAP1* [52, 53, 55, 62, 63]. Genes affected by *de novo* CNVs and/or loss-of-function SNVs converged on networks related to neuronal signaling and development, synapse function, and chromatin regulation [61].

Up to now, estimates suggest that CNVs and SNVs acting in dominant, recessive, or X-linked models might account for a small proportion of ASD (as many as 15% of cases), and common SNP can account for nearly half the variation in autism. Several GWASs [61, 64–66] have been performed to decipher the genetic etiology of autism that is attributable to common variants (i.e., SNPs) with only a few variants having shown significant associations and replicated in an independent population or in endophenotypes. Based on results from individual SNPs and their *en masse* effect on risk, as inferred from the allele score results, it is reasonable to conclude that common variants affect the risk for ASD but their individual effects are modest.

3.2.3. Molecular pathways ASD: “developmental synaptopathies”

As it has been mentioned previously, recent advancements in NGS and WES have enabled the discovery of an overwhelming number of *de novo* mutations (from 500 to 1000 genes) distributed along all chromosomes that confer a risk for ASD demonstrating a heterogeneous genetic landscape [35]. For an up-to-date database of ASD-associated genes, the reader is referred to the SFARI Gene database (Simons Foundation Autism Research Initiative; <https://gene.sfari.org/>). As of June 2016, there were 826 genes and 2163 CNV loci in this database. This resource reviews the evidence for each gene implicated in autism susceptibility and assigns a score, from “Category 1” (“high confidence,” with 16 listed: *ADNP*, *ANK2*, *ARID1B*, *ASH1L*, *ASXL3*, *CHD8*, *DYRK1A*, *GRIN2B*, *POGZ*, *PTEN*, *SCN2A*, *SETD5*, *SHANK3*, *SUV420H1*, *SYNGAP1*, and *TBR1* genes), to “Category 6” (“not supported”). Genes associated with syndromic ASD are categorized separately (“Category S,” including 78 genes, among the best known are: *FMR1* cause Fragile X syndrome, *MECP2* cause Rett syndrome, *SHANK3* is part of a multigenic region that is deleted in Phelan-McDermid syndrome, *UBE3A* present in common 15q11-13 duplications syndrome or deletion in Angelman syndrome, *RERE* in 1p36 deletion syndrome, *PTEN* in Cowden syndrome, etc.).

Fortunately, although the ADS-risk genes are numerous, and many of them have a dual function involved in different neural networks, they seem to converge in a limited number of molecular pathways [67], allowing the study from single gene to a pathway perspective [68]. So, it has been proposed that, rather than as a result of dysfunction of specific genes, ASD

result in dysfunction of specific genetic pathways [69]. The identified several potential genetic pathways to ASD including wnt signaling during development, synaptic function, chromatin remodeling, mRNA translation, and metabolism seem to converge in common functional pathways affecting neuronal and synaptic homeostasis [70]. Just like that, 88% of high-risk genes for autism influence neural induction and early maturation of the neuroblast. In addition, 80% of these same genes influence later stages of differentiation, including neurite and synapse development [71].

Rare mutations-ASD include mutations in synaptic proteins such as ProSAPs/SHANKS proteins (with a crucial role in the assembly of the postsynaptic density during synaptogenesis, in synaptic plasticity and in the regulation of dendritic spine morphology) [72–74]; synaptic vesicle-associated proteins (*SYN2* gene) [75]; and neuroligins/neurexins (synaptic cell adhesion molecules) for excitatory glutamatergic and inhibitory GABAergic synapses [neurexins (*NRXN*) (trigger postsynaptic differentiation), and neuroligins (*NLGN*) (trigger presynaptic differentiation)] [76] and play a pivotal role in synaptic function, especially at GABAergic synapses [77]. It has been shown that many genes associated with ASDs are involved in the neuroligin-neurexin interaction at the glutamate synapse: *NLGN3*, and *NLGN4* on the X chromosome [50, 78, 79], *NRXN1* on chromosome 2p16 [80], *CNTNAP2* on chromosome 7q35 [81], and *SHANK3* on chromosome 22q13 [51, 82]. Autism-associated *NLGN3* mutations commonly disrupt tonic endocannabinoid signaling, providing evidence that alterations in the endocannabinoid pathway may contribute to autism pathophysiology [83]. Besides the neurotrophic factors as well as the neurotransmitter systems are thought to be good candidates for ASD [84]. Thereby, elevated platelet serotonin (5-HT) in 20–25% of ASD cases points to the 5-HT transporter (*5-HTT*; *SERT*) as a strong candidate gene, and also allelic heterogeneity at serotonin transporter locus (*SLC6A4*) may be connected with susceptibility to autism [85]. Also, it has been implicated in the catecholaminergic pathways (allelic variants in the dopamine decarboxylase (*DDC*) gene) [86].

It has been proposed that genes involved in “monogenic” forms of syndromic and nonsyndromic ASD converge on common molecular pathways and mechanisms of synaptic dysfunction (“developmental synaptopathies”): control synaptic protein synthesis and degradation with postsynaptic scaffold architecture and neurotransmitter receptors; and that are involved in synaptic development, plasticity, and signaling [87]. Many studies have suggested that neurites and synapses associated gene products, in fact, may be involved in the early stages of neural differentiation normally recognized, resulting in defects of migration and other indications of disturbance to premigratory cell fate determination [88–90].

However, it is yet unclear whether the same genes may performance through rare, highly penetrant mutations and common genetic risk factors. Additionally, although many approaches have attempted to identify “molecular pathways” implicated in ASDs to unify disparate genes, these data have not converged to provide conclusive and well-replicated evidence. How these mutations lead to ASD phenotypes is poorly understood (for review see [91, 92]). Hence, most cases still remain unknown, but potentially, including additional less penetrant rare variants or complex mechanisms, such as gene-gene interaction or gene-environment interaction. In this way, one might conclude that interactions between multiple genes cause “idiopathic” autism, but that

“epigenetic dysregulation” and exposure to environmental modifiers might contribute to variable expression of autism-related traits [93] and to significant proportion of ASD cases [94–96].

4. Epigenetic and chromatin structure

Definition: Epigenetics is an important aspect of research in current biology, defined as the study of mitotically and meiotically heritable changes in gene function that are not dependent on DNA sequence [97]. So “an epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence.” Epigenetics shows that gene expression undergoes changes more complex than modifications in the DNA sequence; it includes the influence of the environment on the gametes prior to conception. Berger et al. [98] proposed three categories of signals that culminate in the establishment of a stably heritable epigenetic state:

- A signal that we propose to call the “Epigenator,” which emanates from the environment and triggers an intracellular pathway.
- An “Epigenetic Initiator” signal, which responds to the Epigenator and is necessary to define the precise location of the epigenetic chromatin environment. The Initiator could be a DNA-binding protein, a noncoding RNA, or any other entity that can define the coordinates of the chromatin structure to be assembled.
- An “Epigenetic Maintainer” signal, which sustains the chromatin environment in the first and subsequent generations.

The molecular basis of epigenetic processes: is complex and this signal involves many different mechanisms, including major epigenetic silencing pathways: DNA methylation, modifications (like methylation and acetylation) of histones (H), positioning of histone variants, and gene regulation by small noncoding RNAs [99].

The study of chromatin structure has taken an important impetus in recent years and has transcended to the point to understand various diseases in humans including ASD. Chromatin is the structure in which the DNA is organized and packed into the nucleus of the eukaryotic cell. The main components of chromatin are DNA and histone proteins; both units are the target of epigenetic modifications. The primary unit is the nucleosome, formed for a segment of DNA (approximately 200 base pairs) wound in sequence around eight histone protein cores (H2A, H2B, H3, and H4), and linker subunit H1 that connects the nucleosomes. A region of chromatin is in a more or less favorable to gene expression depending on the type of sequences that form and also epigenetic modifications at that level state. The regulation mechanism of transcriptional activity is mainly mediated by DNA methylation and chromatin remodeling.

DNA methylation: Methylation of DNA and mainly 5-methylcytosine methylation is a major mechanism of epigenetic gene silencing, essential for cell division and normal development, and plays an important role of key processes including inactivation of the X-chromosome, genomic imprinting, gene silencing, and repression of repetitive elements. DNA methylation may affect the transcription of genes in two ways:

- Directly impede the binding of transcriptional proteins to the gene.
- Methylated DNA may be bound by proteins known as methyl-CpG-binding domain proteins (MBDs) which later recruit additional proteins to the locus, such as histone deacetylases (HDAC) and other chromatin remodeling proteins that can modify histones, thereby forming heterochromatin.

Chromatin remodeling: Modulate chromatin structure and function can involve covalent modification of histones (acetylation, methylation, or ubiquitination of lysine; methylation of arginine; and phosphorylation of serine), the incorporation of histone variants, and noncovalent ATP-dependent chromatin remodeling complexes. One of the most significant epigenetic processes is to strengthen or weaken the interactions between DNA and histones (“histone code” as epigenetic histone chemical modifications in nucleosomes). The regulation of each histone modification requires specific enzymes that add or remove the methyl or acetyl group. The histone modifications affect interactions between nucleosomes and therefore the degree of chromatin condensation, so a region will behave as euchromatin, lightly packed, and accessible to active transcription; or in reverse when the interaction is strong, the histones are attached firmly to the DNA chromatin (heterochromatin), this means in a closed or dormant state, and it is tightly packed and inaccessible to polymerases and therefore not transcribed [100].

In the transition from the closed conformation to a more open chromatin, the interactions between histones and DNA must be dissolved. This process requires epigenetic “writer” as transferases that add specific small molecules such as the methyl and acetyl groups to DNA, or proteins to alter its packaging proteins—the histones—such as histone acetyltransferases (HATs), histone methyltransferases (HMTs), protein arginine methyltransferases (PRMTs), and kinases lay down epigenetic marks on amino acid residues on histone tails. This modification (e.g., by adding an acetyl group) blocks the ability of the histone to bind DNA and thus making the chromatin more open and is associated with active gene expression. In contrast, epigenetic “eraser” proteins, such as histone deacetylases (HDACs), lysine demethylases (KDMs), and phosphatases catalyze, remove the epigenetic marks from the histones restoring the interaction between the histone and DNA, leaving the DNA once again in a closed conformation tends to be present in transcriptionally silent regions. The epigenetic “readers” (such as proteins containing bromodomains, chromodomains, and Tudor domains) are transcriptional regulators proteins that do not alter the histone but rather detect the acetyl group within the histones.

Proteins that contain reader domains can be usually classified into four groups: chromatin architectural proteins (induce chromatin compaction), chromatin remodeling enzymes (prompt a more open chromatin architecture driven by the energy of ATP hydrolysis), chromatin modifiers, and adaptor proteins that recruit other machinery involved in gene expression. Enzymes which “write” or “erase” epigenetic marks may also contain such reader domains, leading to the coordination of “read-write” or “read-erase” epigenetic processes [101]. The proper gene expression requires the attainment of a balance between the activities of the two opposing systems (writers and erasers) and subsequently the placement of their respective marks, which ensures that the appropriate composition of chromatin is present at

particular gene promoters. Opposite histone systems are susceptible to be dynamic, allowing the cell to quickly respond to changes in environmental signals by altering gene expression at specific loci. Epigenetic machinery is highly redundant, perhaps reflecting the critical importance of maintaining this balance in many different cell types

5. Epigenetic changes involved in ASD

As we mentioned previously, the rates of ADS diagnosis have dramatically increased in the past three decades to constitute “autism epidemic” and have been well documented in public health surveillance studies [102]. New advances in genetic studies (*Linkage and candidate gene association studies and GWAS*) identifying multifarious loci associated with ASD, especially mutations in genes encoding synapse-associated proteins (Shank/ProSAP proteins, synaptic vesicle-associated proteins, synaptic cell adhesion molecules, neurotransmitter...); however so far, genetic analyses have mainly been on the ~1.5% of the genome encoding genes. Although genetic contributions to autism etiology are well accepted, genetics alone does not explain the underlying cause in a substantial proportion of cases. So, the rising prevalence and inconsistent finding from genetic studies suggest a role for interactions between susceptibility genes and relevance of environmental factors and other potential contributors. Now there is convincing evidence that gene interactions with the environment are important in the etiology of ASD. However, the mechanisms by which environmental factors interact with genetic susceptibilities to confer individual risk for ASD remain significant knowledge emptiness in the field. It has been suggested that environmental factors linked to epigenetic mechanisms as DNA methylation and/or histone modifications; they may be involved in ASD by changes into the function of the genome.

A number of rare Mendelian disorders, such as Rett syndrome (by mutation in *MECP2*, locus Xq28), Cornelia de Lange syndrome-2 (by mutations of the *SMC1A* gene locus Xp11.2 encodes a subunit of the cohesin complex, essential for sister chromatid cohesion during mitosis, with broad roles in chromosome condensation, and DNA repair), and Coffin-Siris syndrome (chromatin remodeling complex gene *ARID1B* which encodes an epigenetic modifier of chromatin structure) have pointed to the importance of DNA methylation and chromatin remodeling factors, mainly histones in human brain development. At present the identification of other ATP-dependent chromatin remodelers (e.g., *CHD8*) and transcription factors (e.g., *ADNP*, *ASH1L*, *FOXG1*), which are also typically involved in regulation of gene expression but without alterations in the DNA sequence, have aroused the interest toward the epigenetics of autism and how environmental factors may be playing a key role in transcriptional regulatory influences [94–96, 103, 104].

Recent advances in genomic technologies allow a position to initiate large-scale studies of human disease-associated epigenetic variation, specifically variation in DNA methylation, and highlight the contribution of noncoding variants to the etiology of ASD. Epigenetic state in ASD, mainly epigenetic mark of DNA CpG methylation, can be used as biomarker of disease risk, diagnosis, prognosis, and response to treatments; but also, clues to the causal factors and mechanisms of the disorder [105]. So, aberrant methylation profiles in 1.1% of

ASD cases have been reported [13]. A significant overrepresentation of genes with functions in chromatin regulation and early developmental expression was found in ASD probands but not in unaffected siblings [106]. Also, oxidative protein/DNA damage and DNA hypomethylation (epigenetic alteration) were found in autistic children but not paired siblings or controls [107]. So, nowadays attention has turned to environmental factors but also with epigenetic changes as potential etiological agents predisposing to autism susceptibility. New genetics analyses have displayed a multitude of novel candidate ASD-genes in networks that involve epigenetic change, encoding nuclear factors implicated in chromatin remodeling, histone demethylation, histone variants, and the recognition of DNA methylation [13, 108]. This approach is described as an “epigenome-wide association study” (EWAS) and takes its cue from the association of genetic variability with phenotypes in GWAS. However until today and in contradistinction to GWAS, EWAS are scarce related to ASD (for review see [109]).

Just like that functionally, ASD-risk genes often converge in key molecular pathways early in development, which modulate synaptic transmission but also chromatin remodeling and transcriptional regulation, allowing to define autism as “synaptic and chromatin-remodeling disorders” [110]. All of the above can be added to state that epigenetic changes are potentially reversible, and, therefore, a thorough understanding of these modifications may identify new therapeutic targets for the disease. So finally the question arises: Is autism also an acquired and therefore preventable, treatable, and potentially curable epigenetic disease?

Epigenetic studies besides being keys to the causal factors, also clues to the biomarkers and physiological mechanisms predisposing or resulting from the onset of ASD, and may have an important role in the therapeutic implications. So this chapter will focus on developing the main aspects related to epigenetics of autism, according to the following scheme:

- Congenital epigenetic diseases in ASD:
 - Imprinted-x liability. Sex differences.
 - Classical imprinting disorders.
 - Mendelian ASD disorders of the epigenetic machinery:
 - Mendelian ASD disorders in DNA methylation machinery.
 - Mendelian ASD disorders in the histone machinery.
 - Mendelian ASD disorders in other chromatin remodelers and transcription factors.
- Acquired epigenetics disorders in ASD.

6. Congenital epigenetic diseases in ASD

Congenital epigenetic diseases are a newly delineated group of multiple congenital anomalies, intellectual disabilities, and ASD syndromes resulting from:

- **Classic epigenetic processes:** X chromosome inactivation and genomic imprinting are classic epigenetic processes that cause disease when not appropriately regulated. “Classical imprinting disorders” or epigenetic alterations at specific loci result mainly from disruptions occurring in *cis* (acting in cis).
- **“Mendelian disorders of the epigenetic machinery”** or mutations in genes encoding components of the DNA methylation machinery and histone modifications machinery, which implicate *trans*-acting factors (acting in trans), and are thus expected to have widespread downstream epigenetic consequences (for review see [111–113]).

6.1. The imprinted-X liability: sex differences

X chromosome inactivation (XCI) evolved to solve the problem of gene dosage compensation between XY males and XX females by random inactivation of one of the two female X chromosomes in placental mammals. X inactivation is controlled by a single X-linked *cis*-acting locus called the X inactivation center (Xic), which contains the noncoding RNA encoding X-inactive specific transcript (Xist) locus. However, there are regions on the XCI that sustain active transcription and “escape” inactivation by looping out of the chromosomal territory covered by Xist.

The male predominance universally observed in ASD (male:female ratio approximately 4:1) is one of the best known, and at the same time, one of the least understood characteristics of these disorders. The mechanisms underlying this male preponderance have been associated with genetic, epigenetic, hormonal (e.g., early exposure to androgenic hormones), and environmental factors (e.g., prenatal stress, early maternal immune activation) [114]. It has been proposed that the pronounced sex ratio of ASD could be related to genetic loci expressed on the noninactivated X chromosome modulate the phenotypic expression of autosomal loci conferring risk for ASD [115]. Females have detectably lower global levels of DNA methylation, and increasing the number of X chromosomes further reduces the methylation on autosomes [116]. The mechanism of female protective effect is unknown, yet genome scans have not revealed any predisposing loci on the sex chromosomes [117]. It has been hypothesized as “the imprinted-X liability threshold model,” so imprinted X-linked gene(s) that is ASD protective in nature and raises the threshold for phenotypic expression is expressed only in the X-chromosome inherited from the father. It is normally silenced when transmitted maternally, so because only females have a paternal X-chromosome, the threshold for phenotypic expression is higher in them than in males [118]. Evidence for the existence of the genetic locus was found in a study of females with Turner’s syndrome (X-monosomy), in which females had either a single paternal or maternal X-chromosome [119].

The studies of sex differences in normal human brain development at the genomic level are lacking; a recent large transcriptomic study shows that male-biased genes are enriched for pathways repeatedly implicated in autism like the processes of extracellular matrix formation/glycoproteins, immune response, chromatin, and cell cytoskeleton [120]. X chromosome inactivation status in female human induced pluripotent stem cells (hiPSCs) and their differentiation into neurons have shown complexities. A small number of recent papers have begun to explore cellular phenotypes of autism observed in hiPSC-derived neurons [121–123].

However, at present there are major gaps and inconsistencies in the existing literature regarding XCI status during the derivation and maintenance of hiPSCs (for review see [124, 125]).

Moreover, not all genes on the inactive X chromosome are inactivated, and the genes that “escape” X inactivation in females are revealing some interesting ideas on chromatin and brain transcriptional sex differences in understanding the female protective effect in autism [126]. An example is X-chromosome-linked ichthyosis caused by mutation or deletion of the *STS* gene associated with a deficiency of the enzyme steroid sulphatase, located in the distal part of the short arm of the X chromosome (Xp22.3-pter) and the importance of the region in the higher incidence of neurological disorders among males like attention deficit hyperactivity disorder, autism, and X-linked mental retardation [127]. Other gene that escapes X chromosome inactivation is *KDM5C locus* Xp11.22, encoding O-linked-N-acetylglucosamine (*O-GlcNAc*) transferase (*OGT*) that regulates chromatin remodeling factors (histone demethylase of H3K4, implicated in gene repression), is expressed lower in males than females and further reduced by prenatal stress [128].

6.2. Classical imprinting disorders

As X chromosome inactivation, gene-imprinting process is a selective differential gene expression regulated by epigenetic mechanisms that are different, but in this case, determined by the parental origin of the alleles. Human-imprinting disorders are a group of eight rare but probably underdiagnosed congenital disorders of growth, development, and metabolism, and represent a curious defiance of normal Mendelian genetics. They are caused by similar molecular changes affecting regulation, dosage, or the genomic sequence of imprinted genes, associated with disturbance of parent of origin-specific DNA methylation across the genome. Humans inherit two alleles from mother and father, both are functional for the majority of the genes, but sometimes one is turned off or “stamped” and does not show in offspring, that the gene is imprinted. The term imprinting refers to the differential expression of alleles (the repressed allele is methylated, while the active allele is unmethylated) for a particular gene depending on the parental origin of the allele. Each allele contains a distinct set of epigenetic modifications or marks, which influence chromatin structure and regulate gene expression at particular imprinted loci [129].

At present there are more than 200 genes subject to imprinting in humans, 97 demonstrated and 107 predicted, generally grouped into different chromosomal regions. For an up-to-date database of imprinted genes, the reader is referred to the Geneimprint database (Geneimprint database; <http://www.geneimprint.com>). Nevertheless, it remains to be identified the overall role of human imprinted genes and their regulatory elements implicated in the puzzle of ASD. The regulation of the differential expression of genes at imprinted loci is quite complex and involves DNA methylation, characteristic epigenetic signatures of associated covalent posttranslational modifications of histone tails, noncoding RNAs, and *transacting* factors, all of which play a key role in the process [130]. Epigenetic imbalances in the effects of imprinted genes has been associated with different abnormalities in development: Psychotic spectrum conditions (schizophrenia, bipolar disorder, major depression, PWS, Klinefelter syndrome) have been mediated in part by alterations of imprinted genes with maternal expression or

other genes favoring maternal interests. By contrast, ASD (like Kanner autism, Asperger syndrome, Turner syndrome, AS, and Beckwith-Wiedemann syndrome), commonly engender increased relative effects from paternally expressed imprinted genes, or reduced effects from genes favoring maternal interests [131].

Imprinting disorders may result by different genetic mechanisms: deletion of the critical region, paternal/maternal uniparental disomy (UPD), imprinting center defect, and mutation in the imprinting gene (for review see [132]). For most chromosomes, no obvious phenotypic effect from UPD has been observed. However, UPD of certain chromosomes affecting imprinted region leads to clinically recognizable syndromes (e.g., UPD affecting the chromosome 14q32.2 imprinted region is one that causes a distinct disorder: Temple syndrome (or maternal UPD) and Kagami-Ogata syndrome (or paternal UPD), although different, are two sides of the same coin [133–135]).

The association of so-called “classical imprinting diseases” and autism is well known. For example, **imprinted gene locus on 15q11 region**, results from the loss of function or overexpression of at least 1 imprinted gene [136]. Prader-Willi syndrome (PWS) is caused by a lack of the paternal contribution of genes, and Angelman syndrome (AS) is caused by a lack of maternal *UBE3A* expression resulting from disrupted imprinting via a variety of genetic and epigenetic mechanisms occurring in cis [137]. Duplication of 15q11-q13: As we mentioned previously, microduplication of the 15q11-q13 segment is the most consistently known chromosomal abnormality reported in ASD (1–3% of all autism cases), which occurred on the maternally derived chromosome. It represents a contiguous gene duplication syndrome, and reveals epigenetic alterations in gene expression. The segment contains a cluster of three GABA (A) receptor subunit (*GABR*) genes (imprinted key genes) essential for normal neurodevelopment [138]. Genome-wide analyses suggest that common neuronal pathways can be disrupted in both the Angelman and Dup15q syndromes. mRNA-Seq experiments show that there is substantial overlap of differentially expressed genes between 15q11-q13.1 deletion and duplication neurons. *UBE3A* transcripts can be pharmacologically rescued to normal levels in induced pluripotent stem cell (iPSC)-derived neurons with 15q11-q13.1 duplication [139].

Another **imprinted region 11p15** contains two separate imprinting domains: the *IGF2/H19* locus [imprinting control region 1 (ICR1)] and the *CDKN1C/KCNQOT1* locus (ICR2). When either locus is disrupted, tipping the balance toward increased expression of paternal genes, the Beckwith-Wiedemann syndrome (BWS) overgrowth disorder occurs. This dosage imbalance occurs primarily via epigenetic mechanisms acting in *cis*, most commonly loss of methylation at ICR2, gain of methylation at ICR1, or paternal uniparental disomy (UPD) of the entire 11p15 region [140]. In BWS 6.8% of patients had been diagnosed of ASD, and occurred in children with UPD and ICR2 defects [141]. By contrast, Russell-Silver syndrome (RSS) with growth failure can result from paternal hypomethylation at ICR1, a gene dosage imbalance that leads to increased maternal gene expression with no paternal contribution [142].

We highlighted as BWS and AS occur with an increased incidence in the offspring of infertile couples conceived following assisted reproductive technologies, which involve a multitude of environmental disruptions that could potentially impact malleable epigenetic marks [143].

However, earlier investigations on possible links between artificial reproductive technologies and autism have shown inconsistent findings [144, 145]. More recent epidemiological studies involving larger populations display that IVF is not associated with ASD [146]; but the risk for autism is associated with a small increased risk of intellectual disability, and was significantly higher following intracytoplasmic spermatozoid injection (ICSI) using surgically extracted sperm and fresh embryos compared to *in vitro* fertilization (IVF) without ICSI [147].

6.3. Mendelian ASD disorders of the “epigenetic machinery”

Mendelian disorders of the epigenetic machinery are a newly delineated group of multiple congenital anomaly, intellectual disability, and autism syndromes arising from mutations in genes encoding components of the chromatin remodeling epigenetic pathways.

6.3.1. Mendelian ASD disorders in DNA methylation machinery

- **Rett syndrome (RTT)** is an X-linked dominant (Xq28) and severe neurological disorder caused by mutations in the gene that encode a single polypeptide MeCP2 (methyl-CpG-binding protein 2), a chromatin-associated protein [148] that contains both a methyl-CpG binding domain (MBD) and transcriptional repression domain (TRD) associated with the regulation of gene expression by activating or repressing transcription, or by functioning at a posttranscriptional level. *MeCP2* is capable of binding specifically to methylated DNA and form a complex with histone deacetylase (HDAC/Sin3A complex). *MeCP2*-mediated transcriptional repression may involve two distinct mechanisms one being dependent on chromatin modification by histone deacetylation and the other being chromatin independent (block transcription factors directly) [149–151]. In RTT patients’ lymphocytes compared with controls have been shown to be increased in the density of histone H3, and decreased levels of trimethylation of lysine 4 on histone H3 (H3K4me3), a modification associated with transcriptional activation [152]. *MeCP2* results in the alteration of the chromatin state by suppressing a number of target genes associated with synaptic function and disrupted synaptic plasticity mechanisms (e.g., *BDNF*, *DLX5*, *ID*, *CRH*, *IGFBP3*, *CDKL1*, *PCDHB1*, and *PCDH7*, *LIN7A*) in neurons and other types of brain cells [153, 154]. Thereby controlling excitatory synaptic strength by regulating the number of glutamatergic synapse number [155].
- **Fragile X syndrome (FXS, OMIM #300624)** is the most common known genetic cause of inherited intellectual disability. Despite early controversy, it is now accepted that a substantial proportion (50–75%) of children with FXS meets diagnostic criteria for ASD [156]. FXS is associated with a fragile site at Xq27.3, results from a repeat expansion mutation near the *FMR1* (X-linked gene fragile X mental retardation 1) gene promoter. Full mutations larger than 200 CGG repeats in the 5'UTR (5' untranslated region) of the *FMR1* are able to trigger *FMR1* subsequent heterochromatinization by DNA methylation of the promoter region [157, 158], accompanied by additional epigenetic histone modifications (as DNA hypermethylation coupled with histone H3 and H4 tail deacetylation, and trimethylation at critical residues H3K9 and H3K27) that result in a block of transcription in *FMR1* and absence of the fragile X mental retardation protein (FMRP), involved in multiple

aspects of mRNA metabolism in the brain. Future studies could investigate a therapeutic approach to FXS based on the pharmacological reactivation of the *FMR1* expression [159].

- **MBD5** (methyl-CpG-binding domain 5) is an important factor in methylation patterning and epigenetic regulation and key on the autistic spectrum. Although it was known that the loss of one copy of this gene is the major causative factor in 2q23.1 microdeletion syndrome and point mutations are associated with autism and intellectual disability [160, 161], the duplication of this gene also causes disorders similar to those described in cases with abnormal loss of gene function development [162].
- **Methylation level of specific genes in nonsyndromic ASD:** The postmortem brain tissues from ASD patients show increased DNA methylation within the genes of: oxytocin receptor (*OXTR*) [163], *RORA* [164], *Engrailed-2 (EN2)* homeobox gene [165], *Reelin (RELN)* [166], and *BCL2* gene [167].
 - **Oxytocin receptor (OXTR)** is a G-protein coupled receptor for the peptide hormone and neurotransmitter oxytocin that activates the frontal cortex. *OXTR* is known to be involved in modeling human social behavior (social memory and recognition, anxiety, sexual and aggressive behaviors, and maternal-offspring bonding), and a potential role of the “prosocial” hormone in autism [168]. The involvement of this gene was suggested by its deletion in an autistic patient. The subsequent analysis of a group of unrelated autistic subjects did not show an *OXTR* deletion, but rather hypermethylation of the gene promoter, with a reduced mRNA expression [163]. These findings address two major points of the current debate on the etiology and pathogenesis of autism: the role of the oxytocin-vasopressin pathway and the possible social processes under epigenetic control. Several studies have begun to explore the so-called OXT deficit hypothesis of ASD, but they have yielded conflicting results. Dysregulated OXT biology is not uniquely associated with ASD social phenotypes as widely theorized, but instead variation in OXT biology contributes to important individual differences in human social functioning, including the severe social impairments, which characterize ASD [169].
 - ***RORA*** (retinoic acid-related orphan receptor alpha) is a proposed risk factor for autism because it is reduced in the brain and lymphoblastoid cell lines of multiple cohorts of individuals with ASD, and oppositely regulated by male and female hormones. *RORA* and several of its transcriptional targets might contribute to the sex bias in autism by differentially regulating target genes, including *CYP19A1* (aromatase) in certain regions of the brain of both mice and humans; in a sex-dependent manner that can also lead to elevated testosterone levels. An important sex-dependent difference has been found in the level of *RORA* protein in brain tissues of males and females. Specifically, females without autism have a slightly higher level of *RORA* in the frontal cortex of the brain than males without autism, while the levels of the protein are comparably lower in the brain of both males and females with autism. *RORA*-deficient males may experience greater dysregulation of genes relevant to ASD in certain brain regions during development [164].
 - ***Engrailed-2 (EN2)* homeobox gene:** contributes to neurodevelopmental disorders, especially ASD. *En2* knockout mice (*En2*^{-/-}) display subtle cerebellar neuropathological chang-

es and reduced levels of tyrosine hydroxylase, noradrenaline, and serotonin in the hippocampus and cerebral cortex similar to those ones which have been observed in the ASD brain [170]. The disruption of hindbrain patterning genes can alter monoamine system development and thereby produce forebrain defects that are relevant to human neurodevelopmental disorders [171].

- **Others:** In addition, significant correlations were also identified between the severity of the autistic phenotype and differential DNA methylation at several multiple loci implicated in the pathogenesis of ASDs. Genes that control synaptic molecules are subjected to a specific epigenetic control mechanism, for example, DNA methylation regulates the tissue-specific expression of SHANK3 gene [172]. Other prominent genes regulated by epigenetic mechanisms include *AFF2*, *APC*, *ARHGAP15*, *AUTS2*, *JMJD1C*, *GABRB3*, *KCNJ10*, *MAP2*, *MBD4*, *NLGN3*, *NRXN1*, *PIK3C3*, *SNRPN*, *SLC6A4*, *THAP10*, *TSNAX*, and *UBE3A* [173].

6.3.2. Mendelian ASD disorders in the histone machinery

- **Histone modifications** include acetylation, methylation, ubiquitylation, phosphorylation, sumoylation, ribosylation, and citrullination. Histone lysine acetylation and histone lysine methylation are the most highly studied of these modifications. But histone demethylases are now emerging as important players in developmental processes and have been linked to human diseases such as neurological disorders and cancer.

○ **Histone acetylation**

Brain-derived neurotrophic factor (BDNF) is a protein encoded by the BDNF gene localized to 11p13, whose transcription is controlled by eight different promoters and activity is strongly stimulated by calcium. BDNF is a prosurvival factor induced by cortical neurons that centrally mediates growth, differentiation, and survival of neurons, and essential to promote synaptic plasticity that underlies learning and persistence of long-term memory storage [174]. BDNF binds at least two receptors on the surface of cells: TrkB and LNGFR. It may also modulate the activity of various neurotransmitter receptors, like in serotonergic neurons. Histone modulation of BDNF particularly increased H3 acetylation at the BDNF gene in the medial prefrontal cortex (mPFC), and may be one of the molecular mechanisms that mediated the cognitive dysfunction [175]. Accumulating evidence suggests that BDNF may be implicated in the developmental outcomes of children with ASD. Three recent meta-analysis show as children with ASD have increased peripheral blood levels of BDNF, strengthening the clinical evidence of an abnormal neurotrophic factor profile in this population. Peripheral BDNF levels are a potential biomarker of ASD [176–178].

Proteins involved in the “readout” of lysine acetylation marks, referred to as **BET bromodomain proteins** (including BRD2, BRD3, BRD4, and BRDT), have been shown to be key regulators of chromatin dynamics and disease, and BET inhibitors are currently being studied in several clinical trials. Pharmacological suppression of BET proteins in the brain of young mice, by the novel, highly specific, brain-permeable inhibitor I-BET858 leads to selective suppression of neuronal gene expression followed by the development of an autism-like syndrome.

So, environmental factors controlling BET proteins or their target genes may contribute to the epigenetic mechanism of ASD [179].

○ **Histone methylation**

Recent large-scale exome sequencing studies highlighted dysregulation of histone methylation as a major contributing factor of ASD [180, 108]. Methylation of lysine 4 of histone H3 (H3K4me) is one such modification, which is associated with gene activation. Two families of proteins serve as primary regulators of H3K4me: histone lysine methyltransferases (KMTs) are the “writers,” which place the methyl marks onto histones; and histone lysine demethylases (KDMs) are the “erasers,” which remove them. The mutations in histone lysine methyltransferase KMT2D are a major cause of Kabuki syndrome. Dysregulation of histone methyltransferases and histone deacetylases (HDACs) associated with low activity of methyl CpG binding protein-2 at cytosine-guanine sites in genes may reduce the capacity for condensing chromatin and silencing genes in the frontal cortex, a site characterized by decreased cortical interconnectivity in autistic subjects (for review of mechanisms for altering DNA-histone interactions see [181]).

To date, several genes were found mutated in autism encode histone methylation enzymes such as:

- Methyltransferase complex genes: *KMT2A*, *KMT2C*, *KMT2D*, *KMT2F* encodes H3K4 methyltransferase [182]. *EHMT1*, *EHTM2*, and *WIZ* encode H3K9 methyltransferase [183]. Histone methyltransferase Ash1L mediates activity-dependent repression of neurexin-1 α [184].
- Histone demethylases: *KDM5C* encodes an H3K4-specific eraser enzyme that directly catalyzes the demethylation of mono-, di-, and tri-methylated H3K4, implicated in gene repression; and *JMJD1C*, a demethylase for histone H3K9 implicated in hormone-dependent transcriptional activation.
- **Histone variants: Macrohistones (mH)** are unusual histone variants found exclusively in vertebrate chromatin.
 - The histone H2A subunit has a variant -macroH2A1.1 encoded by the autosomal gene H2AFY. The macroH2A1.1 is generally associated with repressed chromatin, such as the inactive X chromosome [185] and could explain the 4:1 male:female gender distortion present in autism. But because it also contains binding sites for cellular metabolites in through the macrodomain, macroH2A1.1 may have a more dynamic role in the modulation of gene expression in response to environmental signals [186]. Although macroH2A1.1 was identified as an autism candidate gene by a GWAS, no association was found [187].
 - A related family member encoding gene, *MACROD2* (*macro domain containing 2*) on chromosome 20p12 was also found on a separate chromosome in ASD GWAS [65, 188], so *MACROD2* gene is a strong positional candidate risk factor for autistic-like traits in the general population. The macrodomain of MacroD2 binds a cellular metabolite that emerges from histone deacetylation reactions linking both histone variant and histone modification events. There is also evidence that suggests that *MACROD2* could act to regulate the

expression of the phospholipase D2 (PLD2) gene [65]. Phospholipase proteins could play an important role in risk for ASD. The protein derived from PLD2 has been shown to regulate axonal growth [189] and metabotropic receptor signaling [190].

6.3.3. Mendelian ASD disorders in others chromatin remodelers and transcription factors

- **ATP-dependent chromatin remodeling complexes:**

As we have detailed above, the importance of histones (particularly HAT and HDAC complexes) and chromatin structure in the regulation of eukaryotic gene transcription has become much more widely accepted over the past few years. Today it is known as a specific type of chromatin remodeling machine involved in transcription regulation and can use the energy of ATP hydrolysis to alter interactions between histones and DNA within the nucleosome. Changing chromatin states is an active process that requires appropriate external signal, as well as energy in the form of ATP. The engines that carry out the active process are called “ATP-dependent chromatin remodeling complexes”; they utilize the energy from ATP hydrolysis, and are key players in the reorganization and regulation of chromatin accessibility and nucleosome positioning on the eukaryotic DNA, regulating gene expression; and particularly modulates transient extracellular signals to influence neural lineage commitment [191]. These complexes contain an ATPase subunit that belongs to the SNF2 superfamily of proteins. Based on the identity of the sequence homology of their conserved ATPase domains, SNF2 proteins have been into three main groups, the SWI2/SNF2 group, imitation SWI (ISWI) group, and a third class that contain a SNF2-like protein family ATPase and also show deacetylase activity. The diverse subunits together provide a multitude of functions, from early embryogenesis through cell differentiation to organogenesis (for review see [192–194]).

Several components of the ATP-dependent SWI/SNF complex also known as the BAF complex, one of the best characterized ATP-dependent chromatin remodeling complexes, are encoded by genes in which rare autism mutations have been observed, including *ARID1A* and *ARID1B* in Coffin-Siris syndrome, *ATRX* in X-linked alpha-thalassemia/mental retardation syndrome, and *SMARCC1* (BAF 155) and *SMARCC2* (BAF170) genes [195]. A neuron-specific protein ATPase subunit BAF53b defines a neuronal chromatin-remodeling complex that is necessary for long-term memory and synaptic plasticity in mice [196].

Forms of Coffin-Siris syndrome (CSS) have been shown to be caused by mutations in each of five genes encoding subunits of the SWI/SNF complex. These include CSS1 caused by heterozygous mutation in the *ARID1B* gene (614556) on chromosome 6q25; CSS2 (614607), caused by mutation in the *ARID1A* gene (603024); CSS3 (614608), caused by mutation in the *SMARCB1* gene (601607); CSS4 (614609), caused by mutation in the *SMARCA4* gene (603254); and CSS5 (616938), caused by mutation in the *SMARCE1* gene (603111) [197].

In addition, several exome sequencing studies in autism have identified rare mutations in genes encoding the ATP-dependent chromatin helicases CHD8 (CHD8-chromodomain helicase binding protein 8) [198]. CHD8 is a chromatin remodeling ATPase of the SNF2-like protein family and an important regulator of beta-catenin and Wnt signaling pathways in neuronal development, and is typically involved in the regulation of gene expression (e.g., CHD8

insufficiency results in altered expression of 1715 genes, including both protein-coding and noncoding RNAs) [199]. Another novel ATPase of SNF2-like protein family termed ARIP4 interacts with androgen receptors and modulates androgen-dependent transcription [200].

- **Transcription factors:**

Transcription factor (TF) is a protein that binds to specific DNA sequences, just like that controlling the rate of transcription of DNA to mRNA. A defining feature of TF is that they include one or more DNA-binding domains, which attach to specific sequences of DNA adjacent to the genes that they regulate [201]. At present there are numerous transcriptional changes associated with autism, so *MECP2* gene is also a transcription factor.

- **ADNP:** ASD caused by a mutation in ADNP, a transcription factor involved in the SWI/SNF. ADNP is known to be mutated in at least 0.17% of ASD cases, making it one of the most frequent ASD genes known to date [202].
- **FOXP1:** Overexpression of the transcription factor FOXP1 is responsible for the overproduction of GABAergic neurons. Altered expression of gene network modules and FOXP1 are positively correlated with ASD symptom severity, and likely a shift toward GABAergic neuron fate caused by FOXP1 is a developmental precursor of ASD [104].
- **PITX1** (paired-like homeodomain transcription factor 1) is a key regulator of hormones within the pituitary-hypothalamic axis (as ACTH, cortisol, and betaendorphin) and may be implicated in the etiology of autism [187]. The ACTH-cortisol system, which also plays an important role in stress-related responses, is impaired in autistic individuals in whom lower cortisol levels and higher ACTH levels have been reported.

7. Acquired epigenetics disorders in ASD

Compared with the magnitude of genetic studies in ASD, nongenetic biological risk factors were studied to a lesser extent. Even though in the last decade, the number of publications that address epidemiological factors and autism has grown tremendously. Although there are numerous risk factors (prenatal, perinatal, and postnatal risk factors) that have been linked to autism [203–206], in some cases their involvement has been or is still considered controversial, and as a possible hypothesis, postulates that improvements in obstetric and neonatal management have resulted in an increased frequency of survivors with preexisting brain damage, which has subsequently led to autism [203]. For each infant, an environmental challenge during a critical window of development can have particularly serious consequences, causing the abnormal functioning of the CNS, perhaps autism.

Among the most significant environmental factors linked to epigenetic mechanisms in autism include the following (for review see [95, 110]).

7.1. Toxic exposures teratogens and medications

The particular vulnerability of the developing nervous system for low-level exposure to chemicals is well established. Some degree of developmental neurotoxicity was found

for a large number of industrial chemicals and environmental pollutants. However, for only few of these (lead, arsenic, organic mercury, and polychlorinated biphenyls, PCBs), human moderate evidence has emerged for a potential role of environmental pollutants in neurodevelopmental adversity and may, thus, be involved in contributing to neurodevelopmental disorders like autism, ADHD, ID, or cerebral palsy (for review see: [207–209]). However, the mechanisms by which environmental factors produce neurotoxicity during early development are not well stated. Heritable genetic vulnerabilities in ASD may amplify adverse effects triggered by environmental chemicals exposures if genetic and environmental factors converge to dysregulate the same signaling systems at critical times of development.

Among the several major signaling pathways linked to altered neuronal connectivity in the developing brain, as a convergent molecular mechanisms target of gene and environment chemicals interactions, they have been involved in dysfunctional signaling via cytokine dysregulation [210], Ca(2+)-dependent mechanisms, extracellular signal-regulated kinases (ERK)/phosphatidylinositol-3-kinases (PI3K), and neuroligin-neurexin-SHANK (for review see [211]). As well as, exposure to environmental chemicals (using PCBs, lead, and bisphenol A, BPA, as examples) may contribute to adverse neurodevelopmental outcomes of relevance to ASD via effects on DNA methylation in the developing brain (for review see [212]). *In utero* exposure to environmental pollutants increases autism-like behavioral phenotypes in adult animals and induces epigenetic changes, so exposure to heavy metals resulted in multiple behavioral abnormalities that persisted into adulthood. Valproic acid and manganese induced changes in perseverative/impulsive behavior and social dominance behavior, arsenic caused changes only in perseverative/impulsive behavior, and lead induced abnormalities in social interaction in comparison to the control animals. The *Chd7* gene, essential for neural crest cell migration and patterning, was found to be hypomethylated in each experimental animal [213].

- **Heavy metals** like lead and mercury are widespread environmental toxins. Developmental exposure to these compounds is associated with lower IQ, endocrine disruptions, and behavioral disturbances. They also have immunotoxic properties. These features have made them possible, though controversial, candidates in ASD. There are conflicting reports regarding lead in autism. A few studies have documented higher serum lead levels [214] often associated with pica [215], although more recent studies show no difference between autism and control populations [216, 217]. Association between mercury levels and autism is also somewhat contradictory [218]. Ethyl mercury is a component in the vaccine preservative thimerosal, which has received attention in recent years. It has neurotoxic capacities, while *in vitro* studies suggest toxic potential for thimerosal (can alter calcium signaling and cytokine production), a large number of independent epidemiological studies show no link to autism [219, 220].
- **Bisphenol A (BPA)**, an epoxy resin, is an endocrine-disrupting chemical (xenoestrogen), employed to make certain polycarbonate plastic products as food containers and baby bottle, and it is the major estrogenic compound that leaches into nearby water and food supplies. BPA has been detected in 95% of human urine samples, which indicates that environmental exposure is widespread [221]. Exposure to BPA during development may

affect brain organization and behavior, perhaps as a consequence of its actions as a steroid hormone agonist/antagonist and/or as an epigenetic modifier that alters DNA methylation. Fetal and prenatal BPA exposure was suggested to perturb the serotonergic system in rat and mice models. Epigenetic mechanisms are suggested by a mouse study that demonstrated that BPA prenatal exposure had long lasting, transgenerational effects on social recognition [222]. BPA affects the mRNA levels (lower transcript levels) of several genes encoded for estrogen receptors, oxytocin, and vasopressin, recognized as important neuropeptides modulators of various social behaviors. BPA is not metabolized well in children with ASD. The most recent FDA updates (Administration January 2010) points to “some concern about the potential effects of Bisphenol A on the brain, behavior, and prostate gland in fetuses, infants, and young children.” In France, BPA was banned in baby bottles in 2010, and in any food or beverage packaging since January 2015 [223].

- **Other environmental pollutants and toxic products** have also been controversial in its possible relationship with neurodevelopment and ASD. There was weak evidence of an association between nickel exposure during pregnancy and ASD [224]. Also, gestational exposure to inorganic arsenic (an important natural pollutant of water) affected the expression of cysteine/glutamate transporters in the cortex and hippocampus and induced a negative modulation of glutamate receptor N-methyl-D-aspartate (NMDAR) subunits NR2A in the hippocampus. Behavioral tasks showed significant spatial memory impairment in males while the effect was marginal in females [225]. Developmental neurotoxicity and autism risk a positive association with pesticides (organochlorines, organophosphates, pyrethroids) [226, 227]; halogenated aromatic hydrocarbons [PCBs and polybrominated diphenyl ethers (PBDEs)] [210, 228].
- **Exposure to drugs:** Prenatal exposure to valproic acid, ethanol, thalidomide, and misoprostol has been shown to be associated with an increased incidence of autism. These drugs are able to modulate the expression of many genes involved in processes such as proliferation, apoptosis, neuronal differentiation and migration, synaptogenesis, and synaptic activity [229]. Exposure to selective serotonin reuptake inhibitors (SSRI) in depressed pregnant women might be involved in the etiology of autism, as well as the prenatal valproic acid (VPA) exposure, which is also thought to interfere with serotonin levels [230], by disruption of early serotonergic neuronal development [231] or via the reduction of *PTEN* level [232].

Children exposed to valproate *in utero* were seven times more likely to develop autism than those not exposed to antiepileptic drugs. Qin et al. [233] have shown that VPA exposure sequentially activates Wnt signaling and mTOR signaling in rats. Suppression of the Wnt signaling pathway relieves autistic-like behaviors partially by deactivating the mTor-signaling pathway in VPA-exposed rats.

As we have previously redesigned previous research has not been able to prove that administrations of the measles-mumps-rubella vaccine were connected with the autism upsurge [6], but it has been suggested that acetaminophen systematically administered during vaccination, may mediate oxidative stress and neurotoxicity in autism [234], and exposure during pregnancy enhances the probability of appearance of ADHD-like behaviors [235].

These are data from an ecological analysis, not considered optimal as evidence of causality. Nonetheless, there is accumulating clinical and experimental evidence connecting acetaminophen metabolism to biochemical routes known to be relevant for autism and related developmental disorders. Taking into account both ecological and mechanistic evidence, the role of acetaminophen in autism should be formally studied [236]. In rat liver, acetaminophen overdose has been implicated in Id2 (a pleiotropic protein whose function depends on its expression levels) down-regulation via histone-H3 hypoacetylation [237].

7.2. Immune dysregulation or inflammation

The immune system and the nervous system interact widely. Therefore, it is not surprising that immune dysfunction is often observed in neurological disorders. There is accumulating evidence for immune dysregulation playing a role in the pathogenesis of ASD. Early-life infections can skew fetal development, leading to aberrant neural and immune activity. Several infections [238, 239] including measles, cytomegalovirus, and rubella during pre- and perinatal periods have been associated with autism [240–244]. A large-scale epidemiological study showed that infection-related hospitalizations during pregnancy significantly increased the risk of ASD [245], and also worth noting is prenatal inflammation, particularly prenatal urinary tract infection [204]. A multidirectional interaction between immune system activation in the mother during pregnancy and epigenetic regulation in the brain of the fetus may cooperate to produce an autistic phenotype. This interaction includes immune factor-induced changes in epigenetic signatures in the brain, dysregulation of epigenetic modifications specifically in genomic regions that encode immune functions, and aberrant epigenetic regulation of microglia (for review see [246]).

Numerous immune system abnormalities including T and B cell lineages of adaptive immune responses have been described in individuals with autism, for example:

FOXP3 (Forkhead box P3) is a marker of (+) regulatory T cells [T (Reg)] which are potent mediators of dominant self-tolerance in the periphery. A subset of CD4⁺ T cells is primed in early life, to recognize common environmental antigens and inhibit later inappropriate immune responses. The expression of the *FOXP3* locus is intimately linked to its chromatin structure [di- and trimethylation of lysine 4 of histone H3 (H3K4me2 and -3)]. Interestingly, T (Reg) fate determination is an epigenetic event of *FOXP3* promoter demethylation induced by repeated Ca²⁺-mediated signal transduction and prevented by the mTOR pathway [247, 248]. Abnormalities in the ratio of Th1/Th2/Th17 cells and T (Reg) cell-related transcription factor signaling in ASD is characterized by a systemic deficit of *FOXP3*⁺ (TReg) and increased RORγt⁺, T-bet⁺, GATA-3⁺, and production by CD4⁺ T cells [249].

Also immune aberrations, with increased levels of plasma cytokines, have been reported in ASD especially in those autistic children with a regressive form of the disease [250]; and mothers of children with ASD exhibit altered cytokine profiles and autoantibodies indicative of systemic immune activation [210]. There are many reports of cytokine imbalances in autism, including IL-1B, IL-6, IL-4, IFN-γ, and TGF-B. Cytokines act mainly as mediators of immune activity, but they also have meaningful interactions with the nervous system. They take part in normal neural development and function, and improper activity can have a

variety of neurological implications. These imbalances could have a pathogenic role, or they may be markers of underlying genetic and environmental influences (for review see [210]). Histone modifications play critical roles in the regulation of the innate immune response; for example, histone lysine methyltransferase Ezh1 promotes TLR-triggered inflammatory cytokine production by suppressing Toll-interacting protein [251], and histone H3 phosphorylation by I κ B kinases- α is critical for cytokine-induced gene expression [252].

Even though infections and inflammation have profound effects on epigenetic modifications and trigger susceptibility to diseases as ASD, however, very little is known about the epigenetic pathways involved in the modulation of inflammatory and anti-inflammatory genes (for review see [246, 253]).

A novel unifying hypothesis of the etiopathogenesis of ASD for fitting the pieces of the puzzle together (collectively and simultaneously), suggest that ASD are disorders of the immune system that occur in a very early phase of embryonic development. In a background of genetic predisposition and environmental predisposition (like vitamin D deficiency), an infection (notably a viral infection) or food allergy could trigger a deranged immune response which, in turn, results in damage to specific areas of the CNS [254].

mTOR pathway: Postnatal pathogenic exposures and/or Immunological disturbances in autistic individuals have been reported and a role for food allergy has been suggested in ASD. Single gene mutations in mammalian target of rapamycin (mTOR) signaling pathway are associated with the development of ASD and enhanced mTOR signaling plays a central role in directing immune responses toward allergy as well [255, 256]. Multiple ASD syndromes are caused by mutations in genes that act to inhibit mTOR kinase, including Tsc1/Tsc2, NF1, and Pten [257]. In mouse models of ASD mTOR dysregulation causes spine-pruning defects in Tsc-deficient mouse [258]. Increased dendritic spine density with reduced developmental spine pruning in layer V pyramidal neurons are observed in post-mortem ASD temporal lobe, and these spine deficits correlate with hyperactivated mTOR and impaired autophagy [259]. Synaptic mTOR integrates signaling from various ASD synaptic and regulatory proteins, including SHANK3, FMRP, and the glutamate receptors mGluR1/5 [257, 260]. It has been proposed that mTOR pathway plays a central role in directing immune responses toward allergy (e.g., cow's milk allergy, or valproic acid exposure) as well, and may be a pivotal link between the immune disturbances and behavioral deficits observed in ASD [233, 261, 262]. Inhibition of mTORC1 activity by rapamycin improved the behavioral and immunological deficits of cow's milk allergic mice [256]. And also, sulindac (nonsteroidal anti-inflammatory drugs) by suppression of the Wnt signaling pathway relieves autistic-like behaviors partially by deactivating the mTOR-signaling pathway in valproic acid-exposed rats [233].

7.3. Nutritional status

There is consensus that children with autism are nutritionally vulnerable because they have selective or picky eating patterns, food neophobia, limited food repertoire, and abnormal sensory processing ("neurosensorial aversion" can affect both the taste and the texture) that predisposes them to food avoidance and restricted food intake in many children with ASD

[263]. “Insistence on sameness” and compulsive repetitive behaviors reinforce rigid dietary preferences and lead to a limited food repertoire [264].

Although inadequate micronutrient but adequate macronutrient intakes are increasingly reported, there are inconsistent results about the extent and type of nutrient deficiencies (for review see [265]). Micronutrient as essential amino acids, minerals, and vitamins are indispensable for human health, primarily due to their critical function as enzymatic cofactors for numerous reactions in the body, such as the production of neurotransmitters and fatty acid metabolism.

A growing body of literature suggests that certain modifiable risk factors such as maternal metabolic syndrome and nutritional risk factors as certain vitamins, like vitamin D, and folic acid either *in utero* or early life, may be associated with increased risk of autism [266]. Several studies with biomarkers of the nutritional and metabolic status of children with autism have demonstrated statistically significant differences in their nutritional and metabolic status, including biomarkers indicative of vitamin insufficiency, decreased glutathione and increased oxidative stress, reduced capacity for energy transport, sulfation and detoxification, and also impaired methylation [267–269]. Nutrition also influences programming of an offspring's epigenome, so nutritional factors, particularly folate, B vitamins (B2, B6, and B12), and choline, have been studied in ASD, and a likely pathway of this action is the one-carbon metabolism cycle, including the folate metabolism, the methionine-homocysteine remethylation cycle (involving choline and betaine), and the transsulfuration pathway.

7.3.1. Amino acids

The essential amino acids tryptophan and serotonin are precursors of neurotransmitters. It has been seen that plasma-free tryptophan and blood serotonin level are significantly higher in autistic children than in normal control subjects. These results suggest that autistic children have some defects in tryptophan-serotonin metabolism in the brain [270]. And disorders of serotonin metabolism are associated with disturbances of platelet catecholamines, and also with elevated immunoglobulins and enhanced cellular immunity reactions [271].

Multivariate statistical analysis in children with autism from their unaffected siblings and age-matched controls, indicated urinary patterns of the free amino acids glutamate and taurine were significantly different between groups with the autistic children showing higher levels of urinary taurine and a lower level of urinary glutamate, indicating perturbation in sulfur and amino acid metabolism in these children [272]. The metabolomic analyses showed variations in essential amino acid metabolism pathways in children with ASD, related to lowered dietary protein intake or to abnormal catabolism, but their role in the etiology and therapeutic use are contradictory [273]. For body fluid levels of neuroactive amino acids, including glutamate, glutamine, taurine, gamma-aminobutyric acid (GABA), glycine, tryptophan, D-serine, and others, in ASD the results reported in the literature are generally inconclusive (for review see [274]).

7.3.2. Minerals

- **Iron** is critical for early neurodevelopmental processes. Low serum iron occurs more frequently in children with autism compared with children with typical development. The

prevalence of iron deficiency and anemia in subjects with autism is reported between 24 and 32% and 8 and 16%, respectively. Because iron deficiency, with or without anemia, results in impaired cognition and developmental defects, iron deficiency in children with autism could further compromise their communication and behavioral impairments [265]. Thus, ferritin levels should be measured in subjects with autism as a part of routine investigation [275]. Studies consistently show link between maternal supplemental iron and ASD. Maternal intake of supplemental iron (from 3 months before pregnancy through the end of pregnancy and during breastfeeding) was associated with reduced ASD, especially during breastfeeding. Low iron intake significantly interacted with advanced maternal age and metabolic conditions [276].

- **Other minerals:** Yasuda et al. [277] examined scalp hair concentrations of 26 trace elements for 1967 children with autistic disorders and found deficient in zinc (29.7%), magnesium (17.6%), and calcium (5.8%); and 2.0% or less in other essential metals. The incidence rate of mineral deficiency was highly observed in infants aged 0–3 years old. By contrast, individuals were found suffering from high burden of aluminum (17.2%), cadmium (8.5%), and lead (4.8%), and 2.8% or less from mercury and arsenic burden. Findings by other authors also include deficient in: calcium [269, 278], magnesium [269, 279], iodine [269], chromium [269], and selenium [279]. These findings suggest that infantile zinc and magnesium deficiency and/or toxic metal burdens may epigenetically play principal roles as environmental factors in autistic disorders. Also compared with controls and/or reference ranges of healthy children, children with autism have lower concentrations of lithium [269]. Low concentrations of lithium are particularly interesting because its deficiency has been linked to a wide range of psychiatric disorders including autism. The low concentrations of lithium can be translated into decreased activity of enzymes involved in growth factor signaling pathways and regulation of neurotransmitter, and suggest that low-level lithium supplementation may be beneficial for mood stabilization in this group [280].

7.3.3. Vitamins

Children with autism had many statistically significant differences in their nutritional and others vitamin insufficiency, such as low levels of vitamin D, vitamin B6, biotin, vitamin A, and vitamin C, and also trend toward lower levels of vitamin B5 and vitamin E, and total carotenoids, although could not objectify constant data for different populations [269, 281].

- **Dietary vitamin D** could regulate epigenetic machinery. Epidemiologic evidence supporting the role of vitamin D deficiency either during pregnancy [282, 283] or early childhood may be an environmental trigger for ASD in individuals who are genetically predisposed (for review see [254, 284, 285]). Vitamin D and its receptor (VDR) also known as calcitriol receptor NR1I1 (nuclear receptor subfamily 1, group I, member 1) belongs to the family of trans-acting transcriptional regulatory factors, and are involved principally through two main areas: (1) in the brain homeostasis and mineral metabolism the receptor regulates a variety of other metabolic pathways and several genes controlling anti-inflammatory actions, immune response, and neurodevelopment (cellular proliferation, differentiation, and apoptosis); mainly vitamin D might play a role in the regulation of the production of

autoantibodies not only through modulation of T-helper cell function, but also through induction of CD4(+)CD25(high) regulatory T cells. Vitamin D might also protect the mitochondria, and upregulate glutathione, which scavenges oxidative by-products and chelates (captures and excretes) heavy metals. (2) In gene regulation the calcitriol receptor play ball with some chromatin modification enzymes (i.e., histone acetyltransferases and histone deacetylases), taking a role in complex epigenetic events [285, 286].

Recent studies in ASD children have shown that fifty-seven 40–57% of the patients had vitamin D deficiency (serum 25-OHD levels 10–30 ng/mL), and 30–48% had vitamin D insufficiency (serum 25-OHD levels <10 ng/mL); also in patients with severe autism the mean 25-OHD levels were significantly lower than those in patients with mild/moderate autism. Increased levels of serum anti-MAG autoantibodies were found in 70% of autistic patients; and this deficiency may contribute to the induction of the production of serum anti-MAG autoantibodies in these children [287]. Additionally, vitamin D supplementation may have beneficial effects in ASD subject [288].

- **Vitamin B complex**

- **Folic acid and folate** (anionic form) also known as vitamin B9, is a water-soluble vitamin, listed in vitamin B complex, necessary for the formation of structural proteins and hemoglobin and are essential for basic cellular processes including DNA replication as well as DNA, RNA, and protein methylation. Folic acid is essential for the proper development of the central nervous system, and their deficiency during pregnancy has been associated with a wide range of disorders. Randomized controlled trials have found that periconceptional folic acid supplementation reduces the risk of neural tube birth defects up to 70%. So, in several countries, public health policies recommend periconceptional supplementation with folic acid (400 µg/d) to decrease the risk of neural tube defects [289]. But also findings from a large multicenter case-control study, CHildhood Autism Risks from Genetics and Environment (CHARGE), suggest that periconceptional folic acid may reduce ASD risk in those with inefficient folate metabolism [290].

The vitamin folate regulates the metabolic pathway catalyzed by the methylenetetrahydrofolate reductase (MTHFR), encoded by the MTHFR gene (locus 1p36.3), it is the rate-limiting enzyme in the methyl cycle and a key source of the “one-carbon group,” it acts as methyl donor (for homocysteine remethylation to methionine), which critically influences in epigenetic mechanisms to methylate DNA (for review see [291]). But the genomic regions in the offspring that may be sensitive to folate exposure during *in utero* development have not been characterized. Genome-wide DNA methylation profiling identifies a folate-sensitive region of differential methylation upstream of ZFP57-imprinting regulator in humans [292]. The underlying mechanisms of action also included regulation of two microRNAs—let-7 and miR-34—by methylation [293].

The time period at which periconceptional folic acid was added to the diet of women of childbearing age coincides with the seeming onset of a steady increase in the prevalence of autism. Some studies have reported that periconceptional folic acid supplementation, depending on timing and dose, could be associated with a higher incidence of autism. But

nevertheless, a recent meta-analysis shows that the few and contradictory studies present inconsistent conclusions, and epidemiological associations are not reproduced in most of the other types of studies [294]. On the other hand, a well-controlled epidemiological study including a sample of 85,176 children disconfirms this claim and reports a lower incidence of ASD in children whose mothers received prenatal folic acid supplementation around the time of conception (0.10%) compared with those unexposed to folic acid (0.21%) [295]. However, these findings have not been verified in other studies [296]. This could be attributed to periconceptional folic acid, which may reduce ASD risk in those with inefficient folate metabolism (for mothers and children with MTHFR variant genotypes, like MTHFR C677T polymorphism) [290, 297]. Besides, folate receptor autoantibodies (FRAs) that interfere with folate transport across the blood-brain barrier may be important in ASD (a high prevalence to 75.3%) and that FRA-positive children with ASD may benefit from leucovorin (folinic acid) treatment [298]. Also, some studies have reported lower folate levels in patients with ASD [299, 300] and high levels of homocysteine [299, 301]. Folic acid supplementation may have a certain role in the treatment of children with autism, and this treatment also improved the concentrations of folic acid, homocysteine, and normalized glutathione redox metabolism [302]; but the effects of folate-enhancing interventions on the clinical symptoms have yet to be confirmed [294].

- **B-Group vitamins B2, B6 and B12:**

Studies in autistic children report decreased concentrations likely below the reference range of vitamin B-12 and folate by dietary deficits [300]. Vitamin B6 and B12 (or cobalamin) acts as coenzymes in the metabolism, with a close metabolic interrelation in the methylation of homocysteine to obtain methionine (Met). The name “one-carbon metabolism” involving folic acid, vitamins B2, B6, and B12, and folate metabolism does not only generate methyl groups, thus determining epigenetic processes, modifications of the genome and carcinogenesis, it also provides the compounds involved in the DNA synthesis and repair processes, especially the synthesis of purines and pyrimidines [303]. Nutritional supplementation with vitamin methyl-B12, folinic acid, and trimethylglycine might be beneficial on glutathione redox status in children with autism [304].

Elevated and unusually broad vitamin B-6 (pyridoxine) concentrations have been reported in children with autism which may be due to low activity of pyridoxal kinase that converts pyridoxal and pyridoxine into the active form pyridoxal 5-phosphate (P5P), which is the active cofactor for several enzymatic reactions, including the formation of many key neurotransmitters [305]. This explains the benefits of high-dose vitamin B-6 supplementation in individuals with autism with low P5P [269, 306].

- **Biotin** or vitamin B7 (and also H or B₈) is a water-soluble vitamin involved in the metabolism of carbohydrates, fats, amino acids, and purine. Decreased concentrations below the reference range of biotin have been reported in nutritional status of children with autism [269]. Also, it is evident for treatable inborn errors of metabolism patients with ASD. So detailed metabolic revealed biomarkers (urine 3-hydroxyisovaleric acid and serum beta hydroxybutyrate) in 7% of patients for whom biotin supplementation resulted in mild to significant clinical improvement in autistic features [307].

7.3.4. Vitamin-like substances: choline

Other nutrients whose effects are similar to those of vitamins including choline and is among the most prominent associated with ASD. Choline is an essential nutrient soluble in water usually grouped with B vitamins. Choline is an endogenous compound that is synthesized by all cells of mammals, as an intermediary in the major route of transformation of the hill in phosphatidylcholine, an essential phospholipid of the neuronal membrane in the CNS and is essential for proper brain maturation, including astroglia. Choline is a major source of methyl groups needed for methylation of DNA and histones [308]. There is growing evidence that this nutrient also modulates epigenetic regulation of gene expression in both neuronal and endothelial progenitor cells, thereby modifying brain development [309].

Choline is the precursor for betaine and methyl groups derived from betaine, which are used for S-adenosylmethionine (SAM)-dependent methylation reactions including the synthesis of membrane phosphatidylcholine. In this way, choline indirectly serves as a precursor for the synthesis of membrane phospholipids that are essential for normal membrane fluidity, signal transduction, membrane transport, and integrity for synaptic efficiency. Choline is needed by fetal progenitor cells (proliferate, migrate, differentiate, and undergo apoptosis at specific times during fetal development) for membrane synthesis and for methylation (redox-dependent methylation regulates neuronal mRNA). Choline is also a precursor for the synthesis of acetylcholine (ACh), an important neurotransmitter in both the central and autonomic nervous systems [310, 311].

Inadequate choline and betaine can negatively affect folate-dependent “one-carbon metabolism” and in turn downstream methylation and antioxidant capacity (**Figure 1.**) The metabolism of folate, vitamin B12, vitamin B6, choline, and methionine are interrelated and disturbances in one of these metabolic pathways are associated with compensatory changes in the others. So, when humans and animals are fed a diet deficient in choline, it increases the need for dietary folate [312, 313]. Alternatively, if they are fed a diet deficient in folate, dietary choline requirements increase as choline becomes the primary methyl group donor [314–316].

Choline is found primarily in foods that contain fat and cholesterol, and intake of such foods has decreased in recent years. Estrogen induces the gene for enzyme that catalyzes the biosynthesis of the choline-containing phospholipid phosphatidylcholine. Nevertheless, many women have a SNP that blocks the induction of endogenous biosynthesis, which makes longer requirement of choline in the diet. When these women consume diets low in choline, it is likely to be in insufficient supply of this nutrient for the fetus, and may disrupt the progenitor cell proliferation, migration, differentiation, and apoptosis [309].

Low plasma SAM levels and DNA hypomethylation have been shown to be present in children with autism [107]. Choline deficiency has been also shown in animal models to contribute to global and gene-specific DNA hypomethylation and epigenetic abnormalities [317]. There are inconsistent findings on the amounts of free/total choline in autistic individuals. Free choline levels were similar in the autistic and control groups, but total choline was 17% higher in the autistic group ($p < 0.0001$) [269]. Hamlin et al.'s [318] study of 288 children with autism found that 69–93% had less than adequate choline intake, and 18–30% had betaine intake of

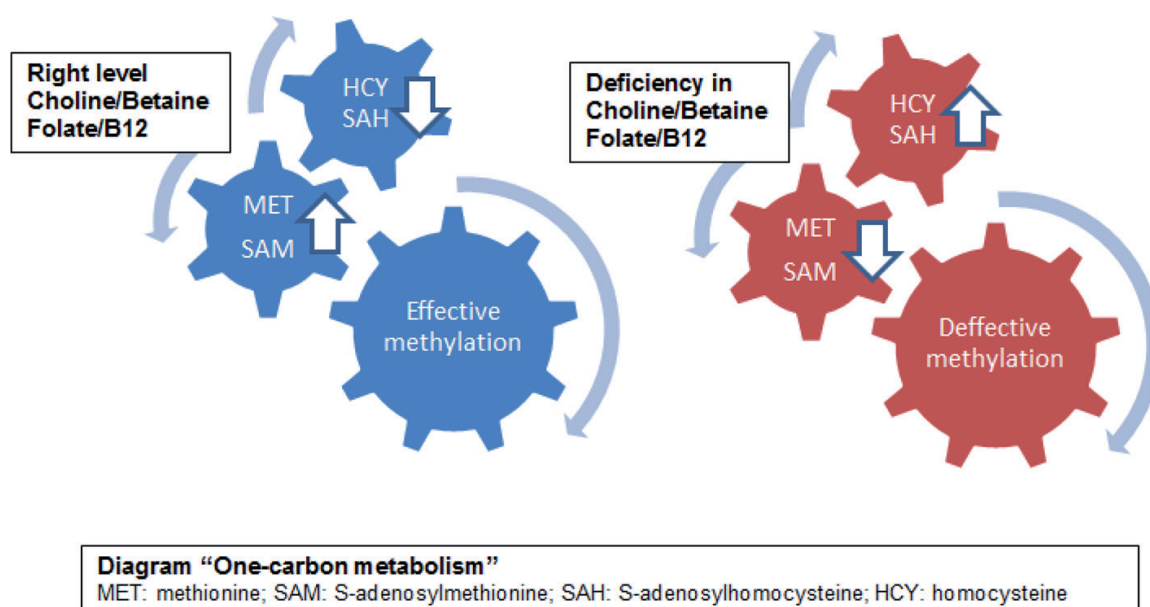


Figure 1. "One-carbon metabolism" implication in epigenetics.

<3.5 mg/Kg. Lower choline and betaine intakes were also correlated with lower plasma choline and betaine concentrations among children with ASD. This suggests that the choline-betaine-homocysteine pathway for Met synthesis may be compromised in children with ASDs.

In a rat model of diet-induced fetal-neonatal iron deficiency, the choline supplementation reduced the effects of iron deficiency, including those on gene networks associated with autism [319]. And also prenatal choline supplementation mitigates the adverse effects of prenatal alcohol exposure on development in rats [320]. A pilot study suggests that an additional evaluation of choline supplementation as an intervention for memory functioning in children with fetal alcohol spectrum disorders is warranted [321]. We have observed improvement after administration of citicoline in a patient with autism and X-chromosome-linked ichthyosis caused by deletion Xp22.3-pte; thanks to the role this nootropic plays in the biosynthesis of structural phospholipids involved in the formation and repair of the neuronal membrane, and a possible contribution of the gene *PNPLA4*, which codes for calcium-independent phospholipase A2 beta, involved in lipoprotein metabolism [127]. Future research should consider whether these metabolic imbalances could be corrected with dietary counseling or supplement interventions.

7.3.5. Fatty acids (FAs)

The inability of the human body to synthesize acid, known as essential fatty acids (EFAs), implies that they must be obtained through diet or supplementation. The EFAs are all omega-3 and -6 methylene-interrupted fatty acids. Polyunsaturated fatty acids (PUFA) are a family of lipids including some subgroups identified by the position of the last double bond in their structure. PUFA omega-3 fatty acids (n-3 or ω -3) include alpha linolenic acid (ALA), eicosapentaenoic acid [EPA, 20:5 (3)], and docosahexaenoic acid [DHA, 22:2 (3)], while PUFA ω -6 include linoleic acid [LA, 18:2 (6)] and arachidonic acid [AA, 20:4 (6)].

The CNS is rich in PUFAs, and in particular AA and DHA are essentials for brain development and play a key role in the maturation and signaling of the brain network. Clinical and animal studies illustrate the importance of long-chain polyunsaturated fatty acids (LCPUFAs) in neural development and neurodegeneration and FAs metabolic pathways may affect proper functioning of the CNS [322]. However, whether or not the levels of these PUFAs are altered in individuals with autism remains debatable.

In female mice maternal ω -3 PUFA deficiency during pregnancy and lactation imprints long-term changes of brain development neurogenesis and apoptosis in adult offspring, associated with DNA methylation of brain-derived neurotrophic factor transcripts [323]. Children with ASD had lower dietary consumption of foodstuff containing DHA, as well as lower serum levels of DHA than controls [324]. In an analysis of the FAs composition of red blood cell (RBC) membrane phospholipids showed that the percentage of total PUFA was lower in autistic patients than in controls; and levels of AA and DHA were particularly decreased ($p < 0.001$) [325]. Reported abnormalities associated with the synthesis of lipid bilayer in ASD as a result of FAs insufficient dietary supplementation or genetic defects can contribute to the symptomatology of autism and individual variety (for review see [326]). A growing body of evidence suggests that individuals who have ASD may have low levels of these PUFAs, particularly DHA and the AA:DHA ratio. Studies also reported significantly higher plasma in the ratios: EPA:AA; linoleic acid:AA; α -linolenic acid:DHA; and ω -3 to ω -6 FA ratios [327–329]. In ASD genetic abnormalities have been reported in the enzymes involved in phospholipid metabolism; e.g., functional abnormalities of the solute carrier 27A (SLC27A) gene family encoding fatty acid transport proteins (FATPs) [330]. But there appears to be no evidence of altered phospholipid-related signal transduction in autism [331].

On the other hand, deficits associated with the release of AA from the membrane phospholipids and its subsequent metabolism to bioactive prostaglandins via phospholipase A (2)-cyclooxygenase biosynthetic pathway have been shown. So, plasma levels of the proinflammatory AA metabolite prostaglandin E2 (PGE2) [325, 331, 332] and leukotrienes (two important lipid mediators) as well as isoprostanes (as marker of oxidative stress) recorded significantly elevated levels in autistics participants compared to controls [333]. The COX/PGE2 pathway and plasma transferrin (an iron mediator related to eicosanoid signaling) play an important role in synaptic plasticity and may be included in pathophysiology ASD [333]. In addition, Wong et al. [334] found that PGE2 activated the canonical Wnt signaling pathway and Wnt-dependent migration and proliferation in neuroectodermal stem cells. In Wnt-induced cells the level of β -catenin protein was increased and the expression levels of Wnt-target genes (Ctnnb1, Ptgs2, Ccnd1, Mmp9) was significantly upregulated in response to PGE2 treatment. This confirms that PGE2 activated the canonical Wnt signaling pathway. Furthermore, the upregulated genes have been previously associated with ASD.

There is strong mechanistic evidence to suggest that vitamin D and n-3 LCPUFAs, specifically DHA, have the potential to significantly improve the symptoms of ASD [335]. But the efficacy of Omega-3 FA supplementation in terms of ASD-symptom management is controversial, so many authors show that DHA supplementation does not improve the core symptoms of

autism [336, 337]. A Cochrane review did not find any evidence of effects of omega-3 FA in ASD [338]; by contrast, a recent study by Mazahery et al. [339] have shown that either vitamin D, DHA, or both are effective; the trial would reveal a noninvasive approach to managing ASD symptoms.

7.4. Gastrointestinal dysbiosis

Gut bacteria are an important component of the microbiota ecosystem in the human gut. The intestinal flora is composed of more than 10¹⁴ bacteria spread over more than 400 species, of which between 30 and 40 are dominant. The profile of an individual's microbiota is continually influenced by a variety of factors, including but not limited to genetics, age, sex, diet, and lifestyle. Although the microbial profile of each person is different, the relative abundance and distribution of bacterial species is similar among healthy individuals. All these bacteria, in continuous competition, produce a huge variety of enzymatic reactions that necessarily interact with the metabolism and physiology of the host (nutritional functions, immunological, toxicological, etc.), in situations of health or disease in the intestine. Activity microbial biochemistry acts collectively like an organ, intervening in the improvement of nutrient bioavailability and degradation of compounds from the nondigestible diet, the supply of new nutrients, and the elimination of harmful and antinutritional compounds. However, they can also be potentially harmful due to the change of their composition when the gut ecosystem undergoes abnormal changes in the light of the use of antibiotics, illness, stress, aging, bad dietary habits, and lifestyle. Perturbations in gut microbiome composition have an emerging role in health and disease including brain function (development of emotional behavior, stress- and pain-modulation systems, and brain neurotransmitter systems); but also may be environmental contributors in neurodevelopmental disorders including ASD. The ability of the intestinal microbiota to communicate bidirectionally with the brain, known as the “gut-brain axis,” in modulating human health by multiple mechanisms, including endocrine and neurocrine pathways, is at the forefront of current research [340, 341]. There is still debate as to whether or not these changes are core to the pathophysiology or merely epiphenomenal [342].

An inconsiderable number of patients with ASD have a history (fetal, neonatal, and infant) of previous antibiotic exposure or hospitalization and GI symptoms; it is important to think about the importance of perturbations in gut microbiome during the first postnatal years of life in modulation of brain development, cognitive functions, and behavior [343]. In the first year of life, decreasing maternal immune protection and child immune system immaturity create an immune vulnerability to disease infection, especially if it is treated with antibiotics, and could facilitate gastrointestinal disorders and dysbiosis. This condition causes a vicious circle between the deterioration of the immune system and increase dysbiosis leading to leaky gut and neurochemical compounds and/or neurotoxic xenobiotics production and absorption. This alteration affects communication “gut-brain axis” that connects the intestine to the CNS through the immune system [344].

The complex carbohydrates (oligo- and polysaccharides) provided by diet are the group of fermentable substrates most abundant. Members gut flora has developed a complex system

of glycohydrolases that allow them to use, thus favoring survival, while generating metabolic energy for enterocytes. Main products of fermentation (enteric bacterial metabolites) are enteric short-chain fatty acids (SCFA) mainly propionic (PPA), butyric acid (BA), and acetic acid, which constitute between 83 and 95% of the total SCFAs. SCFAs represent a group of compounds derived from the host microbiome that are plausibly linked to ASDs. Intraventricular administration of PPA and SCFAs in rats induces abnormal motor movements, repetitive interests, cognitive deficits, perseveration, and impaired social interactions. The brain tissue of PPA-treated rats shows a number of ASD-linked neurochemical changes, including innate neuroinflammation, increased oxidative stress, glutathione depletion, and altered phospholipid/acylcarnitine profiles. These directly or indirectly contribute to acquire mitochondrial dysfunction via impairment in carnitine-dependent pathways; common antibiotics may impair carnitine-dependent processes by altering gut flora favoring PPA-producing bacteria and by directly inhibiting carnitine transport across the gut [345]. GI microbiota and their fermentation products are of potential use as biomarkers for early identification of the etiology and/or symptoms of ASD [346]. Therefore, a significant number of autistic children with chronic digestive disease (most with ileo-colonic lymphoid nodular hyperplasia and inflammation of the colorectum, small bowel and/or stomach) had low serum levels of myeloperoxidase (MPO). However, there was no significant relationship between these levels and severity of GI disease, including the presence of antineutrophil cytoplasmic antibodies (ANCA) [347]. Also, slow intestinal transit contributes to elevate urinary p-cresol level in autistic children [348]. But metabolomics studies in ASD are far to be definitive and univocal [349].

By establishing the hypothesis the role that enteric SCFA, particularly PPA, produced from ASD-associated gastrointestinal bacteria, may be a potential environmental trigger in some forms of ASD. Propionic acid has bioactive effects on (1) neurotransmitter systems, (2) intracellular acidification and calcium release, (3) fatty acid metabolism, (4) gap junction gating, (5) immune function, and (6) alteration of gene expression [350]. SCFA acts as gut-related environmental signals and are capable of altering host gene expression (including CREB-dependent catecholaminergic neurotransmission), partly due to their histone deacetylase inhibitor activity and induced broad alterations in gene expression including neurotransmitter systems (monoaminergic pathways) neuronal cell adhesion molecules, inflammation, oxidative stress, lipid metabolism, and mitochondrial function, all of which have been implicated in ASD [351].

This modulation of the intestinal microbiota is currently a growing area of investigation and it just might be the key to treatment [352]. Studies have also suggested that the intestinal microbiota may be modulated with the use of prebiotics, probiotics, symbiotics, postbiotics, antibiotics, and fecal microbiota transplants and activated charcoal, as an opportunity for therapy in microbiota-associated diseases as ASD [353]. The literature contains information about autism being improved after treatment with common antibiotics (amoxicillin) [354]. Also, ceftriaxone, a beta-lactam antibiotic, increases the expression of the glutamate transporter 1 which decreases extracellular glutamate levels. It is hypothesized that modulating astrocyte glutamate transporter expression by ceftriaxone or cefixime might improve some symptoms of ASD [355].

7.5. Mental stress

Stressor exposure during early life has the potential to increase an individual's susceptibility to a number of neuropsychiatric conditions as anxiety disorders. In the gene-environment interactions underlying ASD, also are implicate early prenatal stress as being especially detrimental to boys with a vulnerable genotype. So, stressful events during pregnancy significantly predicted autistic traits in the offspring in males only [356].

The mechanisms underlying in the nature of this vulnerability have not yet been established. Potentially, etiologic mechanisms of the early postnatal stress including genes involved in the regulation of hypothalamic-pituitary-adrenal axis could be involved. Therefore, in autistic individuals a lower cortisol and higher ACTH levels is described [357, 358]. In experimental animals early life stress increases stress vulnerability through BDNF (brain-derived neurotrophic factor) gene epigenetic changes with decreased levels of acetylated histone H3 and H4 [359]. Recent human epidemiological and animal studies indicate that stressful experiences *in utero* or during early life may increase the risk of neurological and psychiatric disorders, arguably via altered epigenetic regulation and transgenerational epigenetic inheritance (for review see [360]).

7.6. Others prenatal and perinatal environmental factors

7.6.1. Prenatal risk factors

Among the risk factors described for children with ASD those related to the gestational period should be noted. The pregnancy-related risk factors that have been associated with ASD account for few cases, but show the importance of susceptibility of developing brain for certain noxas. As previously mentioned the importance of teratogens toxic, nutritional factors, and immune system, among other factors, has been implicated as prenatal environmental risk. Among these risk factors it should be noted among other things:

- **Maternal chronic disease**, like maternal diabetes, prepregnancy obesity, and pre-eclampsia, is modifiable environmental factor in ASD pathophysiology. Similarly, studies on maternal autoimmune disorders during pregnancy have reported different associated disorders (psoriasis with ASD; thyroid antibodies with ADHD) [361]. Group B streptococcus (GBS) is the most frequent commensal bacterium colonizing or infecting pregnant women. SGB is present in the lower genital tract of 15–30% of healthy pregnant women. In experimental animals, first time gestational exposure to GBS, autistic-like behavior has been reported predominantly affecting male. So, GBS-induced maternal immune activation plays a role in offspring perinatal brain damage (white matter injury) and subsequent neurodisabilities such as autism [362, 363].
- **Maternal smoking during pregnancy**: Growing evidence links prenatal exposure to maternal smoking with disruption of DNA methylation (DNAm) profile in the blood of newborns. An EWAS was conducted using linear regression of methylation values against *in utero* smoke exposure; this study confirms differential methylation in *MYO1G*, *CNTNAP2*, and *FRMD4A* [364]. The association between maternal smoking during pregnancy and ASD risk in offspring has been investigated in several studies, but the evidence

is not conclusive. A recent meta-analysis (including 15 observational studies with 17,890 ASD cases and 1,810,258 participants) indicates that maternal smoking during pregnancy is not associated with ASD risk in offspring [365].

- **Advancing paternal and grandpaternal age and risk of autism:** the father's age is not a factor gene (with genetic implications), which has been associated with ASD, especially in sporadic cases. In men, with age increase *de novo* mutations in their germ cells and there is a greater chance that the children carry a harmful mutation that could increase susceptibility to these disorders [366, 367]. Further, it identifies an association between advanced age (50 or older) males' grandparents when they had their children and diagnosis of autism in the grandsons [368].

7.6.2. Perinatal and neonatal risk factors

Numerous risk factors perinatal and neonatal have been studied in relation to autism, and although there is insufficient evidence to implicate any factor perinatal or neonatal in the etiology of autism, however, there is some evidence to suggest that exposure to a broad class of conditions reflecting general commitments for perinatal and neonatal health may increase the risk [369], with special mention to prematurity [370, 205]. The prevalence (in the range 3.65–12.9%) of ASD among preterm births is 10–12 times more than in the general population [371, 372]. The ASD diagnosis was associated with shorter gestation times and longer hospital stays [373]. Preterm children with very low birth weight (VLBW; 1000–1500 g) or extremely low birth weight (ELBW; under 1000 g) are at increased risk for ASD [374–376]. A number of hypotheses have been put forward to explain these high rates of ASD: prenatal and neonatal complications like sensory impairment associated with prematurity, white matter abnormalities, and cerebellar impairment which occurred more commonly among preterm infants [372, 377, 378]; as well as altered androgen exposure observed in premature infants [379]. Also, gene-environment interactions and prematurity may combine to increase the risk for poor neurodevelopmental outcomes [380]. Thus, fetal membranes from spontaneous preterm birth demonstrate differences in *OXTR* methylation and regulation and expression, but not in *SHANK3*, *BCL2*, and *RORA*, which suggest that epigenetic alteration of *OXTR* in fetal membrane may likely be indicating an *in utero* programming of this gene and serve as a surrogate in a subset of spontaneous preterm birth [381].

Other risk factors involved in the neonatal period include: higher incidence of hyperbilirubinemia [205, 382], birth defect, a birth weight small for gestational age, acute fetal distress, difficult labor and respiratory infection [204], delayed birth cry and birth asphyxia [205], or low Apgar scores [203]. Factors associated with autism risk in a meta-analysis of 40 studies in 2007 [369] were abnormal presentation, umbilical-cord complications, fetal distress, birth injury or trauma, multiple birth, maternal hemorrhage, summer birth, low birth weight, small for gestational age, congenital malformation, low 5-minute Apgar score, feeding difficulties, meconium aspiration, neonatal anemia, ABO or Rh incompatibility, and hyperbilirubinemia. But the authors conclude that there is insufficient evidence to implicate any one perinatal or neonatal factor in autism etiology, although there is some evidence to suggest that exposure to a broad class of conditions reflecting general compromises to perinatal and neonatal health may increase the risk.

8. Conclusion

Although the knowledge about risk factors for ASD increases day by day, the biological basis of ASD remains largely elusive. Up to now, a large percentage of publications have implicated physiological and metabolic abnormalities (examining peripheral biomarkers such as blood and urine) in ASD and other psychiatric disorders. In particular, “four major areas” have been seen to be involved: immune dysregulation or inflammation, oxidative stress, mitochondrial dysfunction, and environmental toxicant exposures. More recent studies have also reported these physiological abnormalities in brain tissue derived from individuals diagnosed with ASD, suggesting that ASD has a clear biological basis with features more related to known “medical disorders” than “psychiatric disorders.”

Genes implicated in ASD alter ratios of excitatory to inhibitory signaling in the CNS (with increased ratio of excitation/inhibition in key neocortical systems) affecting molecular pathways and mechanisms related to synaptic dysfunction, in a process known as “developmental synaptopathy.” The interplay between mutations in different genes can produce “idiopathic” autism, but the exposure to environmental modifiers as well as the epigenetic factors may add up to a varying expression of autistic features, allowing to include autism among the “synaptic and chromatin-remodeling disorders.” In the last years, the role of nutritional and metabolic status in ASD has also gained importance, particularly the “one-carbon metabolism cycle,” including the folate metabolism, the methionine-homocysteine remethylation cycle (involving choline and betaine), and fatty acid metabolism, as well as alterations in gut microbiome composition of children with autism. Furthermore, research in these physiological areas may lead to breakthroughs, in general, as well as subset-specific processes, that could contribute to elucidate the development of ASD. Epigenetic studies besides being useful to discover causal factors, they may also help to unravel the physiological mechanisms predisposing or resulting from the onset of ASD. Therefore, it is also important to follow the epigenetic approach to investigate the possible impairment of metabolic systems in ASD as well as in the search for promising metabolic biomarkers and therapeutic targets.

Nomenclature/Abbreviations

AD	autistic disorder
ADHD	attention=deficit/hyperactivity disorder
AS	Angelman syndrome
ASD	autism spectrum disorder
BA	butyric acid
BDNF	brain=derived neurotrophic factor
BPA	bisphenol A
BWS	Beckwith&Wiedemann syndrome
CDD	childhood disintegrative disorder

CDC	Centers for Disease Control and Prevention
CSS	Coffin=Siris syndrome
CMA	chromosomal microarray analysis
CNS	central nervous system.
CNV	copy number variants
DD	developmental delay
DSM	Diagnostic and Statistical Manual of Mental Disorders
EFA _s	essential fatty acids
EWAS	epigenome=wide association study
FAs	fatty acids
FXS	Fragile X syndrome
GWAS	genome=wide association study
H	histone
HAT _s	histone acetyltransferases
HDAC _s	histone deacetylases
hiPSC _s	human induced pluripotent stem cells
HMT _s	histone methyltransferases
ICR	imprinting control region
ICSI	intra=cytoplasmic spermatozoid injection
ID	intellectual disability
IVF	in vitro fertilization
KDM _s	histone lysine demethylases
KMT _s	histone lysine methyltransferases
LCPUFA _s	long=chain polyunsaturated fatty acids
LCR _s	low=copy repeats
MBD _s	methyl=CpG=binding domain proteins
MCA	multiple congenital anomalies
mPFC	medial prefrontal cortex
NAHR	nonallelic homologous recombination
NDD	neurodevelopmental disorders
NLGN	neuroligins
NGS	next=generation sequencing
NRXN	neurexins
OXTR	oxytocin receptor
PBDE _s	polybrominated diphenyl ethers

PCBs	polychlorinated biphenyls
PDD	pervasive developmental disorders
PDD=NOS	pervasive developmental disorder not otherwise specified
PPA	propionic acid
PRMTs	protein arginine methyltransferases
PUFA	polyunsaturated fatty acids
PWS	Prader=Willi syndrome
RORA	retinoic acid=related orphan receptor alpha
SCFA	short chain fatty acids
SDs	segmental duplications
SNP	single nucleotide polymorphisms
SNV	single nucleotide variants
SWI/SNF	SWItch/Sucrose nonfermentable
RSS	Russell=Silver syndrome
RTT	Rett syndrome
TF	transcription factor
T(Reg)	regulatory T cells
TRD	transcriptional repression domain
WES	whole=exome sequencing
Xic	X inactivation center
XCI	X chromosome inactivation

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