Chapter 16

Etiology of Autism the Complexity of Risk Factors in Autism Spectrum Disorder

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1. Introduction

Autism Spectrum Disorder (ASD), also currently known as autism, refers to a group of complex behavioral disorders of varying severity, characterized by manifestations noted in early childhood, usually before the age of three and defined as impaired social communication and presence of restricted interests and repetitive behaviors, compromising the entire life of the individual (DSM-5). The etiology is very complex and heterogeneous, with numerous causes described, and includes genetic, epigenetic, or environmental factors in isolation or associated. It aggregates in families with the heritability being estimated at 0.50, but the individual risk and to what extent this is caused by genetic factors or environmental factors remains unresolved. These factors probably interact at least in the majority of cases, and thus the assessment of individuals and genetic counseling is further complicated [124].

Valuable information is been gained through the identification of candidate genes, though case-control and association studies and more recently by comparative genomic hybridization and whole exome and genome sequencing. In the epigenetic area, mechanisms such as genomic imprinting, epimutations and methylation have been identified [133]. Copy number variations (CNVs) have gained prominence on the stage of the discovery of the causes of autism. The *de novo* CNVs have been reported in 7% of simplex families and ~2% in multiplex families. Moreover, hypomorphic alterations in some genes suggest oligogenic inheritance. Recently, discoveries employing large-scale whole exome sequencing (WES) showed that one gene alone is not able to confer significant risk for autism. Instead, the most probable hypothesis is the contribution of several risk variants that are scattered in hundreds of genes. There

are approximately 4,000 genes involved in molecular pathways, gene regulation and functional domains that may contribute to neurodevelopmental disorders. It is noteworthy that the same genes can cause different disorders leading to an etiologic overlap [35].

One other issue that has emerged recently is that a significant number of synaptic proteins directly or indirectly affect the structure and function of neurons, dendrites and synapses. Subtle changes in the dendritic and synaptic structures can lead to huge changes in information processing. Dendritic branches and spines are essential for the formation and plasticity of neuronal circuits but are interrupted in many neurologic disorders, such as autism. In many cases the same mutations are observed in unaffected relatives. This suggests the existence of a compensatory mechanism or other genetic or non-genetic causes [140].

New findings on the genetic etiology of autism have pointed to the participation of regulatory regions of transcription factors, the microRNAs (miRNAs), a class of noncoding RNAs of ~22 nucleotides that suppress translation by pairing with miRNA recognition elements present in the 3'untranslated region (3'UTR) of target mRNAs. Candidate genes and sites of miRNA targets have been identified from these. It is known that the expression of many genes involved in autism is regulated by miRNAs. Single nucleotide polymorphisms (SNPs) have been described as modulators or creators of new recognition elements of miRNAs. Therefore, there is a hypothesis that SNPs disrupting the interaction between miRNA and genes can lead to the aberrant expressions of genes implicated in autism, resulting in susceptibility to disease or pathogenesis in at least one subpopulation of affected individuals [149].

Although the involvement of genetic abnormalities in autism is well accepted, recent studies have indicated that there is a similar contribution of environmental factors. However, studies related to the environment, especially those regarding toxic products, have not been systematically reviewed yet, as many studies have limitations, including a lack of reproducibility, small sample size, retrospective design, bias between cases and controls and non-use of an appropriate autism diagnostic tool. Thus, in general, there is a potential involvement of some toxic products in complex genetic-environmental interactions which act synergistically or in parallel on the brain in a way that increases the likelihood of developing autism.

Moreover, although some studies suggest that autistic characteristics are due to central nervous system (CNS) dysfunction, there is evidence of autism-related abnormalities that are not related to the CNS, at least in some individuals. Hence, the metabolic system, immune system dysregulation and oxidative stress have also been implicated in the etiology [107].

Furthermore, other new lines of research also point to the importance of the so-called "brain-gut axis" revealing the central role of the intestinal microbiota in postnatal development and maturation of the immune and endocrine systems that, in turn, control CNS signaling, brain function and behavior [151]. But studies on this line must be carefully analyzed.

The low recurrence related to any one cause is one of the most intriguing aspects of the etiology, as is the difference in the proportion of affected between the genders, because men are four times more affected by ASD than women (CDC, 2014). Additionally, there is an association between increased paternal age and risk for ASD. This finding may indicate that *de novo*

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mutations, which are more common in older men, may play a smaller role in the incidence of autism than the familial genetic load [73].

Investigating and understanding the etiology of autism is extremely important for families because it allows a determination of the recurrence risk, the possibility of detecting other associated medical problems, an assessment of the molecular nature and cellular pathophysiology, and potential therapeutic approaches. In this context, the aim of this chapter is to provide the reader with an overview of these possible causes and others that may contribute to autistic behavior.

2. Common chromosomal alterations: 15q11-q13, 16p11.2 and 22q11.2

In addition to well-established genic syndromes, a lot of cases of ASD present with numerical or structural chromosomal alterations visible by conventional cytogenetic techniques [164]. Due to the high number of cases and the type and location of the genes described, the association of some chromosomal regions is well established in autism including: 1q21, 2q37, 7q11.23, 15q11-13, 16p11.2, 17p11.2, 22q11.2 and 22q13. Rearrangements involving these regions are detected by GTG banding however more sophisticated molecular techniques are recommended. High-resolution whole-genome analysis with array-based technologies have revealed genomic imbalances in at least 10% of cases (Depine et al., 2009).

Chromosome 15 is reportedly the most common site of autosomal abnormalities in autism with the duplication of 15q11-q13 being the most frequently reported alteration. This region contains at least 30 genes, several of which have been associated to ASD, neurobehavioral disorders, cognitive deficits, hypotonia, language delay and seizures [141, 154].This region, known for its genetic instability, contains many low copy repeats and segmental duplications. It is known as a critical region for Prader-Willi/Angelman syndrome and has a complex pattern of paternal and maternal imprinting; it contains at least five paternally expressed genes (*MKRN3*, *MAGEL2*, *NDN*, *C15orf2*, *snoRNAs* and *SNRPN-SNURF*) and two maternally expressed genes (*UBE3A* and *ATP10A*). Furthermore, epigenetic factors regulating 15q11-13 have been implicated in the presence of autism [6].

The phenotype involving recurrent ~600 kb microdeletions and microduplications in the 16p11.2 region is characterized by a spectrum of neurodevelopmental impairments including developmental delay and intellectual disability, epilepsy, autism and other psychiatric disorders which are all subject to incomplete penetrance and variable expressivity. This deletion is observed in ~0.5% of autism patients, making this the second most common abnormality in this disorder [48, 156]. Losses in candidate genes in this region, such as *ALDOA*, *DOC2A*, *HIRIP3*, *MAPK3*, *MAZ*, *PPP4C*, SEZ6L2, and *TAOK2*, seem to contribute to the ASD phenotype (Kumar et al., 2009).

The 22q11.2 deletion is one of the most commonly known interstitial deletions identified in humans, and with a frequency of around 1:4,000 live births in the general population, it is related to DiGeorge Sequence/Velocardiofacial syndrome [98, 132]. Recent studies suggest that

the high prevalence of autistic behaviors in children with 22q11.2 deletions should not be viewed only as ASD but as prodromal symptoms preceding the onset of schizophrenia [9, 132, 153]. Individuals with hemizygosity of the 22q11.2 deletion represent genetically identifiable cases of ASD. However, the 22q11.2 gene(s) responsible for ASD have not been identified yet. *Tbx1* is one of the candidate genes, possibly through its role in diverse cell types, including prenatally and postnatally generated neurons.

Several large chromosomal microarray studies have reported the prevalence of CNV variants in people with particular features (e.g., autism, schizophrenia, and epilepsy) but few studies have investigated the prevalence in the general population. In a screening of 6,813 consecutive cord blood samples from a predominantly French–Canadian population to assess genomic CNVs, 23 children were identified with alterations in 15q11-q13, 16p11.2 or 22q11.2. Longitudinal follow-up studies are needed to determine the clinical consequences of CNVs identified at birth [146]. Anyway, considering the important implications for genetic counseling, these regions must be evaluated in ASD patients.

3. Copy number variations (CNVs)

Dysregulation of gene expression of several genes/loci converging on the same networks (overlay) and/or combinatorial effects of different deleterious genetic variations appear to exceed a threshold and result in the autistic phenotype. In support of these ideas, strategies based on bioinformatics have identified many candidate genes, showing that ASD can be triggered by different types of genetic variations in many different genes, a phenomenon known as non-allelic genetic heterogeneity [74, 138, 105]. This model is more accepted; combines both common and rare variations posing risk for ASD, particularly those involving synaptic genes and genes involved in neurogenesis [138]. Thus it is assumed that people with ASD have a set of genetic variants that predispose them to abnormal development of brain structures involved in processing social information (the "social brain"). But it is known that there is no common pathophysiology in ASD. This may result from mutations in many different genes involved in different functions [138]. The kinds of variants that incline to autism and can involve several genes at the same time are the CNVs.

CNVs are microduplications or microdeletions resulting from insertions, deletions or translocations in the human genome that are observed in the general population and commonly found in genic regions in individuals with neuropsychiatric disorders. They can be inherited or *de novo*, frequent or rare with a frequency of less than 1% of the population. A substantial portion of autism cases appears to result from rare CNVs with variations larger than 100kb; they are more common in individuals with ASD than in the general population [33, 83, 118].

De novo CNVs have been reported in 5-10% of cases of idiopathic ASD. Many studies have revealed that some CNVs occur at significantly higher frequencies than others and some are exclusively observed subjects and not found in normal controls. This has allowed the identification of new candidate genes which have not yet been described in the Autism Chromosome Rearrangement Database, such as *GABRA5*, *GABRA3*, *GABRG3*, *UBE3A*, *E2F1*, *PLCB1*, *PMP22*,

AADAT, MAPK3, NRXN1, NRG3, DPP10, UQCRC2, USH2A, NECAB3, CNTN4, LINGO2, IL1RAPL1, STXBP5, DOC2A, SNRPN, E2F1, AADAT, NECAB3, GPHN, dlg2, HPCAL1, BDNF-OS and *IL1RAPL1*, (http://projects.tcag.ca/autism/). The new risk loci for ASD have functions that suggest an important role in the function and architecture of the brain; one CNV could interfere with normal biochemical pathways and predispose to the disorder [27, 46, 95). CNVs associated with ASD and schizophrenia are also associated with cognitive problems in control subjects showing a probable variable expressivity of these changes [134].

Although structural variations, such as CNVs, play an important etiologic role in the development of ASD as has been proposed by several authors since 2006, most of the results of different studies are not considered in the clinical evaluation of children with ASD, probably due to the rarity of individual variants, the lack of coverage of probes in clinical microarrays, the lack of reproducibility of studies that present different findings and the difficulty to understand the biology corresponding to some variants even when they are significantly associated with ASD. Nevertheless, clinical guidelines suggest that microarray-based tests are the first step in the genetic analysis of children with ASD (128, 68]; however this is not feasible in most cases of low-income countries due to the high cost of these tests.

4. Cellular adhesion molecules (CAMs)

While the majority of genetic mutations currently linked to autism are rare variants that change the protein-coding sequence of synaptic candidate genes, regulatory polymorphisms affecting constitutive and alternative splicing have emerged as risk factors in other diseases, accounting for an estimated 40-60% of general disease risk [131].

Neurons communicate via synapses, mainly mediated by precisely controlled intercellular interactions. Interactions between presynaptic and postsynaptic cellular adhesion molecules (CAMs) drive synapse maturation during development. CAMs provide "bridges", that is, cell-to-cell connectivity between pre and postsynaptic sites. These transsynaptic interactions are regulated by alternative splicing of CAMs RNAs, which ultimately determines neurotransmitter phenotype. Failure to generate the appropriate CAMs can result in loss of activity-dependent neuronal plasticity, and risk for developmental disorders, including autism. However, it remains unclear as to how many and which proteins are involved in the synaptogenesis process [161]. The postsynaptic proteome of excitatory synapses of a mammalian brain contains over 1,000 proteins, indicating complex protein-protein interactions that occur both within and between synapses [15, 30, 32, 47].

Typically, CAMs are located at the center of synapse and contain three domains: an intracellular domain that interacts with the intracellular scaffolding protein, a transmembrane domain and an extracellular domain which interacts with other CAMs [101]. Intercellular interactions in synapses mediated by protein-protein CAMs are involved in recognition and alignment of pre and postsynaptic sites, transsynaptic signaling, the exact location of neurotransmitter receptors and release of synaptic vesicles. There are several families of CAMs that have already been recognized, including neurexins (Nrxs) and neuroligins (NLS), neuronal transmembrane

proteins rich in leucine (LRRTMs), N-cadherin/β-catenin, ephrins and Eph, SynCAM receptors and integrins [161].

The Nrxs and NLS contain an extracellular domain that participates in the pre and postsynaptic interaction and an intracellular domain that is involved in multiple functional interactions and regulatory processes. They interact with high affinity via their extracellular regions [135, 136]. Nrxs create extracellular protein-protein interactions with the intracellular signaling cascade. NLS binds to areas of postsynaptic density (PSD), proteins which are supported by glutamatergic synapses. In postsynaptic sites, the NLS/Nrxs interactions cause an increase in the PSD agglomeration recruitment of postsynaptic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Thus, the binding of Nrxs and NLS helps to align presynaptic and postsynaptic receptor release (Budreck et al., 2013; Mondin, Tessier, Thoumine, 2013).

LRRTM proteins are a group of type I transmembrane proteins containing extracellular leucine-rich repeats and a short cytoplasmic tail. They play a key role in the development and maturation of synapses, but are also directly involved in synaptic transmission and more complex behavior [87]. Contactins (CNTNs) provide a set of glycan phosphatidyl-inositol (GPI) Ig-CAM links containing six N-terminal Ig-like domains and four Fibronectin Type III domains. CNTNs play an important role in the formation of axon connections in the developing nervous system. For example, Cntn-1 and Cntn-2 are involved in axon growth and guidance, and Cntn-6 is expressed in the presynaptic region in the developing nervous system [169].

As most neurological diseases originate as a dysfunction of neural circuits whose function is highly dependent on the accuracy of cell-cell adhesions, there is increasing evidence connecting several neurological disorders with mutations or altered expressions of CAMs. For example, mutations in the *NLS* and *Nrxs* genes are found in patients with autism (Sudhof, 2008). Therefore, research on the role of CAMs will help provide a better understanding of the underlying mechanisms of pathogenic neurological disorders (Frei & Stoeckli, 2013).

5. Synaptic vesicles

Alterations in neurotransmission and its components, such as synaptic vesicles seem to be one of the keys in neurological disorders. Abnormalities in synaptic vesicle endocytosis and recycling may contribute with this type of disorder. Exoendocytic cycling of synaptic vesicles, which are organelles of 40 nm in diameter, is involved in neurotransmitter release. Hundreds of synaptic vesicles, filled with neurotransmitters are found in each presynaptic nerve terminal. When presynaptic plasma membranes depolarize, Ca2þ-channels open and calcium flows into the nerve terminal, triggering the exocytosis of synaptic vesicles and releasing their neurotransmitters into the synaptic cleft. Calcium binds to synaptotagmin, and after exocytosis, vesicles are re-endocytosed, recycled, and refilled with neurotransmitters. Recycling can occur by multiple parallel pathways, either by fast recycling via local reuse of vesicles, or by slower recycling through an intermediate endosomal (Sudhof & Ryzo, 2014). Also, a critical step in presynaptic differentiation is the clustering of synaptic vesicles near neurotransmitter release sites, the active zone, where vesicle fusion and exocytosis of neurotransmitters occur.

Synaptic vesicles at presynaptic terminals store neurotransmitters from presynaptic neurons such as gamma-aminobutyric acid (GABA) and glutamate [133].

Many presynaptic molecules are involved in the regulation of synaptic vesicle release, including CAMs. The family of adaptor protein (AP) complexes, AP-1, AP-2, AP-3 and AP-4 mediates various types of vesicle formation and selection of cargo molecules for inclusion in these vesicles. The synaptic vesicle process involves AP-2/clathrin-mediated endocytosis [133]. The structural composition of synapses may be altered by mutations or deletions in other scaffold proteins, such as those of the Shank family, neurexin and NLS. These changes may result in an altered number of receptors such as mGluR receptors or changes in the composition of NMDA and AMPA, and affect component signaling in PSD. Dysregulation in the synapse morphology induced by structural alterations and disturbed signaling might converge and lead to disrupted long-term potentiation (LTP) formation and plasticity, and a specific decrease in excitatory signaling by various genetic mutations, environmental factors, or immune system alterations, which would lead to an imbalance in inhibition and excitation as a likely cause of autism [26].

The main excitatory neurotransmitter in the brain, glutamate, modulates the neuronal formation and synaptic strength in the early phases of development due to its role in neuronal plasticity and cognitive functioning [88]. Glutamate receptors are diffused throughout the brain, in the cerebellum and hippocampus, regions implicated in ASD pathogenesis [25]. Genetic alterations in glutamate signaling have been found in association with ASD through candidate gene screening and genetic association studies and an imbalance in excitation and inhibition with glutamate signaling is proposed as a mechanism involved in ASD during the early development stages, between one and three years of age [112].

There are two types of glutamate receptors, metabotropic and ionotropic. Metabotropic glutamate receptors (mGluR) are G-protein coupled receptors involved in intracellular signal transduction and can be divided into three groups: Groups I (mGluR1 and mGluR5), II (mGluR2 and mGluR3) and III (mGluR4, mGluR6, mGluR7 and mGluR8). Group I receptors activate phospholipase C. Groups II and III are negatively linked to cyclic adenosine 3′,5′-monophosphate (AMP) production, but they differ in agonist selectivity. Two Group III receptors, mGluR7 and mGluR8, are located within the presynaptic grid, whereas mGluR3 and mGluR2 are located on the preterminal axons. Ionotropic glutamate receptors form ligand-gated ion channels (LGICs) and are labeled according to their prototypical agonists: NMDA, AMPA and kainate [25].

Some studies have focused on the endocytosis, docking, priming, fusion and recycling processes that may play a role in intellectual disability and ASD, but the functions of several vesicle components remain unidentified and more studies are needed to understand these processes [133].

6. Cytoskeletal dynamics

The cytoskeleton forms the backbone of neuronal architecture and is essential for axon growth and synapse formation. The microtubule cytoskeleton has an active role during different

phases of neuronal polarization; microtubules and their stability determine axon formation, they maintain the identity of axons and they regulate the dynamics of dendritic spines. Once the synapses have been formed, the neuronal cytoskeleton supports maturation and maintenance, and so the synaptic cytoskeleton is essential for the stabilization and remodeling of synaptic connections [40]. Actin filaments are the predominant component of the cytoskeleton in dendritic spines [20]. Changes in these key molecules mediating that bind to actin and members of the Rho family of small GTPases, such as RhoA, Rac and Cdc42 can disrupt this process [69]. These proteins play important roles in synaptic functions, dendritic branching, the formation and maintenance of dendritic spines and the growth and differentiation of neurites [133]. Their genes include *OPHN1, MEGAP, OCRL1, ARHGEF6, ARHGEF9, FGD1, LIMK1, PAK3*, and *IQSEC2* [133].

It is known that genetic alterations in the pathways controlling local protein synthesis in neurons contribute to diverse intellectual disabilities and ASD. A set of cytoskeletal proteins has been reported as mutated in these individuals. The resulting disorders are called synaptopathies with dysgenesis of dendritic spines being a recurrent anatomical feature. These include factors that regulate the dynamics of the actin cytoskeleton, such as GAPs and guanosine factors (Ba; van der Raadt, Nadif Kasri 2013). Mutations in the tumor suppressor genes, *TSC1* and *TSC2*, are also connected to the ASD mutant proteins that seem to disturb the dynamics of the cytoskeleton and the structure of dendritic spines [61]. Moreover, the microtubule-associated protein, *KATNAL2*, has emerged as a risk factor for ASD [105]. But the best example of dendritic spine defects is Fragile X syndrome. This disease results from a loss of function of the RNA-binding protein, the fragile X mental retardation protein (FMRP), which regulates dendritic targeting of mRNAs and controls protein synthesis and mRNA decay in neuronal soma and at synapses. High-throughput screenings have revealed that a wide array of neuronal mRNAs is targeted by FMRP, suggesting that simultaneous dysregulation of many proteins contributes to the syndrome, including cytoplasmic FMRP-interacting protein 1 (CYFIP1) [38].

New research has revealed many interactions associated with brain disorders, opening up new perspectives to define regulatory pathways shared by neurological disabilities characterized by dendritic spine dysmorphogenesis.

7. Translational regulation and the process of ubiquitination

It has been seen that many synaptic proteins are critical to the formation and maintenance of proper synaptic function. The expression level of many of these proteins may be tightly controlled by the balance between translation and turnover. The growing number of developmental cognitive diseases, whose underlying cause is a defect in the regulation of either translation or turnover, suggests that the equilibrium between these opposing processes is a sensitive point in establishing normal cognition and behavior [36].

Ubiquitination, the covalent attachment of ubiquitin to a target protein, regulates most cellular processes and is involved in several neurological disorders. Many genes in the ubiquitin

pathway and neuronal proteins that are targeted by the ubiquitin-proteasome system have been linked to cognitive deficits [57, 82].

Studies have highlighted an important role for protein degradation by the ubiquitin proteasome system (UPS) in synaptic plasticity [126, 91]. These observations suggest that changes in synaptic transmission involve extensive regulation of the synaptic proteome. The synaptic proteome is also affected by nonsense-mediated mRNA decay (NMD) that provides a quality control linked to translation. NMD has a role in degradation of aberrant mRNAs with a premature termination codon and the regulation of the transcriptome [109]. CNVs and mutations in several genes associated to NMD such as *UPF3B, UPF3A, SMG6, EIF4A3, RNPS1* and *RBM8A* have been identified as probable causes or predisposing factors for neurodevelopmental disorders such as autism [5, 76, 108].

UPS consists of a group of enzymes, an ubiquitin activating enzyme (E1), an ubiquitin conjugating enzyme (E2) and an ubiquitin ligase (E3), which are associated with ubiquitin ligases and proteasomes to mediate protein degradation. The ubiquitinated target protein is subsequently shuttled to a protease complex known as the 26S proteasome and subjected to degradative proteolysis. They also play a role in the regulation of cell signaling and cell cycle progression, and are associated with cytoskeletal elements. Thus, posttranslational ubiquitination modifies protein function and triggers the subsequent degradation of ubiquitinated proteins by the 26S proteasome. Several components of the UPS are required for proper brain development, axon guidance, and the development and plasticity of synapses. It has been shown that protein degradation via the UPS controls the appropriate synaptic balance, maintaining optimum levels of the protein, thereby promoting functional balance [23].

Several studies have shown a crucial role of UPS in neuronal transmission. For example, mutations in *UBE3A* have been associated with ASD. *UBE3A* encodes an ubiquitin E3 ligase that contains a domain that catalyzes the ubiquitination of target proteins. A reduction in density results in defects in synaptic plasticity [57]. UBE3A regulates the development of excitatory synapses by controlling the degradation of activity-regulated cytoskeletal protein (Arc or Arg3.1). Arc is critical for long-term memory formation and essentially every form of plasticity, including LTP, long-term depression (LTD), and homeostatic scaling (Greer et al., 2010). It has been shown that UBE3A-deficient mice, express high levels of Arc in response to synaptic activity, which coincides with severely impaired hippocampal LTP [78, 133]. Also, Arc regulates the ionotropic glutamate receptor (iGluRs) expression and trafficking. Findings from various experimental systems implicate iGluR dysfunction in ASD [148].

8. miRNAs

As previously reported, CNVs are recognized as important genetic factors in ASD, with a high prevalence of *de novo* CNVs in sporadic and familial cases compared with control subjects. However, studies conducted so far have highlighted a pathogenic role of CNVs in terms of changes in dosage of encoding protein genes without bearing in mind the potential involvement of non-encoded RNAs, particularly miRNAs, even with the inherent difficulties of this

type of study [120, 150]. Generally these two themes (CNV and miRNAs) are investigated separately.

A few studies have investigated the transcriptome in ASD samples of postmortem brains and some of them used mRNA from peripheral blood of patients [1, 54, 71, 110]. More recently, disruption of miRNA expression has been repeatedly reported in microarray studies and it is believed to be linked to the pathogenesis of autism (Sarachana et al., 2010; [28, 55]. However, lymphoblastoid cells are not representative of neural tissue and very few miRNAs exhibit consistent deregulation between studies.

According to [93], miRNA loci are underrepresented in highly polymorphic and well-validated CNV regions. One study investigated the pathogenic role of miRNAs in autism by checking associations with *de novo* CNVs. Twenty-four miRNA genes likely to play a pathogenic role in autism were identified on chromosomes 1, 2 and 22. Two, *mir-HSA-4436b-1 and 4436b-HSA-mir-2*, appear to be strong candidates. Unfortunately, the targets of these miRNAs were not identified [94]. The difference in penetrance of the deleted/duplicated genes may be explained by a variety of factors including: (i) pre-natal exposure to environmental risk factors; (ii) the presence/absence of functional SNPs in genes that encode proteins related to susceptibility for autism; (iii) epistasis; (iv) epigenetic factors and (v) the number and type of genes encoding proteins co-existing in different CNVs and overlapping in the same miRNA.

The *HEY1, SOX9*, *miR-486* and *miR-181b* are some candidate genes. All of these are involved in the development and function of the nervous system, and some, such as *HEY1*, are involved in Notch signaling networks [55]. However, a systematic analysis of CNV-miRNAs based on their interactions with target genes identified other miRNAs such as hsa-miR-590-3p, hsa-miR-944, miR-HSA-570, hsa-miR-34a, hsa-miR-124, hsa-miR-548f, hsa-miR-429, miR-HSA-200b, hsa-miR-195 and miR-497-HSA. Moreover, the miRNAs related with CNVs can explain the difference in levels of the important genes that are controlled by them. These CNV-miRNAs can also harm the overall biogenesis and processing of all miRNAs by targeting key molecules in the miRNA pathway [150].

On the other hand, dysfunction of neuronal miRNAs can result in a number of neuropathological conditions. It has been reported that neural miRNAs and their target mRNAs are co-expressed, suggesting their participation in feedback mechanisms to connect the transcriptional activation with the control of local dendritic protein synthesis [145]. Interestingly, the functions attributed to miRNAs overlap with growth abnormalities, delays and the disruption of neuronal maturation observed in the brains of autistic individuals. Aberrations in the translational control of multiple mRNAs mediated by targets of each miRNA may lead to the difference in phenotypes observed in ASD. Moreover, multiple miRNAs may target the same mRNA leading to phenotypes resulting from the converging of several loci in CNVs. Thus, a change in expression or level of miRNA will affect the expression of target genes and might have a pleiotropic effect that would produce a more severe autistic phenotype. However, one can not underestimate the clinical relevance of the deregulation of a single or a subset of CNV-miRNAs. Based on this, it is clear that the characterization of this relationship may illustrate the complexity of the underlying neuronal development, function and dysfunction that will eventually help in the understanding and treatment of autism [150].

9. Chromatin remodeling

Chromatin is defined simply and collectively as genomic DNA associated to proteins within the nucleus. There is a vast assortment of chromatin factors dedicated to the DNA packaging and the enzymatic functions involved in changing chromatin states. Nucleosomes are the primary unit of chromatin organization with a histone core (H2A/B, H3, and H4) and linking subunit H1. They keep DNA condensed and regulated by only releasing genes into the open conformation when their accessibility is needed (Lasalle, 2014). Interestingly, disruptions in chromatin regulator genes are frequently the cause of neurodevelopmental and neuropsychiatric disorders. Chromatin regulators are widely expressed in the brain, yet symptoms suggest that specific circuits are altered when they mutate [143]

Chromatin regulator genes are also altered by *de novo* mutations in a small proportion of ASD cases. They are involved in various cellular processes such as transcriptional regulation, cell cycle regulation, genomic stability and DNA damage repair, but it is still unclear how mutations in chromatin regulators lead to behavioral phenotypes. Following deletion models, it is suggested that mutations that affect chromatin regulators can lead to ASD because they are "global regulators" that are placed on top of a hierarchy of regulators. The regulators interact with many genes and pathways, and thus disruption can simultaneously affect multiple target genes. Furthermore, it is proposed that these genes may also act as phenotypic capacitors, protecting processes of genetic development and environmental perturbations [138].

Methylation of the genome in certain areas appears to remodel chromatin and consequently the genes that are in this region. The regulation of each histone modification requires specific enzymes that add or remove the methyl or acetyl group. Several mutated genes associated to ASD encode histone demethylases, including *KDM5C*, a demethylase of histone *H3K4* implicated in gene repression and *JMJD1C*, a demethylase of histone H3K9 implicated in hormone-dependent transcriptional activation. Furthermore other genes involved in specific chromatin remodeling, such as *SMARCC1*, *SMARCC2*, *ARID1A*, *ARID1B*, *CHD8*, *CHD1*, *CHD3*, *CHD75*, and *ATRX*, have been described as mutated in rare cases [66, 77, 105, 114, 111]Lassalle, 2013).

10. Epigenetic: genomic imprinting, epimutations, histone and DNA methylation

Epigenetic is a term used to refer to features of organisms, such as DNA and chromatin modifications, that do not involve changes in DNA sequence. The effects of environment on the phenotype are generally mediated through epigenetic mechanisms. These mechanisms, such as DNA methylation, can become programmed (e.g. imprinted). Genomic imprinting is a unique phenomenon wherein genes are expressed in a monoallelic way, and the choice of which allele is expressed is determined by the parental origin of the allele. Disruptions of the epigenome are called epimutations. Some of these appear to be corrected by normal germline-

specific epigenetic reprogramming and are therefore not transmitted transgenerationally, but others are not corrected and are transmitted over multiple subsequent generations [97].

Epigenetic mechanisms act on chromatin accessibility to transcriptional regulation. Then, they regulate DNA structure and gene expression that can be influenced by exposure to environmental factors. The most studied are the methylation of genes and modification of histones. Interestingly, epigenetic abnormalities are associated with several neurodevelopmental diseases. The connection between ASD and epigenetic comes from the identification of genetic mutations in imprinted regions and genes that control epigenetic processes. As cited before, among the most common chromosomal alterations in ASD are duplications of the imprinted region 15q11–13, which is maternally inheritable [96].

There are several possible explanations for the involvement of imprinted genes in autism. The imprinted brain theory of autism suggests that autism is a disorder of the extreme imprinted brain and would be caused by imbalances that involve increased effects of the 'paternal brain' relative to the 'maternal brain'. Imprinting has been hypothesized to explain the gender difference through the proposed action of unknown paternally imprinted loci on the X chromosome. Also, as mentioned, autism has been strongly associated with chromosomal abnormalities in the imprinted region of chromosome 15q. This includes the Angelman and Prader-Willi syndromes, as well the 15q duplication syndrome, which occurs in up to 5% of individuals with ASD. Imprinted genes may also contribute to autism indirectly as targets of other genes such a regulatory connection between *MECP2* (the gene associated with Rett syndrome) and the imprinted gene *UBE3A* (associated with Angelman syndrome). Besides this, imprinted genes are candidates for association with autism because of their functional haploid state. This feature may make them extremely vulnerable to rare mutations because the gene may be inactivated. Moreover, a single epigenetic change may lead to loss of imprinting, leading to biallelic expression and to gene dysregulation (Ben-David, Shohat, Shifman, 2014). Both genetic and environmental factors can affect the imprinting process and alter the level of expression of genes. But the full contribution of genomic imprinting to the risk for autism is still unclear.

The imprinted genes are expressed in several types of tissues but are highly expressed in the brain. Human neurons require extensive methyl modifications throughout development and postnatal life. Several important posttranslational modifications of histone core subunits within nucleosomes involve methylation, an epigenetic mechanism. Recent unbiased genome-wide analyses have turned up a multitude of novel candidate genes that encode nuclear factors implicated in chromatin remodeling, histone demethylation, histone variants, and the recognition of DNA methylation. Both histone and DNA methylation patterns are highly dynamic processes in the early development phase that correlate with dynamic changes in cell lineage and differentiation events. Interestingly, mutations in autism have been found in several genes encoding proteins involved in demethylase reactions, that is, reactions that remove methyl groups from histones or DNA (Lasalle, 2014).

The mechanism of action of the *SHANK3* gene (also known as ProSAP2) is an example of this phenomenon in ASD. The three members of the SHANK family, *SHANK1*, *SHANK2* and *SHANK3* are expressed in different regions of the brain. *SHANK3* is strongly reported to be

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involved in the etiology of autism since several mutations have been identified in a particular phenotypic group of patients. *SHANK3* regulates the structural organization of dendritic spines and is a binding partner of NLS. It codes a synaptic scaffolding protein enriched in PSD of excitatory synapses and plays important roles in the formation, maturation and maintenance of synapses. Haploinsufficiency of this gene is related to the 22q13.3 deletion syndrome (known as Phelan-McDermid syndrome), a developmental disorder which is characterized by severe language and speech delay, hypotonia, global developmental delay and autistic behavior. It is possible that loss of one copy of this gene makes the nervous system more vulnerable to degeneration in the long term and less able to recover after psychiatric and somatic events. Five CpG-islands have been identified in this gene, and tissue-specific expression is epigenetically regulated by DNA methylation. Much evidence in animal models has shown that *SHANK3* variants are expressed in the developing rodent brain with expression being regulated by DNA methylation of intragenic promoters [39, 147].

[75] reported that mothers of autistic children show significantly lower levels of methylfolate and methionine, two essential precursors for methylation in DNA compared to a control group, but methylation-inhibiting protein levels and S-adenosylmethionine (SAM), adenosine, and homocysteine were elevated. SAM has a role in the DNA methyltransferase reaction, which produces S-adenosylhomocysteine (SAH) and methylated DNA. The SAM/SAH ratio is considered to be an indicator of DNA methylation potential.

Additionally, oxidative stress in brain cells caused by environmental and genetic factors leads to decreased activity of the methionine synthase enzyme which participates in DNA methylation processes. When the activity of this enzyme is impaired, affected individuals can exhibit attention deficits and other signs, including autistic behavior symptoms due to defects in the expression of genes controlled by this epigenetic mechanism [42, 104]. Therefore, environmental factors may also activate intracellular pathways during embryonic development, causing epigenetic changes in neural function that would explain the relationship between environmental signals and the genome in the regulation of individual differences in behavior [166].

A study in Sweden with 208 autistic children showed an association between advanced paternal age and an increasing risk for ASD in offspring. Autistic-like traits in the normal population are associated to both young and advancing paternal age and the autistic similarity in twins seems to increase with advancing paternal age. Exposure to toxic agents during life, *de novo* mutations in germ lines and epigenetic alterations are correlated factors [72, 90]. Advancing age in mothers has also been reported as a risk factor for ASD [116].

Thus, epigenetic alterations may be the biological targets through which environmental factors can cause autism. Imprinted genes may be associated with autism because they are involved in brain development and also because they may be more vulnerable to genetic or epigenetic mutations. Some features of ASD are highly consistent with epigenetic dysregulation such as the discordance between monozygotic twins, parental origin and the gender-dependent effects of some alterations. The cause of autism is not just by congenital genetic defects but can also be caused by environmental factors via epigenetic factors, with epigenetic modifications being affected by environmental factors including fetal exposure to drugs.

11. Environmental factors

11.1. Toxicity during neurodevelopment

Although the involvement of genetic alterations in ASD is clearly accepted, new studies point to a similar or even greater contribution by environmental factors, particularly environmental toxicants. Toxic chemicals cause dysregulation of the developing human brain by interacting with the genome or through direct toxicity.

The developing human brain is exceptionally sensitive to injury caused by toxic chemical exposure with several developmental processes being highly vulnerable. The dissemination of chemical industrial agents in the environment is important contributor to the call "global silent pandemic of neurodevelopmental toxicity" [63]. An estimative by U.S. National Academy of Sciences (NAS) shows that 3% of all neurobehavioral disorders including ASD, attention-deficit hyperactivity disorder and dyslexia are caused directly by exposure to environmental toxics and that another 25% are caused by interactions between environmental factors and inherited susceptibility [103].

About 80,000 new synthetic chemicals have been developed in the past 50 years, but only about 20% was screened for potential toxicity in early neurodevelopment. The human fetus is susceptible and not well protected against industrial chemicals, because the placenta does not block the passage of all toxicants from the maternal to fetal circulation. The individual's brain is unique and individual variability in genetic susceptibility can influence responses to environmental toxicants [62, 79, 106].

Exposure to many environmental toxicants has been correlated to autism including pesticides, polychlorinated biphenyls, solvents, toluene, toxic waste, and heavy metals such as mercury, lead and arsenic, besides exposure to automotive air pollution [115, 132, 152]. On the other hand, there was a false indicative association between these disorders and vaccines such as the measles, mumps, rubella vaccine and thimerosal-containing vaccines against diphtheria, tetanus and pertussis vaccine. One meta-analysis, combining the results of five previous studies involving 1,256,407 children with five case-controlled studies with a further 9,920 children show that there was no relationship between vaccination and autism [144].

There is thus strong evidence that the relation between environmental and genetic factors may converge to neurotoxic mechanisms that could lead to behavior disorders. But what kind of biological mechanisms would be involved in the causation of autism due to toxic agents? Epigenetic mechanisms are known to affect the subsequent gene expression in the brain as has already been described in this chapter. Toxics can lead to a modification in epigenetic gene expression, resulting in methylation, histone modification or changes in non-protein-coding RNA [60]. However, possible individual susceptibilities to toxicants implicated in ASD, including altered detoxification, genetic factors, oxidative stress, altered neuronal development, epigenetic mechanisms, synaptic function, hormonal factors, etc., may amplify the effects of toxicants during the prenatal and early postnatal periods [123]. The susceptibility to effects of environmental toxicants needs more attention and studies.

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11.2. Oxidative stress

ASD also can be characterized by some physiological abnormalities, such as oxidative stress and immune dysregulation/inflammation. Many of the behavioral and cognitive features of ASD appear to arise from dysfunctions of the brain but studies have documented physiological abnormalities in organs other than the brain [122].

Many studies have reported genetic variations in glutathione-related pathways associated with autism, such as lower concentrations of reduced glutathione (GSH), higher levels of oxidized glutathione (GSSG) and a decrease in the GSH/GSSG redox ratio, along with a lower mitochondrial GSH reserve in individuals with ASD compared to controls [19, 2952, 65, 75 , 121]. Moreover, ASD severity has been correlated with lower GSH levels and markers of increased oxidative stress [2, 56, 123]. Oxidative stress studied in postmortem brain samples from individuals with ASD demonstrated low levels of GSH, the major cellular antioxidant, oxidative damage to proteins, lipids and deoxyribonucleic acid (DNA) as well as alterations in the activity of enzymes important for redox metabolism [123, 142].

Adaptive responses to oxidative stress were shown by [102], when significantly lower methionine synthase mRNA levels and lower homocysteine and cystathionine concentrations were observed in the frontal cortex of the brains of ten individuals with autism compared to ten controls.

Also it is possible that the reduced transportation of folate into the brain as a consequence of the folate receptor alpha autoantibody or mitochondrial dysfunction could reduce the methylation process and glutathione metabolism. Many findings reported for the brain (oxidative damage to lipids, protein and DNA, glutathione abnormalities and reduced function of enzymes essential in oxidative stress regulation) have been found in the blood, immune cells and cell lines from individuals with ASD, raising the question of whether these findings are specific to the brain or whether they represent a more general process (Frye et al., 2013; [123].

11.3. Immune system

Dysfunctional immune activity, both innate and adaptive, can impact neurodevelopment, cognitive function and behavior, suggesting a profound effect on neurodevelopment. Some studies in individuals with ASD have reported evidence of immune dysregulation and inflammation related to ASD, including disruption of genes of the immune system with elevations of tumor necrosis factor-alpha (TNF-α) in lymphocytes and amniotic fluid, alterations of immune proteins, and increased levels of cytokines, expression in genes related to immunity and microglial cell activation in brain tissue [113]. The expression of inflammatory genes in brain tissue was studied by [163], who reported that nuclear factor-kappa β expression in the orbitofrontal cortex was increased in individuals with autism compared to controls.

Microglias, a type of glial cell, are the resident macrophages of the brain and spinal cord. They therefore act as the first and main line of active immune defense of the CNS and participate in immune surveillance of the CNS and synaptic pruning in normal neurodevelopment. They are activated to eliminate some agents and damaged cells via phagocytosis, but, they may

increase inflammation if chronically activated by releasing proinflammatory cytokines and free radicals [41]. Changes in microglial morphology and gene expression are seen in many psychiatric disorders and neurodegenerative diseases, such as Alzheimer's, Parkinson's, Multiple Sclerosis and autism. [100] and [165] reported that microglias in the dorsolateral prefrontal cortex were frequently located closer to neurons in ASD individuals compared to controls. Another study with autism showed microglial activation in the cerebellum, brainstem, corpus callosum, fusiform gyri, superior temporal gyri, anterior cingulate, orbitofrontal, and parietal lobes [139].

Proteomic analysis indicates that the levels of many immune proteins in plasma, such as cytokines, chemokines, complement proteins, adhesion molecules and growth factors are altered in ASD [113]. Increases in plasma levels of pro-inflammatory cytokines [interleukin (IL)-1β, IL-6, IL-8 and IL-12p40] as well as macrophage migration inhibitory factor (MIF) and platelet-derived growth factor (PDGF), have been reported in ASD [11, 64].

Alterations in immune mediators can occur at an early stage of development and might alter NMDA and NMD receptor-mediated excitatory synaptic transmission and plasticity, which is relevant to ASD (Escobar et al., 2011). These immune mediators may be related in LTP which is involved in synaptic plasticity in learning and memory processes. IL-1 influences many molecular components of LTP, such as NMDA and AMPA receptor signaling and glutamate release. IL-6 is involved in the generation of LTP and an increase in IL-6 leads to a reduction of insulin-like growth factor (IGF)-binding protein 3 and IGF1 [43, 117]. Furthermore, other cytokines, such as IL-18, IL-1b, and TNF-α, act on AMPA and NMDA receptors in different ways [25].

Increases of pro-inflammatory cytokines (including TNF-α, IL-6 and granulocyte-macrophage colony-stimulating factor), a T helper cell type 1 cytokine (interferon gamma) and a chemokine (IL-8) were reported in postmortem frontal cortex brain samples of autistic individuals, but no significant differences in T helper cell type 2 cytokines were identified (IL-4, IL-5, and IL-10) [86]. The cytokines IL-2 and IL-4 have been shown to influence repetitive and cognitive behaviors. Mice treated with IL-2 showed an increased "climbing behavior" that is thought to denote repetitive behavior, a pattern of behavior that is characteristic of ASD.

There is evidence suggesting a role of immune dysfunction in ASD symptoms, but further investigations should be carried out to clarify these findings because it is still unknown whether these changes are a primary cause or a secondary consequence of neuronal deficits.

11.4. Microbiota and autism

Gastrointestinal (GI) disorders are a comorbidity of autism and interestingly have a strong correlation with disease severity [7, 16]. Thus, a growing number of papers point to the importance of the so-called "brain-gut axis", revealing the central role of the intestinal microbiota (the new name of "microflora") in postnatal development and maturation of the immune and endocrine systems for control signaling in the CNS, brain function, and behavior [51; 158].

Individuals with ASD often suffer from GI illnesses, e.g., diarrhea, constipation, bloating, and gastroesophageal reflux [22, 3]. The composition of the microbiota is influenced primarily by

genetic factors, age and diet [80, 168], although the interactions between these are multifactorial and not well defined yet [21].

Thus, several studies have been conducted in an attempt to profile the microbiota in ASD compared with healthy siblings and controls [4, 51, 59]. While some authors have reported little or no differences in the composition of the intestinal flora between children with ASD and their unaffected siblings, the imbalance in the intestinal microbial composition in samples of ASD has been identified, especially when compared to controls [49, 50].

Analyses of the feces of patients with ASD have revealed that the microbiome of ASD is significantly different to controls and consists in over 1,000 different species compared to unaffected children. Of the phyla, patients with autism have underrepresented *Firmicutes* and actinobacteria, especially bifidobacteria, and over-represented *Bacteroidetes* and proteobacteria compared to control individuals [49, 51, 119].

Interestingly, non-autistic siblings often presented intermediate microbiota profiles between ASD and controls [49, 155], possibly due to genetic factors, but also as a reflection of shared environmental conditions. These studies are complicated by the fact that autistic individuals often receive medications such as antibiotics and are often on special diets or have repetitive eating habits, both of which can alter the composition of the microbiota [89].

Interestingly, although *Bacteroidetes* are over-represented in ASD feces, the presence of the genus *Prevotella* and other fermenters, albeit in lesser amounts, has been described in the gut of children with ASD. *Prevotella* do not only have the ability to synthesize vitamin B1, which attenuates the ASD symptoms, but it is also considered a central niche to maintain the structure of the intestinal microbial community of healthy humans [10, 13, 162]. Moreover, *Propionibacterium* and *Clostridium* that are over-represented in the intestine of ASD, produce propionic acid, a short-chain fatty acid capable of passing the gut-blood-brain barrier thereby changing neurophysiological processes by binding to acetyl-CoA and acetyl-carnitine which are involved in mitochondrial lipid transport. Recent experiments have shown that administration of propionic acid in young mice causes mental retardation with cognitive disabilities, innate neuroinflammatory response and restricted, repetitive behavioral, symptoms consistent with autism [53, 92]. Propionic acid induces behavioral and functional alterations in the brain of animals. Most likely, the presence of this acid alters mitochondrial function. Taken together, these reports support a view that the metabolism of proteins and the host/pathogen relationship is altered in patients with autism [99].

Few studies have considered a deregulated metabolism of fecal levels free of amino acids in autism. Certain amino acids, in particular glutamic acid (Glu), act as neurotransmitters in the center of the CNS. An excess of Glu leads to neuronal cell death and plays an important role in the pathophysiology of several neuropsychiatric disorders [127]. High levels of Glu were found in fecal samples of autistic children [37]. As it has a role in brain development, the findings support the hypothesis that glutamatergic neurotransmission is involved in ASD [130].

The microbiota might also be involved in the etiology of the disease via interactions with the immune system. Some of the possible mechanisms described above are likely to involve

changes in the global balance of the entire microbial community, while others may be exercised by certain bacteria. More studies are needed to clarify whether the gut microbiota in fact plays a role in ASD. These include prospective studies to address the issue of cause and consequence, and intervention studies aimed at modulating the microbiota with probiotics or dietary interventions. The right profile, the categorization of patients and control groups, with the application of molecular techniques to verify the profile of fecal microbiota and urinary metabolome will corroborate the underlying relationship between gut microbes and the host [34].

Some authors believe that the action of microbiota may have much wider effects on the physiology of the host than originally thought, and emerging evidence shows that it may include modulation of brain activity and behavior. Differences in the composition of the microbiota between individuals with ASD and healthy controls were identified in several studies, both based on bacterial cultures and on molecular methods [18]. However, changes in bacterial diversity that were reported in one study [51] were not confirmed by another [158]. The direct comparison between studies is complicated because different methodologies are employed and study groups may not be directly comparable due to the heterogeneous nature of ASD [89]. However, if the differences in gut microbiota between autistic children and controls are one of the causes of the disorder or the result, could have implications on the diagnosis, treatment and prevention [37].

Nevertheless, despite the substantial amount of data, it is not possible to clarify whether the results represent the presence of a causative agent, or reflect a consequence of treatment, or whether they are nothing more than confounders. This has to be considered and caution is needed before recommending "miracle diets" to the child and family that may only increase the anxiety in respect to symptom improvement.

12. Etiology and mechanisms of sexual differences

It is well known that ASD affects more males at a ratio of 4 boys:1 girl. Some studies suggest that gender differences in phenotypic presentation, including less severe manifestations of restricted and repetitive behaviors in girls, seem to affect this ratio. Genetic studies show that girls seem to be more "protected" from the effects of inherited and *de novo* variants that cause ASD than boys. This suggests that genes that participate in sexual development and/or sex hormones, particularly testosterone, may modulate the effects of genetic variations in the autistic phenotype [157].

Despite the fact that ASD is more prevalent in boys *de novo* mutations are also more frequent in girls with ASD. The female protection theory fits in with the model of loss of robustness. According to this model, besides the variations between individuals, there is a difference in the average degree of robustness of the brain between males and females. According to this hypothesis the development of the female brain is more robust, and this may explain the higher rate of severity of behaviors when *de novo* mutations are present in girls with ASD. The brain can also be more vulnerable compared to other organs because it is a complex organ that

develops later in life and is mainly composed of terminally differentiated cells. In the first year of life, the brain may be particularly sensitive to reduced robustness because social development depends on signals from the environment [138].

Although neurodevelopmental systems evolved to be robust, they can be vulnerable to disturbances in a specific subset of genes called phenotypic capacitors [85]. Phenotypic capacitors are genes that act against disturbances and thus contribute to the robustness of the phenotype. Thus, phenotypic capacitors, when operating normally, can prevent the development of diseases such as ASD, even in patients exposed to genetic and environmental risks. This means that girls are less likely to develop ASD, but when they have, they tend to have a more severe phenotype [138].

There seems to be unknown factors that "protect" females from ASD [157]. Recent genome studies showed that, on average, women with ASD have more mutations than men, including single nucleotide variants (SNVs) and CNVs [74, 84, 105]. Furthermore, *de novo* CNVs in females were larger and included significantly more genes than males [84].

Moreover, not all genes in the inactive X chromosome are inactivated, and the genes that escape X inactivation in females are revealing some interesting insights into gender differences related to chromatin. *KDM5C* and *JARID1C* are genes that escape X chromosome inactivation (Xu, Deng, Disteche, 2008). In addition, the gene encoding O-linked N-acetylglucosamine (O-GlcNAc) transferase (*OGT*) which regulates chromatin remodeling factors is less expressed in males than in females and the expression is reduced further by prenatal stress [70]. These sexual and epigenetic differences must be investigated further in respect to the protective effect of the female gender in autism [81].

Recently, gender-specific gene expression obtained from the transcriptome of normal human brain development using a bioinformatics approach suggested that male-biased genes are enriched for the processes of extracellular matrix formation/glycoproteins, immune response, chromatin, and cell cytoskeleton. These pathways have been repeatedly implicated in autism and demonstrate that autism candidate genes are also enriched for these pathways. Furthermore, the development of the male brain may be naturally more susceptible to environmental factors as its normal development is more strictly dependent on the immune system [167].

A gender-dependent difference in the incidence of neural tube defects has been described. It was speculated that these defects are due to a synergistic effect between the gene expression of *SOX9* and *Barx1*. *SOX9* is an essential transcription factor for skeletal development, but it is also involved in the development of the male phenotype (Barriounuevo & Scherer, 2010), thus contributing to the increased risk of autism in males. Interestingly, a study showed up-regulation of *SOX9* in autism [55].

On the other hand, altered behaviors in ASD are often related to a “more masculine” pattern of behavior linked to testosterone. However, although current levels of steroid hormones appear to be altered in patients with autism, the data indicate that prenatal testosterone by itself does not seem to be sufficient for the disorder to develop [125].

Differences between individuals with an atypical karyotype (monosomy X or Y or X-polysomy) and those with a typical karyotype are often interpreted as being significant for the

difference in susceptibility between genders. However, it is likely that many of the differences arise because of hyperexpression or hypoexpression of genes in pseudo autosomal regions that escape X inactivation, thereby being similarly expressed in men and women with a typical karyotype. The addition or loss of an X or Y chromosome, which leads to altered protein levels that are atypical or typical for both men and women, cannot explain the gender difference in ASD. However, some genes on the X chromosome are not in these regions and have no corresponding functional alleles on the Y chromosome, and escape X inactivation in some circumstances [125].

Moreover, the *SRY* gene is mapped in a region on the Y chromosome and can also directly regulate the gene monoamine oxidase A (MAOA) that is located in the Xp11.3 region and encodes a key enzyme in the breakdown of catecholamines and other monoamines. SRY can directly affect transcription in the brain [159]. As individuals diagnosed with ASD are found with changes in catecholamine and metabolite levels in the dependent activity MAO-A and autism severity is associated with child and maternal MAO-A genotypes, the disruption of the synthesis of catecholamines may be modulated by the gender-specific *SRY* gene associated with ASD. Several genes on the Y chromosome are expressed in the brain. Since this leads to a specific expression of gender in the brain, these genes, some of which play a role in catecholaminergic functions, are candidate genes for the increased susceptibility to ASD in males. An investigation of the role of these genes will possibly clarify the gender-specific mechanisms underlying ASD and thus help in the understanding of the etiology of ASD [31, 125].

Another factor that may contribute to the skewed gender ratio in ASD is parental age. Increased parental age is known to increase the risk of a child with ASD [45] and there are some indications that parental age affects the gender ratio of children diagnosed with ASD [125]. The male-female ratio dropped from 6.2:1 in under 30-year-old parents to 1.2:1 in parents older than 44 years old [8]. This finding may be related to the higher frequency of *de novo* mutations which are more common in older men and seem to play a minor role in the gender bias in the incidence of familial ASD. However, it is still not clear whether this effect is true for simplex families, which are most representative of ASD in the general population [125].

13. Conclusion

Thus, considering all that has been studied about ASD, it is difficult to conduct a comprehensive analysis about the etiologic complexity, without running the risk of over or under estimating some factors. On consulting the available literature on ASD, there is an impression that all can cause autism. The truth is that everything that affects the CNS system can interfere with mental health and this opens up a universe of possibilities. The gene expression, immune susceptibility and environmental stressors, even those that affect men more than women, need to be organized using a multidisciplinary approach in order to explain what actually happens in normal CNS development within the first three years of life. From there, the goal is to have a method of comprehensively analyzing each particular case to try to obtain specific treatment. While this is not available, it is interesting to investigate the most common risk factors and give support to the patients and families in order to improve their quality of life.

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