Abstract: Autism spectrum disorders (ASD) are a group of neurodevelopmental diseases. The cause of ASD is unknown, but several genetic and non-genetic risk factors have been characterized that, alone or in combination, are implicated in the development of ASD. Currently, no diagnostic biomarkers are available, and the diagnosis of ASD is based on typical features that include repetitive behaviors, and impaired social communication and interaction. Several pathomechanisms such as alterations in brain development and function, and synaptic defects have been proposed to contribute to these behaviors. In addition, processes outside the central nervous system may contribute to, or modify, the clinical phenotype and severity. This chapter summarizes the clinical features of ASD, highlights the important genetic and non-genetic risk factors for ASD, and introduces the current knowledge around the pathological processes within and outside the brain.

Keywords: autism spectrum disorders; etiology; genes; incidence; prevalence; symptoms

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INTRODUCTION

While “autism” as a term was initially introduced to describe a behavioral symptom of self-withdrawal in schizophrenic patients, psychiatrists Dr. Leo Kanner and pediatrician Dr. Hans Asperger adopted the term autism in the 1940s to introduce a syndrome in children with behavioral differences in social interaction and communication, and restrictive and repetitive interests (1, 2). Nowadays, the umbrella term “autism spectrum disorders” (ASD) is used to describe a clinically heterogeneous group of neurodevelopmental disorders that share common behavioral core features affecting social communication and include restrictive and repetitive stereotypic behavioral patterns and interests. The term incorporates several conditions such as idiopathic forms, including autism, Asperger syndrome and Pervasive Developmental Disorder - Not Otherwise Specified, and Childhood Disintegrative Disorder and certain genetic disorders like Rett syndrome, which can exhibit autistic traits (3). With the establishment of the DSM-V (diagnostic and statistical manual of mental disorders, fifth edition), these formerly separate diagnoses were brought under one unifying umbrella as ASD (3) (see chapter 2). Thus, spectrum disorder classification is reflective of the heterogeneous nature of ASD.

CLINICAL FEATURES

The clinical features historically associated with ASD are the triad of impairments in speech, social interaction, and the presence of repetitive or restricted behaviors. However, ASD are also associated with many co-morbidities, which can be psychological and physiological.

ASD typically start to manifest in childhood and is diagnosed by age 3 in most cases. Early indicators of ASD in childhood (before age 3) include lack of response to one's name and discomfort with maintaining eye contact (4). Most symptoms of ASD persist into adulthood, especially with regards to social functioning and cognitive ability. Symptoms, particularly communication skills, can improve over time as the individual reaches adolescence and adulthood. Intellectual functioning and IQ tend not to change over time. While some features of ASD remain relatively stable, the quality of life of an individual with ASD can be improved by creating a strong social support system or other accommodations and interventions (5).

The clinical diagnosis of ASD is based on the presence of some key features, such as impaired social development, and repetitive behaviors and interests (see chapter 2). Delay in speech development, learning impairment, and difficulties with social interaction are common in ASD. Executive function and organizational skills are notably impacted in individuals with ASD. They commonly tend not to initiate interactions with their environment, particularly social interactions. They also often suffer from difficulties with processing stimuli and planning out the steps of an activity and may display ritualized or rigid behavior patterns. Thus, difficulty with performing independent behaviors and initiating tasks are notable features of ASD. Another area often impacted in ASD is the capacity to generalize skills across different settings. Individuals tend to have difficulty adapting to new environments, persons, and materials, likely due to highly specific stimuli.
The third core feature of ASD is the presence of repetitive or restricted behaviors. These can be stereotyped actions such as hand flapping and rocking of the body, and verbal behaviors such as repetitive words and phrases. Individuals with ASD also tend to have specific or restricted interests. For example, intense interest in a certain topic, sensory fixations on a particular object, or adherence to a specific routine or method of doing a task (6).

Secondary symptoms of ASD include aggression, hyperactivity, impulsivity, and the occurrence of co-morbidity such as anxiety and depression. A major feature of ASD is the heterogeneity of its clinical features. A diversity of symptoms along with many psychological and physiological comorbidities may be present. Figure 1 illustrates the diversity of these symptoms and comorbidities.

Psychological comorbidities include attention-deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), anxiety, and intellectual disability. There is a notable co-occurrence of ADHD with ASD, and the two conditions share many neurological and behavioral similarities. For example, hyperactivity and social deficits are found in both ASD and ADHD. About 30–50% of persons diagnosed with ASD show symptoms of ADHD as well. The 5th edition of the Diagnostic and Statistical Manual of Mental Disorders takes this into account and now allows for a dual diagnosis of ADHD and autism (7). Conditions such as anxiety and depression often co-occur because of the challenges persons with ASD face in day-to-day life, such as social isolation and difficulty in communication. However, the co-occurrence may also be based on overlapping biological processes disturbed in these disorders. Schizophrenia is also thought to be linked with ASD, and ASD were once considered a childhood form of schizophrenia. The two diseases appear to share some similar features regarding behavior and brain biochemistry, and certain risk factors. Other abnormalities, including aggression and self-harming behaviors, may also be observed in the individual.

Physiological comorbidities of ASD include epilepsy, sleep disorders, and gastrointestinal (GI) problems. Epilepsy and ASD co-occur at a relatively high rate for reasons that are not fully clear, though they are likely due to similar risk factors (for example, prenatal environment) and brain pathologies such as synaptic defects. Interestingly, ASD individuals with intellectual disability are more likely to develop epilepsy than those without intellectual disability (8).

Immune system dysfunction is another comorbidity of ASD with reports showing increases in cytokine levels and inflammation (9). An association between ASD and some autoimmune disorders has long been recognized in scientific literature. Besides, the transfer of maternal autoantibodies to the developing fetal brain, thereby affecting neurodevelopment, was raised as possible contributing factor to the pathogenesis of ASD. In addition, maternal immune activation seems to be a significant risk factor for ASD (10).

The presence of a comorbidity is frequently correlated with more severe autism core symptoms, which, in general, vary from profound, where an autistic person may be non-verbal and unable to function without considerable support, to relatively “high-functioning.” Asperger’s syndrome was previously considered a high-functioning form of autism, although this is not believed to be the case now. DSM-V lists Asperger’s syndrome under the broad category of ASD. Symptoms of Asperger’s tend to be less severe than typical autism, and Asperger’s syndrome may manifest later, with one study reporting average age of diagnosis as seven years (11). Individuals with Asperger’s usually do not experience a delay in speech.
The three core features of ASD are deficits in social communication, restricted and fixed interests together with speech deficits, and language delays. The first two features are used to diagnose ASD according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. In addition, individuals with ASD may present further symptoms and comorbidities such as cognitive deficits (intellectual disability), anxiety, depression, attention deficits and hyperactivity, impulsivity, seizures, gastrointestinal problems, sensory dysfunction, aggression, metabolic disorders, sleep disorders, motor dysfunction, and altered immune responses. However, not all symptoms and comorbidities are present in individuals with ASD, and those that are, vary in severity. This results in a considerable heterogeneity of clinical features of individuals with ASD.

Figure 1. Clinical features of ASD. The three core features of ASD are deficits in social communication, restricted and fixed interests together with speech deficits, and language delays. The first two features are used to diagnose ASD according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. In addition, individuals with ASD may present further symptoms and comorbidities such as cognitive deficits (intellectual disability), anxiety, depression, attention deficits and hyperactivity, impulsivity, seizures, gastrointestinal problems, sensory dysfunction, aggression, metabolic disorders, sleep disorders, motor dysfunction, and altered immune responses. However, not all symptoms and comorbidities are present in individuals with ASD, and those that are, vary in severity. This results in a considerable heterogeneity of clinical features of individuals with ASD.

development and have average to above-average intelligence. However, symptoms such as significant impairment in social interaction and restricted behaviors and interests are shared with other ASD (12).

Taken together, there is considerable variability and heterogeneity in ASD regarding its clinical features and co-occurring conditions. These comorbidities can affect different areas of functioning and body systems, ranging from physiological to psychiatric.
ETIOLOGY

The etiology of ASD is likely to be multifactorial, with both genetic and non-genetic factors playing a role (Figure 2). ASD can be syndromic or non-syndromic. Syndromic ASD is often associated with chromosomal abnormalities or monogenic alterations. Such examples include Rett syndrome, fragile X syndrome, and MECP2 duplication syndrome (13). Contrary to syndromic ASD, the etiology of non-syndromic ASD is still relatively undefined due to its genetic heterogeneity. A collaboration of de novo mutations and prenatal plus postnatal environmental factors are likely to play a role.

Distribution

According to the Centre for Disease Control and Prevention Agency, USA, figures in the United States show that 1 in 54 children are currently identified with ASD. According to a study undertaken during 2009–2017, approximately 17% of children between the ages of 3–17 have an established developmental disability, such as autism and attention-deficit disorder (14). European studies have had fluctuating results throughout the years due to alterations in diagnostic criteria. A large study carried out titled “Autism Spectrum Disorders in Europe” included 14 countries. Amongst them were Spain, Italy, Ireland, France, Belgium, and Poland. The priority was to estimate the prevalence of ASD in children ranging from 7–9 years in 2015 using nationwide registries and regional statistics. Results varied dramatically with a prevalence of 4.76/1,000 in South-East France and 31.3/1000 in Iceland. Ad hoc analyses with shared protocols were carried out in multiple countries, including Ireland and Italy. As of now, only the results gathered from Italy have been published, estimating an ASD prevalence of 7.99/1,000 (15). Furthermore, a meta-analysis focused on the prevalence of ASD in the South, East, and Western parts of Asia has identified East Asia (including South Korea and China) with the highest prevalence with 51/1000, followed by West Asia with 3.5/1000 and South Asia with 3.1/1000 (16).

However, the prevalence of ASD is still not well defined, with multiple studies across the globe identifying significantly higher figures in different countries and continents. Additionally, case figures in low-income countries are not definitive due to a lack of assessment and diagnostic tools. The latest statistics from 2020 suggest Hong Kong, South Korea, United States, Japan, and Ireland hold the highest prevalence of cases, whereby 372 in 10,000 children in Hong Kong have an ASD (17). An increase in ASD diagnoses has been reported in several countries over the last decade. For example, studies on autism carried out in the 1960s throughout Europe and the United States portrayed ASD as a rare disorder with the prevalence ranging from 2 to 4 children out of 10,000. While this may be linked to increased awareness and a change in diagnostic criteria, the contribution of environmental factors, although not well characterized, cannot be excluded.

Further to this, a gender difference was observed, with boys more likely to have a form of autism than girls. Reports suggest ASD is four times more frequent in boys than in girls (18). However, current studies suggest that this ratio is at least partly due to underdiagnosis of females with ASD, especially at young age. In line with this, the average age of diagnosis is higher in females.
Figure 2. Model for the etiology of ASD. A. ASD are caused by genetic or environmental factors or a combination of these. For some individuals, ASD is solely caused by the presence of the genetic mutation(s) (red line). Exposure to a certain environment may cause ASD independent of the genetic background in some individuals (green line). In most cases, the genetic setup of an individual will, in combination with the exposure to environmental factors, determine the risk for ASD. For example, while a person with genome A may be at low risk in environment A, the same person may have high risk in environment E. B. Each genome and each environment is a combination of many genetic factors (upper panel) and non-genetic factors (lower panel), respectively. Whether a genome confers a high risk of developing ASD may be determined by a single mutation, i.e., as seen in many syndromic forms of ASD (red line), or a combination of gene variants, where ASD-associated variants may act in a simple additive way (green line) or are in a more complex relationship. For example, the ASD-associated gene 3 may result in low risk if in combination with gene 6, but the risk increases in the presence of gene 5. Thus, multiple variants will increase ASD risk, but specific gene variants may also decrease the overall risk. Similarly, the presence of one particular environmental factor may be such a strong trigger that it acts independently of other environmental factors (red line). In many cases, however, a combination of environmental factors will determine the overall risk contributed through non-genetic factors in an additive or more complex relationship. C. In case the risk of developing ASD surpasses a threshold, the genetic factors, environmental factors, and their interplay will determine the severity of ASD symptoms and the presence and severity of comorbidities. This results in considerable heterogeneity among ASD.
Gender differences in the presentation of ASD have been heavily studied with few significant results, although some comorbidities such as depression, sensory issues and seizures may be more frequent in females, further obscuring ASD diagnosis (19).

**Genetic factors**

ASD is considered a complex genetic disorder with high heritability. Epidemiological twin studies support the strong genetic component of ASD. The concordance rate for identical twins is 70–90% and fraternal twins is 0–10% (20). In families with existing cases of ASD, familial clustering can be observed. Younger siblings of family members with an ASD diagnosis face an increased risk for ASD, even more so for younger male siblings (21, 22). In 20–25% of children or adults with ASD, genetic causes can be identified in the form of de novo mutations, common and rare genetic variations, and ASD-associated common polymorphisms (20, 23, 24). Overall, the SFARI (Simons Foundation Autism Research Initiative) gene database, a database of autism candidate genes, lists about 1000 genes associated with ASD.

Genes entered into the database are scored based on their strength of association with ASD risk, and are sorted into four different categories: S syndromic, category 1, category 2, and category 3 (25, 26). The 126 genes currently listed within category S include mutations that pose a substantial risk for ASD and additional characteristics outside of the requirements of present symptoms for an ASD diagnosis. Genes found in category 1 have a high confidence rate for their role in ASD. Their implication in ASD is based on the presence of at least three de novo mutation according to literature, inclusion in the SPARK list (27), and for most a false discovery rate threshold under 0.1. To date, 207 genes are included in category 1. The 211 genes ranked in category 2 are implied to be strong candidates for an association with ASD. To be scored as a strong candidate ASD gene, at least two de novo mutations have to be reported in the literature. The gene of interest should have a genome-wide significance threshold (a threshold that determines the statistical significance of a reported association between a common variant and a given trait), of $p \leq 5 \times 10^{-8}$, or evidence of a functional effect of the mutation in genome-wide association study (GWAS). Category 3 includes the largest number of genes, 506, ranked as suggestive candidates associated with ASD. For these genes, only a single de novo mutation has been reported, or the gene has been implicated in a GWAS that has not been replicated to date.

**Environmental factors**

Non-genetic factors mediating ASD risk could include parental age, maternal nutritional and metabolic status, infection during pregnancy, prenatal stress, and exposure to certain toxins, heavy metals, or drugs. Parental age may be a contributor of genetic mutations. Increased paternal age has been shown to increase the risk of ASD in offspring (28); however, some studies have rejected this hypothesis (29). Maternal nutritional status throughout pregnancy is a critical component for normal brain development. An excess, or a deficit, of micronutrients such...
as folic acid, zinc, iron, vitamin D, and omega-3 may lead to impaired neurodevelopment. Comparative studies have found a higher risk of ASD in children who develop under folic acid deficient conditions (30). However, an excess of folic acid also leads to neurocognitive impairments. Trace metal dyshomeostasis has also been shown to affect brain development and is linked to ASD (31). In particular, the accumulation of toxic metals such as mercury and lead, and the lack of the essential metal zinc during pregnancy has been linked to ASD in epidemiological and animal studies.

Another non-genetic factor increasing the risk for ASD is maternal infection (32). Following the association between congenital rubella infections and ASD development, the role of immune activation and infections has been under constant review. However, the focus has shifted to the maternal immune response an infection may provoke, including inflammation and cytokine activation. Maternal immune activation studies are currently carried out on animal models such as mice and rhesus macaques. Several hypotheses have been proposed whereby maternal cytokines may shift to the placenta leading to inflammation and excess cytokine production in the fetus and may result in gene dysregulation. Maternal antibody exposure studies on rhesus macaques have corroborated a common endophenotype of ASD through maternal immune activation, leading to increased brain growth and total cerebral volume in the offspring (33). Studies show that inflammatory abnormalities are not restricted to gestation but persist outside of the womb into adulthood, with multiple studies reporting abnormal adaptive and innate immune function in individuals with ASD. For instance, elevated chemokine and cytokine levels have been detected in the central nervous system and peripheral blood (34–36). Activation of astroglia and microglia cells alongside increased proinflammatory cytokine levels throughout life suggests a neuroinflammatory phenotype in ASD that might play a significant role in the induction of ASD behavior (36).

Maternal medicinal use during pregnancy, especially those used to counteract epilepsy and depression, has been correlated with the risk of ASD. Studies have implicated maternal valproate use with several neurodevelopmental alterations in the offspring, including ASD, though indications suggest it is dose related. Antidepressant use during pregnancy, including selective serotonin reuptake inhibitors, can increase the risk of ASD development; however, conflicting results have also been found (37).

While most of the data on paternal factors are based on epidemiological studies, maternal immune activation and prenatal zinc deficiency have been modeled in animals and identified as potentially causative factors, and the underlying mechanisms have been investigated (38). Additionally, various environmental factors increase the risk of co-morbidities, for example, gastrointestinal disorders, epilepsy, and obesity (30).

**PATHOLOGY OF ASD**

For many years, brain development and function have been the focus of research in ASD. Experimental and postmortem studies have identified central nervous system (CNS) pathologies at gross morphological level and cellular level, for
example, in neurons and glial cells. From these studies, it can be concluded that
neuropathologies are evident in ASD. However, research in recent years on
immune responses and gut-brain signaling (38) have revealed that pathologies in
ASD also exist outside the CNS.

**Neuropathology**

Several studies have reported abnormalities such as increased head circumfer-
ences and intracranial volumes in children aged 1–4, who were later diagnosed
with ASD (39). A meta-analysis of volumetric studies investigating the brain
structure of young individuals with ASD found alterations in the lateral occipi-
tal lobe, the pericentral region, the medial temporal lobe, the basal ganglia,
and proximate to the right parietal operculum (40); however, in older indi-
viduals with ASD, the previously reported anatomical abnormalities such as
larger intracranial volumes, smaller cerebellar volumes, larger amygdala vol-
umes, or altered volumes of the corpus callosum and hippocampus were not
confirmed using large sample sizes obtained from the Autism Brain Imaging
Data Exchange (ABIDE) database (39). Thus, it has been proposed that early
brain overgrowth in ASD is followed by a phase of growth arrest during devel-
opment or even degeneration (41). In addition, the reported gross anatomical
abnormalities cannot be directly linked to clinical phenotypes of individuals
with ASD.

Brain connectivity was reported to be different in individuals with ASD. Lower
connectivity between distal brain regions and increased connectivity within prox-
imal brain regions has been found. However, there is conflicting data, and several
studies could not confirm this finding (42).

Abnormalities have also been reported in the cytoarchitecture of the brain of
individuals with ASD. For example, a decreased number of cerebellar Purkinje
cells was found in ASD brains (43). However, the clearest neuropathology of ASD
is synaptic dysfunction. Many of the 207 SFARI genes associated with high risk
(syndromic and category 1) for ASD encode for proteins that play a crucial role in
synaptic function in the brain. Widely recognized and well-published high-risk
candidates for ASD susceptibility include members of the **SHANK** (SH3 and mul-
tiple ankyrin repeat domains) family of postsynaptic scaffolding proteins (i.e.,
SHANK2/3) (43–46), the cell adhesion family of neurexins (i.e., NRXN1) (47–49),
and neuroligins (i.e., NLGN2, NLGN4X) (50–53). Based on in vitro and in vivo
models with genetic manipulations, numerous ASD-associated genes were found
to be involved in pathways responsible for protein synthesis and degradation,
chromatin remodeling, and synaptic function, ultimately converging in their role
in synaptic homeostasis and synaptic plasticity (54–56). Therefore, ASD is also
classified as a synaptopathy (57). At synapses, two signaling pathways seem to be
critical to the ASD pathology. The mTOR/PI3K pathway that is particularly associ-
ated with syndromic ASD and the NRXN-NLGN-SHANK pathway (58, 59).
Together, these pathways are key regulators of synaptogenesis. However, their
presence is vastly limited to excitatory synapses, which ultimately may result in an
imbalance between excitatory and inhibitory transmission. It has been speculated
that many genetic and non-genetic risk factors for ASD mechanistically converge
at synaptic level (60).
Extracerebral pathology

Beyond the brain, in recent years, researchers started to investigate the role of other organ systems altered in ASD. One system that has been heavily implicated in ASD is the GI system, with many individuals with ASD reporting GI dysfunction. Reports about the prevalence of GI disturbances within the autistic population vary between studies, with estimates ranging between 20–86% (61). Besides GI dysfunction being more prevalent in individuals with ASD than in non-autistic individuals, the severity of GI abnormalities seems to be correlated with the severity of ASD (62), pointing towards a potential role of the GI system as modifier of ASD behavior and a factor in ASD etiology (63). GI symptoms like abdominal pain, bloating, diarrhea, constipation, or gastroesophageal reflux are the most commonly reported GI problems (64, 65).

Furthermore, increased intestinal permeability in ASD individuals and animal models of ASD has been reported (66–68), giving rise to the hypothesis of “leaky gut” contributing to chronic inflammation in ASD. Interestingly, barrier dysfunction is also a common feature in inflammatory disorders of the gut such as Crohn’s disease, inflammatory bowel disease, and celiac disease, further implicating the role of GI disorders and immune function. In certain genetic forms of autism, mutations in genes affecting the CNS directly affect the gut’s enteric nervous system, causing gut dysmotility (69, 70).

The gut-microbiome-brain axis has been recognized to modify behavior and play a role in neurodevelopment (71). Dysbiosis of the gut microbiome in individuals with ASD and animal models has been described in many studies. Investigation of the microbiome in ASD subjects versus non-autistic individuals discovered differences in microbial diversity in stool and fecal samples (72–76). Besides abnormal bacterial diversity, individuals with ASD present with an abnormal microbial composition which might exacerbate GI pathology and inflammatory processes (77). Despite conflicting reports due to differences in general methodology, sample size, exclusion or inclusion of participants with known GI dysfunction in the study, and likely difference in diets due to different countries of origin, several studies report changes in Bacteroides and Firmicutes microbiota on phylum level (72, 78–83). Furthermore, on phylum level, Actinobacteria in individuals with autism are different compared to control subjects (73, 78, 83). Changes to gut microbiota and abnormal intestinal epithelial barrier function (“leaky gut”) directly or indirectly elicit inflammatory processes that impact cerebral function, thereby contributing to the neuropathology of ASD (84, 85).

CONCLUSION

ASD are very heterogenous due to complex underlying pathomechanisms triggered by various factors. Some children and adults with ASD are fully able to perform all activities of daily living, while others require substantial support to perform basic activities. To date, the etiology and pathogenic mechanisms remain incompletely understood. However, the presence of common behavioral features that are the basis of ASD diagnosis hints at a core pathology shared by the majority of individuals with the condition. Given that ASD are associated with a substantial
socioeconomic burden (86), more research investigating the etiology and pathology of ASD is needed to identify possible biomarkers and treatment and prevention strategies, and improve existing therapies. Due to the large heterogeneity, individuals with ASD will highly benefit from a personalized medicine approach.

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