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REVIEW ARTICLE

# Autism spectrum disorder

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Autism spectrum disorder (ASD) is a complex heterogeneous condition that is characterized by impairments in social interaction, communication, and behaviour which mostly co-exist with several comorbidities. The current prevalence of ASD in the general population is estimated to be that of 1 in every 54 children in USA. The accurate diagnosis involves detailed assessments at age specific intervals and finally a comprehensive evaluation by specialists. Although genetic and environmental factors contribute to cause ASD, the precise mechanisms underlying ASD are poorly understood. Concerning management, early interventions are always recommended, as they lead to better outcomes. However, despite the availability of multiple medications, no definitive cure currently exists and the management of the disease remains poor, posing significant problems to life perspectives. Therefore, further studies are required to fully understand the pathogenesis and the possible resultant identification of more effective treatment options for ASD. This overview on autism covers its causes, presentation and therapies.

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## Introduction

ASD is a complex and heterogeneous neurodevelopmental disorder, which manifests itself with a variety of signs and symptoms.[1] The *Diagnostic and Statistical Manual of Mental Disorders* (DSM V) defines ASD as an incessant neurodevelopmental disorder that exhibits poor social skills, essentially in terms of social-emotional reciprocity, verbal and non-verbal communication along with restrictive and repetitive behaviour which are present from early developmental age. According to DSM V, several related diseases such as Asperger's disorder, childhood disintegrative disorder also known as Heller syndrome or pervasive developmental disorder, which are not otherwise specified and, others are now diagnosed as ASD. However, the notion that mental disorders can be classified into distinct, discrete categories has been challenged and scientists are re-examining the theories underlying brain illnesses, significantly. Indeed, it appears that these disorders shade into each other, as there are no hard dividing lines and, changes in the brain's decision-making systems could be involved in many different conditions. This new perspective is further supported by genetic evidences showing that the same genes are associated with seemingly distinct disorders, such as autism and schizophrenia.[2-3]

It has been proposed that the use of new diagnostic criteria could be responsible for the rise in the number of cases of autism, rather than a true rise in the prevalence of the disorder.[4,5] However, it seems unlikely that this assumption could account for the diagnosis of a child with autism with every 54 new-born in US[6] and the higher incidence rates reported in distinct countries such as Hong Kong and Japan. Psychiatrists have long observed differences also between women and men in terms of their susceptibility to certain brain disorders. Autism is among those, as boys are more affected than girls (4:1 ratio)[7] and females are diagnosed with ASD at later age compared to males.[8] Interestingly, it has been reported that estrogens can rescue ASD phenotypes in animal models of autism supporting the "*female protective theory*".[9]

To address ASD-related issues and compile this review we selected the literature published from 1995 till 2020 using the PubMed, Scopus and Google Scholar databases and the keywords autism spectrum disorder, etiology, diagnosis and treatment. Particular emphasis was given to the evaluation of evidence based research and clinical practice trough systematic review of high quality publications.

## Presentation

The presentation of ASD tends to vary from one individual to another. This variation in the clinical symptoms could be explained by alterations in the heritable background, epigenetics, and environmental factors.[10] The following three main core symptoms should be observed to enable one to properly diagnose ASD in a subject: (a) continuous difficulty in communication and interaction which are social and reciprocal; (b) difficulty in using or understanding language, tending to focus attention and conversation on a limited number of topics, frequently repeating phrases, and have very limited speech ability; (c) restrictive and repetitive behavior. The severity of ASD can be graded from Level 1 to Level 3 in two domains: social communication and restrictive stereotyped behaviour. Regarding communication, severity ranges from problems with starting social interactions to verbal and non-verbal communication

resulting in impaired functioning. Concerning behavioural deficits, the phenotype can range from the inflexibility of behaviour in one context to extreme difficulty in coping with changes in daily routine, significantly interfering with proper functioning in all spheres.[11] Autism coexists with other disorders in nearly 95% of the cases and its occurrence alone is rare. Indeed, several comorbidities are often associated with ASD such as epilepsy (up to 30%), intellectual disability (~40%), sleep disorders (50–80%), gastrointestinal problems (up to 70%) and motor deficits (~80%).

## Screening and diagnosis

The *American Academy of Pediatrics* (AAP) policy recommends surveillance and screening to identify children who are at a risk of ASD at an earlier stage to ensure implementation of the effective interventions. The guidelines recommend developmental surveillance at 9, 15 and 30 months of age and specific screening for autism at 18, 24 and 30 months of age.[12-13] Surveillance includes: *a*) maintaining a developmental history; *b*) making accurate and informed observations of the child; *c*) eliciting and attending to parents' concerns; *d*) identifying the presence of risk and protective factors; *e*) documenting the process and findings. It should be performed at every preventive visit throughout childhood. Further standardized developmental tools should be used for screening if surveillance raises concerns. Notably, the AAP recommends that all children should be screened during visits to a primary care provider in an outpatient setting with the same time schedule, regardless of whether any concerns have been raised or not. The screening of the general pediatric population is essential to the timely identification of children at risk or exhibiting signs suggestive of ASD. In addition, AAP recommends using a standardized autism-specific screening tool on all 18-month old children at a preventive care visit with repeated evaluations for those who regress after the initial screening.[14] Selection of the autism-specific screening tool is done according to the age of the child. If the screening raises concerns, a referral specialist (pediatric neurologist, developmental-behavioural pediatrician, child psychiatrist, licensed child psychologist) should be consulted for a definitive diagnosis and comprehensive assessment.[15] The comprehensive examination includes: *a*) detailed pediatric history along with parental concerns; *b*) physical examination including assessment for dysmorphic features, head circumference, Wood's lamp examination of the skin (tuberous sclerosis) and full neurologic examination; *c*) direct observation of the child's current cognitive, language, and adaptive functioning by a clinician experienced with ASD according with the DSM V criteria.[16] However, making an ASD diagnosis is just the beginning. Further in-depth evaluations should be performed to understand the child's unique strengths and challenges. This evaluation is crucial for defining what kinds of medication, educational programs and behavioral therapies would be most beneficial. Usually, this process involves a number of specialists, such as child neurologists, developmental behavioral pediatricians, speech-language pathologists, child psychologists and psychiatrists, nurse practitioners, educational specialists and occupational therapists.

## Genetic factors

Over the last few decades, it has been shown that the genetic component of ASD can range

from 40 to 80%.[3,17] Large variation in the genetic mechanism underlying ASD exists depending on the inheritance pattern, chromosomal aberration and mode of action.[18-20] The advanced paternal age has been implicated in neurodevelopmental disorders due to increased mutation occurrence in spermatogenesis at a later age.[21-22] A seminal study performed by Filstein and Rutter showed that monozygotic twins had a concordance of 36% meaning that over a third of both pairs had autism while no concordance was found between dizygotic twins. More recently concordance of ~60% has also been reported.[3] A Swedish study demonstrated that monozygotic co-twin always had another neurodevelopmental disorder disconcordant for ASD.[23] It is proposed that more than half of the risk of developing ASD is linked with genetic variability that is evident by an increased prevalence of ASD in families of individuals with autism.[24] Notably, these genes are also found in other neurodevelopmental and psychiatric disorders.[25] To date, hundreds of risk genes have been identified by means of large-scale genetic screenings of ASD patients and their family members.[3] The majority of reproducible hits point to proteins involved in synapse pathology (synapse formation and transmission), transcriptional regulation, chromatin-remodeling pathways and neural network formation (*e.g.* neuroligins, cadherins, synaptic vesicle cycling proteins synapsin-1 (SYN1), synapsin-2 (SYN2), *MeCP2, UBE3A, FMRP, FXRP1, SHANK3*, *GABRG3, etc*). Genetic defects in sodium, calcium and potassium channel types plays an important role in the pathogenesis of autism (*e.g. SCN2A, CACNA1E, KCNJ10, KCNQ3, KCNQ5, KCND2*)[26]. Investigations carried out at the University of Malta showed that dysfunction of the inwardly-rectifying potassium channels Kir4.1 results in autism associated with epilepsy (*autism-epilepsy phenotype, AEP*) [26-29]. Indeed, an international collaborative research team identified germline heterozygous variants in some affected children where epileptic spasms were major issues emphasizing the role of variants in *KCNJ10* (Kir4.1) and *KCNJ2* (Kir2.1) in AEP.[30-32] In the previous seminal studies the functional properties of Kir4.1 mutant channel were characterized demonstrating that the identified mutations produced gain of channel function.[31] In another study on monozygotic twins with autism and short QT interval on ECG as a comorbidity, it was demonstrated the presence of a novel *KCNJ2* variant that increased the surface expression of Kir2.1 channels (*gain of function*). This study pointed to the involvement of Kir2.1 channels in AEP and the necessity to perform neuropsychiatric assessments in patients with short QT syndrome (SQT3) to identify the presence of subtle autistic traits.[32] Recordings from surgical specimens of patients with intractable epilepsies showed a remarkable reduction of Kir conductance in astrocytes, impairing their ability to perform potassium clearance. It could be inferred that either the enhancement or the reduction of Kir4.1 activity leads to epilepsy possibly causing an alteration in the excitatory-inhibitory balance in the brain. Nevertheless, the mechanisms involved in this apparently contradictory dual effect is unclear.[33] Scientists from the Department of Physiology & Biochemistry at the University of Malta have contributed prominently to these discoveries and are currently clarifying the mechanisms responsible for the development of AEP that may render valuable benefits to autistic individuals. Indeed, a genetically modified mouse model of autism was generated and is currently under investigations in the above mentioned

laboratories to further understand the pathogenic relevance of *KCNJ10* mutations, clarify the underlying mechanisms and identify potential treatments.

## Environmental factors

The possibility that the environment contributes to the causation of autism has arisen from our current understanding of the exquisite vulnerability of the developing human brain to toxic substances in the environment and studies that specifically linked autism to prenatal exposures to environmental factors or medicines.[34] The antiepileptic medication Valproic acid represents the typical example of drug-induced autism which does so through different mechanisms.[3] Hallmayer and colleagues showed that a moderate genetic component combined with considerable environmental factors may cause ASD.[35] In terms of maturity, preterm infants are at greater risk of adverse neurodevelopmental outcomes in comparison to the full term infants. Thus, it is recommended that premature individuals should be closely observed in order to implement effective interventions if the need arises.[36] The proposed factors that may play major roles include hypoxia, oxidative stress, inflammation, endocrine disturbance and immune activation. Several autoantibodies such as anti-$MAP_2$, anti-MBP, anti-NFP, anti-MAG and anti-Tau were found in higher levels in children exhibiting ASD with their mothers having similar levels. In contrast, control children and their mothers had negligible amounts of auto-antibodies against neuronal and glial proteins, implying the involvement of the maternal immune system in the development of ASD in offspring.[37] Maternal intake of folic acid during perinatal period reduces the risk for the development of ASD. Also, maternal intake of polyunsaturated fatty acids decreases the risk for ASD, whereas, very low levels of Omega 3 fatty acids increase the risk for ASD.[35,38] Heavy metals may else play an important role in autism. Indeed, newborns from mothers exposed to high levels of mercury, lead, nickel, and manganese were at higher risk of developing autism.[39] The risk for ASD was doubled by gestational exposure to nitrogen dioxide ($NO_2$), particulate matter less than 2.5 (PM 2.5) or 10 (PM 10) micrometers in diameter.[40] In Malta, high concentrations of airborne PM are reported particularly in heavy-traffic areas with the consequent relevant health implications.[41] Organochlorine exposures in the first trimester of gestation showed a strong association with ASD. Whereas, exposures to pyrethroid or bifenthrin during the overall gestational period showed moderate association.[42] The observed heterogeneity in symptoms severity and prognosis of ASD patients also suggests that a combination of genetic predisposition, gut microbiota (GM) dysbiosis and, the alteration of metabolites produced by microbes may represent a critical "environmental factor" impacting brain function and behaviour, thus potentially promoting the development of autism.[1] Several observations strongly support the role of GM in ASD, such as the high occurrence of gastrointestinal (GI) abnormalities in ASD patients, the amelioration of symptoms upon short-term treatment with antibiotics and probiotics and the improvement of GI function and behaviour in autistic children after Fecal Microbiota Transplant (FMT).[43-48] Abnormal GM composition has been widely reported both in animal models with behavioral traits relevant to autism and in human pre-clinical investigations of autistic patients.[49-52]

## Treatment

In spite of considerable economic costs caused by autism, there are limited treatment options to ameliorate the typical symptoms associated with ASD, and the relevant comorbidities known to exacerbate the severity of the phenotype. There are numerous challenges for the identification of effective treatments for ASD. Systematic reviews highlighted the possibility that the high heterogeneity in the genetic, environmental, cognitive, social and ASD phenotype reduce the overall validity and efficacy of potential interventions.[53] Anthropological differences, which propose the deviation from typical behaviour in one culture but not in another culture, further contribute to obscure treatment strategies.[54] Aripiprazole and Risperidone are the most widely studied medications used to manage behavioral symptoms. Aripiprazole, is an atypical antipsychotic drug.[55] The US FDA particularly indicated aripiprazole and risperidone for children and approved them for the treatment of behaviours associated with ASD.[56] Such medications control irritability, aggressive and self–injury behaviours.[57-58] Despite some beneficial effects, these drugs present with adverse effects such as extrapyramidal symptoms, tremors and sedation.[59] Parents and health care professionals must closely monitor a child's progress and reactions while he or she is taking a medication to be sure that any negative side effects of the treatment do not outweigh the benefits. Apart from medications, early intensive behavioral therapy is considered to be beneficial for school-aged children diagnosed with ASD.[60] Behavioral interventions can be classified as early intensive behavioral and developmental interventions, social skills interventions, parent training, play/interaction-focused interventions, interventions targeting symptoms commonly associated with ASD such as anxiety, and other general behavioral approaches. The Agency for Healthcare Research and Quality (United States) reviewed several studies reporting statistically significant evidence and showed that early intensive behavioral therapy over extended timeframes was associated with improvement in cognitive functioning and language skills of young children with ASD. A notable treatment approach that is used in many schools and treatment clinics for people with ASD is called *applied behaviour analysis* (ABA) which uses principles and techniques to understand, treat and prevent challenging behaviors such as anxiety and to promote new, desired behaviors. There are different types of ABA: A) Discrete Trial Training (DTT) that uses a series of trials to teach each step of a desired behavior or response. Lessons are broken down into their simplest parts and positive reinforcement is used to reward correct answers and behaviors. Incorrect answers are ignored; B) Early Intensive Behavioral Intervention (EIBI) that is used for ASD children younger than five, and often younger than three; C) Pivotal Response Training (PRT) that aims to increase a child's motivation to learn, monitor his own behavior, and initiate communication with others; D) Verbal Behavior Intervention (VBI) that focuses on teaching verbal skills. The interventions used in the early intensive behavioural therapy are outlined in the University of California, Los Angeles (UCLA)/ Lovaas-based approach, the Early Start Denver Model (ESDM), and parent training approach.[61] The UCLA/ Lovaas-based approach applies ABA procedures that focus on teaching new skills and reducing interfering behavior in children with ASD. It relies on one-on-one therapy sessions where a trained therapist adopts discrete teaching trials with a

child to practice target skills. The therapy is tailored to each individual in order to benefit the needs of the child.[62] The ESDM is an approach for preschool-aged autistic children that incorporate ABA with developmental and relationship-based approaches. This therapy is delivered by trained therapists and parents.[63] The Building Block program provides early interventions for young children with autism and their families. The Building Block model includes various approaches such as positive behavior support, naturalistic play-based intervention, assessment of sensory processing issues, and extensive use of visual supports, behavioral and developmental theory, structured teaching and the development of functional communication skills.[65,66] Notably, children attending this program showed significant improvements on some social and communication skills. A randomized control trial involving parent training was conducted in Australia[64] and compared two variations of Building Block program that was performed at home or center based. This trial showed that children receiving centre based intervention had greater improvement in language comprehension.[64] Social skill training improves social interaction in school-aged children.[61] A meta-analysis of early intensive behavioral intervention for children with autism supported that this should be an intervention of choice for children with autism. Regrettably, the costs are excessive, require several resources to be implemented and not all patients may benefit from these interventions.[67] Other approaches include occupational therapy that teaches skills to help the person live as independently as possible. Skills might include dressing, eating, bathing, and relating to people. The speech therapy helps to improve the person's communication skills. Some people are able to learn verbal communication skills. For others, however, using gestures or picture boards is more realistic. Indeed, the Picture Exchange Communication System (PECS) uses picture symbols to teach communication skills. The person is taught to use picture symbols to ask and answer questions and have a conversation. Some dietary treatments have been developed by reliable therapists, although these treatments do not have sufficient scientific support needed for widespread recommendation. Complementary and alternative treatments (e.g. special diets, chelation of heavy metals from the body, secretin treatment, deep pressure, etc.) are used by some parents and health care professionals despite the fact that they are outside of what is typically recommended by pediatricians.

## Concluding remarks

ASD is a complex disorder that has several etiologies involving genetic and environmental factors. Remarkable advances in the discovery of factors leading to autism have been achieved in the past years. However, the different types of modifiers that may exacerbate or ameliorate disease severity have not been identified, clearly. Such modifiers could include epigenetics, sex-linked modifiers, or environmental factors. Furthermore, the key architecture of ASD development which could be targeted for treatment remains still an uncharted territory. A better understanding and awareness of autism by the general population and health care professionals is also essential as it allows prompt diagnosis and early interventions that influence positively the development of the child affected by this invalidating disease. New hopes for children with ASD may result from the accomplishment of the Research Domain

Criteria project by the National Institute of Mental Health that aims to explore the biological and psychosocial causes of ASD and identify new treatments strategies for autism.[68] Thus, further work is imperatively needed to broaden the horizons on the causes and accomplish new therapeutic options for ASD.

## Abbreviations

Autism spectrum disorder (ASD), Diagnostic and Statistical Manual of Mental Disorders (DSM V), American Academy of Pediatrics (AAP), autism-epilepsy phenotype (AEP), inwardly-rectifying potassium channels (Kir4.1), microtubule-associated protein-2 (MAP-2), myelin basic protein (MBP), neurofilament triplet proteins (NFP), myelin-associated glycoprotein (MAG), particulate matter (PM), gut microbiota (GM), gastrointestinal (GI), Fecal Microbiota Transplant (FMT), applied behaviour analysis (ABA), University of California, Los Angeles (UCLA), Early Start Denver Model (ESDM)


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