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A Review

Autism spectrum disorder (ASD): A current review of assessment, risk factors and prevention

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Autism spectrum disorders (ASDs) are extremely heritable neurodevelopmental disorders observed in 1-2% of children, usually seen before they reach 3 years of age. ASD is characterized by impaired social behavior, poor communication, stereotypic behavior, abnormal sensitivity to sensory stimuli and self-injurious behavior. The exact causes of ASD are unclear but increased oxidative stress; hyperserotonemia and loss of purkinje cell integrity in the cerebellum are some pathological findings in ASD. This article reviews trends in assessment of this neurodevelopmental disorder, autism risk factors, and preventions for autism. Screening tools for ASD, such as checklist for autism in toddlers (CHAT), modified checklist for autism in toddlers (M-CHAT), autism behavior checklist (ABC) and the autism spectrum screening questionnaire (ASSQ), social communication questionnaire (SCQ), australian scale for asperger syndrome (ASAS), are available for use by general pediatricians. Pregnant ladies exposure to some toxic chemicals and selective serotonin reuptake inhibitors (SSRIs) may increase the risk of ASDs. Mother and child cohort study (MoBa) found prenatal folic acid supplements reduce the risk of autism spectrum disorders in children, but these findings cannot determine whether they protect against other neurodevelopmental disorders.

Keywords: Autism, Risk factors, Screening tools, Serotonin reuptake inhibitors

Autism spectrum disorders (ASDs) are extremely heritable neurodevelopmental disorders observed in 1-2% of children with varying degree of symptoms and severity[1]. Autism was originally defined by Leo Kanner in 1943 as an innate inability to create normal, biologically determined, emotional contact with others[2]. Autism is usually seen in children before they reach 3 years of age. According to the first precise estimate of the country's autism prevalence is approximately 23 in 10000 children in India. This comparison of, about 0.23 percent, is far less than 1.47% prevalent in the United States[3,4] (Fig. 1). Autism is characterized by impaired social behavior, poor communication, stereotypic behavior, abnormal sensitivity to sensory stimuli and self-injurious behavior[5]. The exact causes of ASD are unclear but increased oxidative stress[6,7] hyperserotonemia[8,9] and loss of Purkinje cell integrity in cerebellum[10] are some pathological findings in autism. Some children with ASD or ADHD (Attention-deficit/hyperactivity disorder) exhibit no obvious symptoms, making it difficult to reliably diagnose the disorders during childhood[11]. Brain alters in ASD include a reported 67% more neurons in the prefrontal cortex, more than 17% increase in brain weight and abnormal cortical patterning. Additional transcriptomic analysis of postmortem brains from human ASD individuals revealed altered expression of proteins which are important for functional synaptic activity in the prefrontal cortex and cerebellum[12].

Various factors like genetic aberrations, environmental offences, and social factors contribute to the development of autism. Chemicals like mercury, ethanol, thalidomide, and misoprostol produce a generation of reactive oxygen species (ROS) which are responsible for deficits in the development of the cerebellum, limbic system and of brain[5]. Several studies have reported on ASD-associated abnormalities in the peripheral nervous system, enteric nervous system, and neuroimmune system. ASD patients of Postmortem brains showed increase microglia and astroglia activation in the cerebellum and cerebral cortex, along with increased levels of proinflammatory cytokines in the cerebrospinal fluid and cortical regions

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of the brain[11]. The main aim of behavioral and educational treatment strategies is to reduce ASD symptoms and other associated behavioral and emotional problems, thus increasing the psychological functioning of the patient. Although there are advances in early diagnosis and intervention, no therapy has yet confirmed to completely reverse the core symptoms of autism[13].

This article reviews trends in assessment of this neurodevelopmental disorder, autism risk factors, and prevention of autism.

Assessment of potential autism

American psychiatric association published DSM-IV-TR in 1994 for the diagnostic and statistical manual of mental disorder. After long time, version 5 (DSM-5) published in 2013, has made significant changes in the proposed criteria for autism diagnosis and classification, although most of the core features from the DSM-IV TR still prevail[14]. Screening tools for ASD, such as checklist for autism in toddlers (CHAT), modified checklist for autism in toddlers (M-CHAT), autism behavior checklist (ABC) and the autism spectrum screening questionnaire (ASSQ), social communication questionnaire (SCQ), australian scale for asperger syndrome (ASAS), are available for use by general pediatricians[15,14].

The INCLEN Diagnostic Tool for autism spectrum disorder (INDT-ASD) is the new tool proposed and it has been developed in India. This tool is to be used by trained personnel and is based on both history from primary caregivers and direct observation of a child aged between 2 to 9 years. This tool was first developed in English then, forward and backward translated to Hindi and Malayalam by two teams with two independent, bilingual translators in each, to achieve the proximity of the source and target versions. Apart from English, the instrument was modified in Hindi version and then forward translated to English, Malayalam and six additional Indian languages (Odia, Konkani, Urdu, Khasi, Gujarati, and Telugu) and backward translated in a similar manner[14]. The indian scale of assessment of autism (IASS) has been developed which provides the detailed assessment procedure and tools for assessing the extent of disability for persons with autism beyond 6 years of age[16].

Risk factors

Genetic risk factors are some epidemiological findings in ASD, even though; there is presently no definitive biomarkers[17]. 10% of children affected by ASD have certain genetic or chromosomal conditions (*e.g.*, Down's syndrome or fragile X syndrome)[17]. Fragile X syndrome (FXS), Cornelia de Lange syndrome (CdLS) and Tuberous Sclerosis Complex are the three most common genetic syndromes associated with ASD. List of some genetic syndromes associated with autism is reported in (Table 1)[18,19].

Ten chromosomal regions, on chromosomes 1p, 4p, 6q, 7q, 13q, 15q, 16p, 17q, 19q and 22q have been

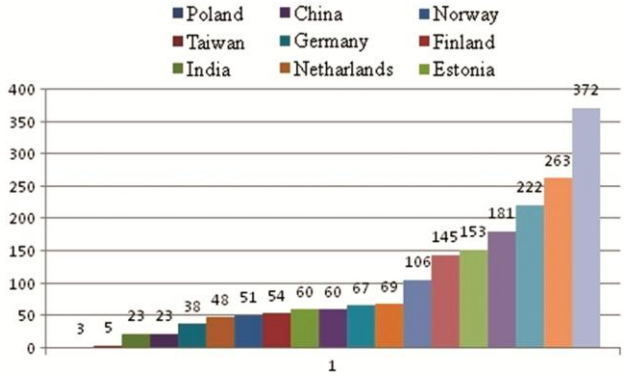

Fig. 1 — The number of autism diagnoses per 10000 children in selected countries in 2014

Table 1 — List of genetic syndromes associated with autism

Fragile X syndrome (FMR1 gene)	Apert syndrome
Rett syndrome (MECP2 gene)	Williams syndrome
Angelman and Prader-Willi syndromes	Joubert syndrome
(15q11-q13 deletions or rearrangements)	
Smith-Lemli-Opitz syndrome	Noonan syndrome
Smith-Magenis syndrome (17p11.2 deletion)	Down syndrome
Tuberous sclerosis	Turner syndrome
PTEN-gene-mutation-associated disorders	Neurofibromatosis
(Cowden and Bannayan-Riley-Ruvalcaba syndrome with extreme macrocephaly)	
Shprintzen/velocardiofacial syndrome (22q11 deletion)	Myotonic dystrophy
Sotos syndrome	Duchenne muscular dystrophy
CHARGE syndrome	Moebius sequence
Hypomelanosis of Ito	Cohen syndrome
De Lange syndrome	Oculoauriculovertebral spectrum
Mitochondrial dysfunction	Untreated or poorly treated phenylketonuria (PKU) Adenylate succinase deficiency

analyzed in a total of 17 multiplex families with autism originating from the isolated Finnish population by pairwise linkage analysis and sib-pair analysis. These ten chromosomal regions have potentially been linked to autism. Mild evidence of putative involvement was found only with the 1p chromosomal region in the susceptibility to autism[20]. Children with ASD are decrypted with microdeletions and duplications using newer techniques involving chromosome regions 1q24.2, 2q37.3, 3p26.2, 4q34.2, 6q24.3, 7q35, 13q13.2-q22, 15q11-q13, 15q22, 16p11.2, 17p11.2, 22q11, and Xp22[21].

A clinical study reported that nearly one-third of autistic subjects have platelet hyperserotonemia. An additional study indicated direct support showing the treatment with selective serotonin reuptake inhibitors (SSRIs) was effective in reducing autistic traits such as rituals and aggression. SLC6A4 is human serotonin transporter gene which has been studied extensively in human genetic studies of autism, it possesses several polymorphic loci affecting its expression or function (Ile425Val, Gly56Ala, intron 2 VNTR and the 5-HTT-linked polymorphic region [5HTTLPR]) (Ma *et al.*, 2010). However, these results do not necessarily dismiss the SLC6A4 gene as a susceptibility factor for a subset of autistic patients[22]. Gamma-aminobutyric acid (GABA) is a main inhibitory neurotransmitter in the brain. GABRB3 might be associated with ASD of rare variants and increased GABRB3 expression may contribute to the pathogenesis of ASD in some patients[23].

The paraventricular and supraoptic nucleus of the hypothalamus synthesized the Oxytocin and is released into the bloodstream by axon terminals in the posterior pituitary. Oxytocin (OXT) is a nine-amino-acid peptide and its effects on women of reproductive age are in stimulating uterine contractions during labor and in regulating lactation[24,25]. Lee *et al*., 2009; Harony, 2010; Caldwell, 2008 have reported oxytocin and arginine vasopressin (AVP) plays a key role in regulating social affiliative behaviors, including sexual behavior, and social recognition[24,26,27]. Katherine *et al.*, 2010 have examined 18 SNPs at the oxytocin receptor (OXTR) gene for association in three independent autism samples from Ireland, Portugal, and the United Kingdom. They investigated *cis*-acting genetic effects on OXTR expression in lymphocytes and amygdala region of the brain using an allelic expression imbalance (AEI) assay and on investigating found the correlation between RNA levels and genotype in the amygdala region. No marker survived multiple corrections for association with autism in any sample or in a combined sample[28].

Tarabeux *et al.*, 2011[29] have studied the systematic sequence alterations in all NMDARs genes in ASD and SCZ and reported several potentially damaging and/or de novo mutations. Here, potentially damaging de novo mutation was recognized in GRIN2A and GRIN2B associated with sporadic cases of SCZ and ASD, respectively, and truncating mutations in GRIN2C, GRIN3A, and GRIN3B, whereas neither truncating nor de novo mutations were found in GRIN1 and GRIN2D. The most consistently reported genes among the common variants include engrailed homeobox 2 (EN2); integrin, β3 (platelet glycoprotein IIIa, antigen CD61; ITGB3); met proto-oncogene (hepatocyte growth factor receptor; MET); and contactin-associated protein-like 2 (CNTCAP2) genes[30].

Prominently, two current large exome studies, involving 3871 and 2508 cases revealed multiple de novo LoF variants occur in coding genes – ADNP, ANK2, ARID1B, CHD8, DYRK1A, GRIN2B, KATNAL2, POGZ, SCN2A, and TBR1[31]. Interestingly, many frequent variants were observed in ASD probands. These include different types of DNVs (De novo variants) that occur in coding regions of SCN2A (Sodium Channel, Voltage-Gated, TypeII, Alpha subunit) and were found in four different studies[32-35]. Several gene families include voltage-gated calcium channel (CACNA), catenin (CTNN), and chromodomain helicase DNA binding protein (CHD) gene that appears to contribute to specific ASD phenotypes[36-38].

Reelin (RELN) is a large secreted extracellular matrix glycoprotein that helps proper brain development and synapse function[39]. Ashley *et al*., 2007 have reported APOE (Apolipoprotein E) is not the main cause of autism in patients, nor is there any evidence of a joint effect of APOE with RELN. RELN, though, remains a good suspect for autism susceptibility[40]. Neuroligin, a type I membrane protein, is a cell adhesion protein on the postsynaptic membrane that mediates the formation and maintenance of synapses between neurons. Some patients with autism spectrum disorder (ASD) and other neurodevelopmental impairments could have been caused by mutations in neuroligin-4 (NL4; gene symbol: NLGN4)[41]. Jackson *et al*., 2009 reported the rs1858830 C variant in the MET gene found to be promoter associated with autism[42]. Ramoz *et al.*, 2004

have been reported SLC25A12 was originally associated with autism[43].

Glutamate, a major excitatory neurotransmitter in the brain and may be a key neurotransmitter involved in autism[44]. Reduction of GluRs glutaminergic gene dosage can decrease ASD-like features in fragile X-mice[45]. GluR6 or GRIK2 coding of glutamate receptor 6 has been suggested as a prospective gene for autism based on its localization in the autism-specific region on chromosome 6q21 and the action of the receptor protein in cognitive functions like learning and memory[46]. Dutta *et al*., 2007 have carried out genetic analysis of three markers of GluR6 (SNP1: rs2227281, SNP2: rs2227283, SNP3: rs2235076) for feasible association with autism through the population, and family-based (TDT and HHRR) approaches[46]. List of some SNPs in ASD related genes reported in (Table 2)[28,46,42,43,47-50]. Rodenas *et al.*, 2014, and Martin *et al*., 2015 reported disruption of heterozygous CNTNAP2 found in individuals with intellectual disability (ID), seizures, signs of ASD such as repetitive behaviors, and language problems, including dysarthric language, delayed or absent speech or language acquisition[51,52]. Genetic studies have implicated polymorphisms in GLO1 in humans, such as panic disorder, depression, autism, schizophrenia and restless legs syndrome (RLS)[53]. The TPH1 and TPH2 genes control serotonin biosynthesis, and serotonin is clearly altered in autism[54].

Lai *et al.*, 2016 and Setiawan *et al*., 2015 have reported increased levels of pro-inflammatory cytokines in serum, plasma, and blood cells, suggesting that inflammation might be involved in the mechanisms of psychiatric disorders[55,56]. The activation of astrocytes and microglia, increased expressions of transforming growth factor-β (TGF-β) and monocyte chemotactic protein-1 (MCP-1) in the brains of subjects with ASD have been compared to typically developing children (TDC)[57]. Chez *et al.*, 2007 and Li *et al.*, 2009 have investigated that pro-inflammatory cytokines such as interleukin-1 β (IL-1 β), IL-6, tumor necrosis factor-α (TNF-α), granulocyte macrophage colony-stimulating factor, interferon-g, and interleukin-8, significantly increased in the brains of ASD subjects[58,59]. Manabu M *et al*., 2016 have studied TNF-α mRNA expression in PBMCs without any stimulation and found significantly lower TNF- α expression in subjects with ASD[60]. Although, mice were subjected to acute stress can improve working memory by inhibition of TNF- α[61]. However, TNF- α molecular mechanism has not been elucidated on behavior[60].

Table 2 — List of SNPs in ASD related genes

Genes	SNPs	Chromosomes	Reference
SLC6A4	5-HTTLPR STin2 3'UTR-SNP	17q11.1–q12	47
GABRB3	rs878960	15q11-q13	48
OXTR	rs237897	3p25-3p26	28, 49
rs237895			
	rs13316193		
GluR6	rs2227281	6q21	46
	rs2227283		
	rs2235076		
RELN	rs727531	7q22	50
	rs2072403		
	rs2072402		
	rs362691		
	rs362719		
	rs736707	(This SNPs not association with ASD	
	in the Indian population)		
MET gene	rs1858830	7q31	42
SLC25A12	rs2056202	2q24-q33	43
	rs2292813		

Lakshmi Priya and Arumugam Geetha investigated the proteins from hair and nail samples of autistic children with different grades of severity low functioning autism (LFA), medium fun functioning autism (MFA), and high functioning autism (HFA). Both high and low sulfur proteins were significantly low in the hair and nail extracts of autistic children by using the SDS-PAGE analysis and the Western blot analysis showed an increased percentage of nitration of low sulfur proteins in autistic children when compared with normal children. The concentration of enzymatic and nonenzymatic antioxidants level decreased whereas the increased concentration of TBARS and NO were also observed in the blood of autistic children when compared to normal children[62]. Apart from this, Lakshmi Priya and Arumugam Geetha examined the urinary levels of oxidative stress markers and antioxidants as biomarkers in autistic children. The Urinary oxidative stress markers can be correlated with increasing order of severity (LFA > MFA > HFA), whereas antioxidants, showed

a negative correlation. The level of antioxidants excreted in urine was significantly low in autistic children. This study also discloses that urine samples can be used as a source for the examination of oxidative Stress markers and antioxidants, such as sulfhydryl groups, to monitor the level of oxidative stress in autistic children[63]. Nuclear transcription factor κB (NF-κB) is a stress-inducible transcription factor. A recent study has demonstrated that there was a significant increase in the amount of NF-κB in samples from children with autism when compared with those from age-matched controls[64].

The Glial fibrillary acidic protein (GFAP) is a brain-specific protein taken out from severely gliosed human tissue[65]. GFAP has been investigated in many neurological diseases, such as neuromyelitis optica attacks, stroke, traumatic brain injury [TBI], glioblastoma and Parkinson's disease[66]. Jingwei W *et al.*, 2017 have studied that the mean serum GFAP level was significantly higher in autistic children as compared to age-matched controls, apart from this, serum homocysteine (HCY) and C-reactive protein (CRP) were also higher in autistic children. Hence, the serum GFAP levels may be related to severity of ASD among Chinese children. This recommended the assumption that increased serum levels of GFAP could also be concerned with the pathophysiology of autism in Chinese children[66].

Histone deacetylases (HDACs) enzymes play crucial roles in numerous biological processes, mostly through their suppressive influence on transcription[67]. In utero exposure to the HDAC inhibitor valproic acid (VPA) which is linked to autism spectrum disorder in humans and has been observed to cause social cognition deficits in rodents[68,69]. This recommends that HDACs play a key role in brain development mechanisms leading to autism[70].

Ugur C and Gurkan CK *et al.*, 2014 have examined the serum levels of vitamin D, calcium (Ca), phosphorus (P), alkaline phosphatase (ALP) and folate in 54 young children, aged 3–8 years, with autism spectrum disorders (ASD) and in 54 age and gender-matched normal controls. Although, Vitamin D, Ca, P, ALP and folate levels in children with ASD were not much different from control group[71].

Shen *et al*., 2013 have investigated that high-risk infants who subsequently developed ASD had increased extra-axial cerebrospinal fluid (EA-CSF) volume during 6-24 months, which was correlated with autism severity at 36 months[72]. Synaptic cell adhesion molecules (SCAMs) are localized at synaptic terminals at which they connect pre- and postsynapses during the process of synapse formation, maturation and modification by homophilic or heterophilic interaction through their extracellular cell adhesion domains[73]. CASK may be one of the molecules seeking a hint for a common intracellular signaling pathway for several SCAMs. CASK is recommended to be one of the strongest risk genes for X-linked mental retardation as well as the moderate association with ASDs[74-76].

Geier DA *et al.*, 2013 reported an association between increasing organic-Hg exposure from Thimerosal-containing childhood vaccines and the subsequent risk of an ASD diagnosis[77]. The Vaccine Adverse Event Reporting System (VAERS) database was analyzed in the first phase for hypothesis generation, and it indicated a significant relationship between increasing organic-Hg exposure from Thimerosal-containing DTaP vaccine administration in comparison to Thimerosal-free DTaP vaccine for the risk of ASD. The vaccine safety datalink (VSD) database was analyzed in the second phase for hypothesis testing, and it indicated that those cases diagnosed with an ASD were at significantly higher risk of exposure to increasing doses of organic-Hg from Thimerosal-containing hepatitis B vaccines administered at three specific intervals within the first six months of life in comparison to controls. Hooker B *et al.*, 2014 has reported there is no increased risk of autism from exposure to organic Hg in vaccines. Nevertheless, some studies have reported that exposure to Thimerosal appeared to decrease the risk of autism[78].

Several pesticides interfere with acetylcholine (Ach) and g-aminobutyric acid (GABA) neurotransmission, thus contributing to the etiology of ASD. This inhibition of acetylcholinesterase (AChE) by Organophosphates (Op) was reported to induce a plethora of toxicological effects due to overstimulation and consequent desensitization of, cholinergic transmission. More current research recommended that some OPs cause developmental neurotoxicity by mechanisms independent of AChE inhibition. Some pesticides interfere with the type A family of GABA receptors (GABR) and block their ability to mediate chloride fluxes. These changes implicated in autism and associated co-morbidities including sleep disturbances, anxiety, and epilepsy[79].

Table 3 — Some chemicals may be concern causes the autism disorder

Chemical	Sources of Exposure	Adverse Health Effects
Mercury	Fish Frequently enters the food chain from coal combustion	Reduced IQ, including autism. Neurodev- - developmental disorders
Lead	Paint (in houses built pre--1978). Occupational exposure occurs in battery manufacturing and recycling, car repair, and welding	Behavioral disorders; reduced IQ; increased risk of preterm labor
Pesticides	Food residues; agricultural settings; in-home use	Impaired cognitive and neurodevelopment; impaired fetal growth; increased susceptibility to testicular cancer; childhood cancer
PCBs (banned substance)	Certain fish. Fish absorb PCBs that has been dump into waterways	Development of attention deficit and hyperactivity disorder; increased body mass index; reduced IQ
Bisphenol-A (BPA)	Polycarbonate plastics; food, consumer products and packaging	Birth defects; neurodevelopmental disorders; possibly obesity, diabetes
Solvents	Industrial workplaces; used in numerous consumer products including plastics, dyes, detergents, food containers, carpeting and cleaning products, nail salons	Fetal loss; miscarriage
Phthalates	Plastics; cosmetics; cleaning products; medical devices; toys and many other everyday products	Birth defects; shortened gestational age; impaired neurodevelopment in girls
Perfluorochemicals	Food wrappers, stain-resistant and nonstick surfaces	Reduced birth weight; birth defects.
Chemicals in cigarette smoke	Cigarette smoking; second-hand smoke	Cigarette smoking; second-hand smoke

Sinha SN *et al.*, 2015 study showed a possible correlation between exposure to OP pesticides in relation to impairment in some of the neurobehavioral and immunological parameters that might be useful in assessing OP poisoning[80].

Preventions

The solution suggested is that pregnant ladies must take the following secure and proactive steps to potentially save them from harm and their babies.

Air pollution and toxic chemicals

Exposure to air pollutions such as NO and NO_2 during pregnancy was reported to increase the risk of ASD[81]. A similar effect was reported with other toxic chemicals also[82]. Hence, reduce pregnant women exposure to air pollution and toxic chemicals. Following are some chemicals indicated in the Autism disorder (Table 3) which are causes of concern for ASD.

Medications

Some results suggest that prenatal exposure to selective serotonin reuptake inhibitors (SSRIs) during the first trimester may increase the risk of ASDs. Antidepressants may be sequestered in lipophilic fetal tissues such as the brain, prolonging the period of exposure for the developing fetus after ingestion of the medication has to be discontinued by the mother[83]. Hence, diminish pregnant women exposure to antidepressant medications.

Folic acid intake

Folic acid is a B_9 vitamin and referred to as folate which is needed for healthy cells and blood[84]. Mother and child cohort study (MoBa) found prenatal folic acid supplements reduce the risk of autism spectrum disorders in children, but these findings cannot determine whether they protect against other neurodevelopmental disorders[85].

Conclusion

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that appears as a major public health problem, especially observed in 1-2% of children and usually seen before children reach 3 years of age. Screening tools should be used for assessing the Autism. The exact causes of ASD are not fully clear but increased oxidative stress, hyperserotonemia, genetic, neurologic, immunological, environmental (Air pollution and Toxic Chemicals) risk factors may be the cause of Autism. Some toxic chemicals and selective serotonin reuptake inhibitors (SSRIs) may increase the risk of ASDs. Folic acid supplements reduce the risk of autism spectrum disorders in children, but these findings cannot determine whether they protect against other neurodevelopmental disorders.

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