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Abstract:
Autism Spectrum Disorders (ASDs) are a group of complex neurodevelopmental conditions characterized by abnormal patterns of attention, and impaired social and communication skills. ASDs are also associated with a number of functional challenges and potentially harmful deficits, including restricted and repetitive behaviors, anxiety, irritability, seizures, and self-harm. Although the exact causes of ASDs are currently unknown, it is suggested that genetic, epigenetic and environmental factors play critical roles. Recent findings support evidence for synaptic defects and impairments in brain information processing that are linked to social and perceptual skills. Owing to the clinical heterogeneity and lack of precise diagnostic tools, current therapeutic approaches aimed at managing ASD-associated conditions are not definitive. In this review, we seek to provide a contemporary account of the key pathological events pertaining to autism: the theory of oxidative stress and inflammatory causes; ideas of immune dysfunction; the probable biomarkers that can be used for diagnostics - and the use of pharmaceuticals and stem cells as possible candidates for the treatment of ASDs.

**Keywords:** Autism spectrum disorders; Autism; Epigenetics; Stem cells; Nanoparticles; Neuroscience
Autism spectrum disorders (ASDs) comprise a variety of neurodevelopmental conditions, characterized primarily by impaired verbal and non-verbal communication skills, a lack of social reciprocity, and repetitive or restrictive behaviors and interests (APA’s DSM-5th Edition), likely due to anatomical and physiological abnormalities in brain connectivity. The ASDs are mainly grouped as follows: classical autism, atypical autism and Asperger’s syndrome. The two other closely related ASDs – Rett syndrome (RTT) and childhood disintegrative disorder (CDD) – show considerable symptom variation from the main ASD category. In both RTT and CDD, affected children initially demonstrate normal development, but over time show an impaired development of skills associated with interaction, language and coordination.

The prevalence of ASDs is 1 – 1.5% in the US. Males are more frequently affected than girls, with a one in 42 boys being diagnosed with an ASD compared to one in 189 girls. Despite the myriad studies conducted on ASDs thus far, the exact etiology and pathophysiology of these disorders remain poorly understood. For instance, although increasing paternal and maternal ages at birth appear to increase the risk of a child developing autism, the underlying molecular processes linking this association to disease pathogenesis remain elusive. In addition, the clear heterogeneity of symptoms between affected individuals very likely reflects the heterogeneity of pathophysiological mechanisms in ASDs. This heterogeneity provides a significant hurdle to the development of broadly applicable diagnostic tools and therapies.

However, recent studies have identified broad abnormalities such as synaptic connectivity problems, excitatory/inhibitory imbalances and white matter disorganization that may account for the impaired development of social and communication skills in children with autism. Moreover, there is growing evidence that various genetic, epigenetic and environmental factors could account for a wide range of neurodevelopmental abnormalities seen in ASDs.

In this review, we will outline the main genetic, epigenetic and environmental factors implicated in the etiology of ASDs, followed by an overview of various biomarkers that may prove useful in the diagnosis of ASDs. We will end by discussing the
potential of certain drugs, stem cells and nanoparticles as therapeutic options in ASDs (Figure 1).

**Genetic/Epigenetic Factors in ASDs**

Advances in genetic tools have helped to clarify potential causative factors in autism by enabling the identification of genes associated with ASDs. 22-25 This genetic approach to elucidating the pathophysiology of ASDs has been adopted by various groups, who utilize a diverse array of investigations, including: copy number variation (CNV) 26-32; single nucleotide polymorphisms 33-36; tandem repeat polymorphisms (TRPs) 37; and twin births. 38,39

These investigations have revealed ASD-associated mutations in a wide variety of genes that ultimately converge on several common biological pathways. For instance, many AD-associated genes appear to be linked directly or indirectly to synaptic plasticity and efficient neurotransmission. In a review of genome-wide association studies conducted so far, Glessner et al. highlighted that the risk for autism was significantly influenced by variation in a number of gene families encoding components in excitatory synapses, such as the neurexin (NRXN), CACNA, GRM, CNTN gene families. 40

Specifically, mutations in the neurexin (NRXN), 41,42 as well as in the related neuroligin (NLGN) and ProSAP/Shank gene families, 43-48 have been shown to increase the risk for ASDs by altering synaptic structure and function. NLGNs are postsynaptic adhesion molecules that bind presynaptic NRXNs with high affinity. By connecting pre- and post-synaptic neurons, they facilitate trans-synaptic signaling and shape neural network properties. At the same time, members of the ProSAP/Shank family such ProSAP2/Shank3 appear to be involved not only in synaptic transmission through AMPA and NMDA receptors, but also in regulation of the density of presynaptic and postsynaptic proteins via neurexin–neuroligin trans-synaptic signaling. ASD-associated mutations in any of these components (NRXNs, NLGNs, or ProSAPs/Shanks) could thus interfere with efficient trans-synaptic signaling and with maturation of excitatory synapses within the CNS. 48
In addition to neuronal adhesive proteins, variation in genes involved in neurotransmitter function may significantly alter the risk for autism. For example, studies have proposed a link between the dopamine transporter (DAT1) gene and ASDs, and between the dopamine D4 receptor (DRD4) gene and autistic symptoms in children with attention-deficit/hyperactivity disorder (ADHD)\(^{49}\). The former DAT1 gene encodes presynaptic membrane protein is responsible for the high-affinity reuptake of synaptically released dopamine. The DAT protein is thus an important regulator of dopaminergic tone in the CNS. A \textit{de novo} missense mutation in the DAT1 gene, which results in a Thr to Met substitution at site 356 (T356M), have recently been identified in patients with ASDs. This T356M variant is characterized by anomalous protein function, in which there is a persistent reverse transport of dopamine out of neurons. This process, known as substrate efflux, effectuates a major derangement in dopamine homeostasis, which in turn is thought to predispose to the development of ASDs and other psychopathologies.\(^{50}\)

There is now growing recognition that variation in the epigenetic state of DNA across individuals may be a significant source of variation in the risk for autism. Briefly, epigenetics describes the molecular factors at regulatory regions of DNA that modulate gene expression without changing the primary DNA sequence itself. DNA methylation is perhaps the best understood epigenetic modification, where a methyl (-CH\(_3\)) group is covalently attached to the cytosine of a CpG dinucleotide to downregulate gene expression. In this way, the expression level of a gene is hugely influenced by the epigenetic features of its surrounding regulatory regions.

Some of the genes thought to be epigenetically different in ASDs are involved in important hormonal pathways. One such gene is the retinoic acid-related orphan receptor alpha (\textit{RORA}) gene, a nuclear hormone receptor RORA that has dual roles in immune function and neurodevelopment. The RORA protein has been shown to transcriptionally regulate a number of genes, including NLGN1, NTRK2 (which encodes a catalytic receptor for neurotrophins), RBFOX1 (which encodes a splicing regulator for neuronal transcripts important for synaptic transmission and membrane
excitation), ITPR1 (which encodes a calcium channel implicated in the neurodegenerative disease infantile-onset spinocerebellar ataxia) as well as aromatase (or CYP19A1). It is thus conceivable that expression of all these downstream genes would be dysregulated by reduced expression of RORA, potentially leading to impairment of multiple neural pathways in autism.\textsuperscript{51}

Epigenetic regulation of RORA expression via differential DNA methylation has been observed in lymphoblastoid cell lines from discordantly diagnosed monozygotic twins and their nonautistic siblings. In the same study, the cerebellum and frontal cortex of autistic and age- and sex-matched control subjects revealed decreased expression of RORA in the autistic brain.\textsuperscript{52} The observation of similar methylation profiles in the brain and peripheral blood could suggest that epigenetic reprogramming is an early developmental event that occurs prior to germ layer specification. In this way, low levels of RORA during neurodevelopment due to DNA methylation could at least partially account for the defects in connectivity that thought to underlie the disease.

Interestingly, the differential expression of RORA in male and female brains could explain the strong 4:1 male bias in ASDs \textsuperscript{53}; estrogen is known to increase RORA levels in the brain while testosterone decreases it. Since aromatase, which is activated downstream to RORA, is itself responsible for aromatizing testosterone to estrogen, this sets up a positive feedback loop of decreased RORA expression - which may predispose children with high testosterone and low estrogen levels to ASDs. This relationship is depicted in Figures 2 and 3.

There is also evidence that methylation of the oxytocin receptor (OXTR) gene is associated with autism, high callous-unemotional (CU) traits, and differential activation of brain regions involved in social perception.\textsuperscript{55} In one study, DNA methylation analysis of a CpG island known to regulate OXTR expression found that several CpG dinucleotides show increased DNA methylation in the peripheral blood cells and temporal cortex in independent datasets of autistic individuals, as compared to control samples. In agreement with these findings, the same study found decreased OXTR mRNA levels in the temporal cortex tissue of age- and sex-
matched autistic individuals compared to controls.  

At present, our understanding of the chronology, regulation and reversibility of epigenetic programming during fetal development is limited. Future studies are necessary to determine whether loci such as the \textit{RORA} and \textit{OXTR} loci are epigenetically labile and susceptible to certain exposures \textit{in utero} that alter the methylation status of these genes. A more thorough understanding of epigenetic changes during the first few weeks of pregnancy would help clarify how genes and the environment interact in the pathophysiology of ASDs.

**Environmental Risk Factors in ASDs**

It has been established that genetic, epigenetic and environmental causes are closely interlinked in non-syndromic ASDs.  

As would be expected, many of these environmental challenges are driven by maternal factors. Examples include: intake of prenatal and perinatal analgesics; teratogenic/toxic agents such as thalidomide, valproic acid, ethanol and misoprostol; frequent prenatal ultrasound recordings inducing birth defects in early pregnancy; parental occupational hazards such as varnish, xylene and asphalt compounds; pre-term accumulation of trace metals such as lead, nickel, and arsenic; potential chemical hazards like organophosphorus pesticides, polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs); and highly mutagenic synthetic perfumes and cosmetics. However, it is important to note that there are inherent difficulties in forming definitive links between environment influences and ASDs. For instance, although several lines of study have associated numerous trace metals with an increased risk for autism, a recent study cast doubt on this association by suggesting that only mercury is related to autism. Hence, the matter of labeling heavy metals as a risk factor for ASDs is contentious and remains unresolved.

**Immune Dysregulation, Oxidative Stress and Neuroinflammation**
It has been established that problems with immune function are likely to contribute to the development of ASDs.\textsuperscript{77-80} In fact, immune dysfunctions, oxidative stress, mitochondrial abnormalities and neuroinflammatory processes appear to be mutually reinforcing in patients with ASDs.\textsuperscript{81-84} Results from peripheral biomarker studies emphasize the key roles of both innate and adaptive immune system deregulation in ASDs.\textsuperscript{85,86} Deregulated immune system genes in autistic subjects seem to be important causes of behavioral problems,\textsuperscript{85,87} as well as gastrointestinal complications.\textsuperscript{88,89} Loss of blood brain barrier (BBB) integrity is one of the key pathological steps in the progression of immune dysfunction associated with ASDs.\textsuperscript{90,91} Notably, mast cell activation may lead to gut-blood-brain barrier disruption, as well as brain inflammation.\textsuperscript{92}

Using a mouse model of autism, drastic changes in various immune molecules have been observed \textsuperscript{93}, whilst clinical data suggest that pro-inflammatory cytokine levels can often be correlated with autistic behaviors.\textsuperscript{94,95} Inflammatory cytokine alterations in subjects prone to environmental risks have been evident in several studies,\textsuperscript{79,93,96} and it is plausible that increased neuroinflammation provoked by elevated cytokines could contribute to observed behavioural dysfunction in autistic individuals.\textsuperscript{97-100} Th1-like (IFN-gamma, IL-2) cytokines have been found to be decreased and Th2-like (IL-4, IL-6, and IL-10) cytokines have been found to be increased in autistic children as compared to control groups.\textsuperscript{101} An elegant transcricomic analysis by Gupta et al, found that a gene expression module corresponding to M2-activation states in microglia was elevated in autism cases compared to controls. In addition, this M2-activation state is negatively correlated with a neuronal module corresponding to synaptic transmission genes that appears to be differentially expressed in autism. The enrichment of genes corresponding to an M2-activation state and simultaneous attenuation of genes implicated in synaptic transmission implicating dysregulated microglial responses in concert with altered neuronal activity-dependent genes in the pathophysiology of autism during early development.\textsuperscript{102}

Furthermore, maternal IgG antibodies such as those produced in autoimmune disease (e.g. systemic lupus erythematosus) can cross the placenta to the fetus.
While these antibodies are less likely to cross the adult BBB, the developing BBB in the fetus is more permeable and hence more vulnerable to antibody-mediated damage, as shown in Figure 4. 96,103 In fact, Braunschweig et al. found a significant correlation between maternal IgG reactivity to fetal brain proteins and a childhood diagnosis of autism. 104 Altogether, the evidence suggests that environmentally driven peripheral immune dysfunctions and associated neuropathological consequences form a series of interlinked events, which can manifest in autistic features.

As such, although there are a wide variety of genetic, epigenetic and environmental factors that have been implicated in ASDs, these factors are likely to eventually contribute to a specific underlying pathogenic mechanism – such as immune dysfunction – that may be targeted for diagnosis or therapy.

**Diagnostic Biomarkers for ASDs**

Within ASDs, a complexity of genetic polymorphisms, observed symptoms, and phenotypic variants exist, making it difficult to pinpoint a single biomarker with any real predictive value. Although a number of studies have attempted to find such biomarkers for ASDs, any promising and accurate diagnostic measures have yet to be established – and this remains a crucial, as of yet unresolved, step in the construction of targeted therapies for ASDs. Nonetheless, various categories of biomarkers have already been suggested to identify those at risk. At the hormonal level, plasma oxytocin (OT) and whole-blood serotonin (5-HT) levels are found to be consistently altered in individuals with ASD 105; whilst increased androgen (androstenediol, dehydroepiandrosterone, androstosterone and their polar conjugates) and steroid hormones (both C21 and C19) in the saliva have also been found to be indicative of anxiety and other features witnessed in autistic children, 106 and could be considered for diagnostic purposes. The latter has interesting wider correlates with neuropsychological theories of autism – such as the ‘hyper-male’ expansion of the empathizing versus systemizing dichotomy. Studies on thyroid hormone alterations are yet to establish significance among autistic subjects, though this remains a field of considerable interest. One review article implicates changes in basic biochemistry
(blood count, trace metals, thyroid and sex hormones and cholesterol tests) and transsulfuration biomarkers.\textsuperscript{107} It further describes decreases in levels of methylation, glutathione, transferrin, ceruloplasmin, carnitine and vitamin D, as well as increases in urinary 8-hydroxyguanine and isoprostane. Biomarkers of immune dysregulation such as altered serum antibodies to brain endovascularature, markers of cell-mediated immune activation (neopterin and biopterin), and increased levels of urinary N-methylhistamine seem especially pertinent but lack accuracy. Decreased blood levels of essential fatty acids (EFA), 5-oxoproline and lactic acid, and increased blood levels of arginine and taurine metabolites are also considered as biomarkers.\textsuperscript{108} Moreover, studies focusing on heavy metals as diagnostic biomarkers (increased blood and urinary levels of toxic metals and xenobiotics, RBC tests on toxic metals such as mercury, arsenic and lead) and gastrointestinal biomarkers (inflammation, urinary organic acids, gluten intolerance, food allergies) have also been reported. In other recent studies, variations in thyroid hormone (T4) levels and decreased tryptophan metabolism also offer clues for developing a potential diagnostic assay for ASDs.\textsuperscript{109,110}

miRNAs, which are small endogenous molecules that bind mRNA and silence genes through the RNA-induced silencing complex, may also be useful diagnostic biomarkers for autism. Circulating miRNAs in the serum and plasma are known to be stable and are increasingly recognized as potential noninvasive biomarkers for neurological and neurodevelopmental disorder. One study found that thirteen miRNAs were differentially expressed in autistic and non-autistic children, as shown in Figure 5.\textsuperscript{111} Using receiver operating characteristic curve analysis to evaluate the trade-off between sensitivity and specificity in diagnostic tests,\textsuperscript{112} the study authors found that five of these miRNAs (miR-181b-5p, miR-320a, miR-572, miR-130a-3p and miR-19b-3p) were highly sensitive and specific for autism.

In keeping with the theme of minimally invasive, peripheral biomarkers for the central neuropsychiatric abnormalities in ASDs, microarray work in peripheral blood mononuclear cells of infants have revealed an abnormal gene expression profile in these cells. The identification of sensitive and specific alterations in gene profiles
could form the basis of a simple, efficient and cost-effective blood-based biomarker for diagnosing autism. Moreover, since epigenetic profiles undergo reprogramming during very early gestation (from fertilization to implantation), one would expect that differential epigenetic profiles in patients in autism would be observable in tissue of both ectodermal origin (nervous tissue) and mesodermal origin (peripheral blood cells). The occurrence of a consistent and global epigenetic profile in cells of the CNS and the periphery is a crucial consideration in developing diagnostic tests based on tissue from more accessible sites in the body. In fact, future work on the safe and simple blood-based biomarkers may even culminate in the development of a routine blood spot test during childhood development to detect ASDs in a prompt and efficient manner. This would ensure adequate medical, psychological and social support is provided as early as possible to children who develop autism, which in turn may help to alleviate symptom severity, alter the developmental trajectory, and improve the quality of life in autistic patients and their families.

The use of minimally invasive neuroimaging approaches to observe the disease process or outcome in autistic patients is another promising field of study. Brain imaging and monitoring methods such as structural magnetic resonance Imaging (MRI), functional MRI (fMRI), electroencephalography, and magnetoencephalography are expanding in use, and are already identifying potentially significant alterations in brain architecture and function in ASDs (such as atypical lateralization revealing both grey and white matter defects). Figure 6 illustrates deviant auditory processing in response to an unexpected auditory stimulus in two autistic individuals. In this study, it was proposed abnormal cortical activation in the superior frontal gyrus, posterior temporal lobe, and superior and inferior parietal lobule may form a neural basis for the resistance to unexpected change in the environment in autism. In addition, another group found that multi-voxel pattern analysis (MVPA) by fMRI could be used to predict the severity of clinical symptoms of autism. Here, patients with ASD showed fusiform gyrus hypoactivation when presented with unfamiliar faces, compared to controls. The subnormal level of activation in the area of the brain primarily responsible for facial
recognition might underlie the defective face processing in individuals with ASD. More importantly, a reliable correlation was observed between MVPA classification performance and standardized measures of symptom severity, a finding which could support the use of fMRI in diagnosing and classifying ASDs.\textsuperscript{120}

**Thinking Small: Small Molecules for the Treatment of ASDs**

The progress in our understanding of the mechanistic underpinnings of ASDs has undoubtedly guided efforts to design effective treatments. To this end, many potential small molecule drugs have been tested for their potential to manage symptoms in ASDs. Unfortunately, there has been limited success with any of the compounds screened in pilot studies and clinical trials so far. Hence, it seems reasonable that a more systematic and methodical approach to search for potential treatments is necessary – for instance, priority should be given to developing accurate biomarkers, before screening a wide range of compounds. This sequential approach would not only reduce fiscal cost, but also save time and manpower.

Drugs that modulate neurotransmitter function have been extensively used in neuropsychiatric conditions. Recently, alpha\textsubscript{2A} adrenoceptor agonists such as clonidine and guanfacine have shown promise in improve symptoms of hyperactivity in autistic patients\textsuperscript{122}. In fact, an extended-release formulation of guanfacine (GXR) has already entered phase III trials in children with ADHD. Similarly, small, controlled studies have indicated a possible improvement in symptoms of hyperactivity in autistic patients given the psychostimulant methylphenidate.\textsuperscript{123} Methylphenidate is a noradrenaline-dopamine reuptake inhibitor that increases catecholamine levels in the brain, and is being extensively studied as treatment in ASDs, either in its own right or in conjunction with other drugs. In fact, an ongoing trial is investigating the concomitant use of methylphenidate and guanfacine in autistic patients.

It is worth noting that the mechanism of action of many promising drugs, as well as their effected changes in neurotransmitter levels of such, are diverse and in some instances contradictory. For instance, guanfacine is thought to decrease overall
noradrenergic tone while methylphenidate increases it. This observation reflects the fact that our understanding of the underlying deficits in ASDs, and of how exactly these drugs achieve their therapeutic effect, is still unclear. What is clear, however, is that because these drugs alter crucial signaling pathways within the brain, adverse effects are almost inevitable. For instance, alpha\textsubscript{2A}-agonists are associated with sedation and cardiovascular effects due to the decreased noradrenaline levels within the locus coeruleus and vasomotor center of the brain, respectively. In addition, selective serotonin reuptake inhibitors such as fluvoxamine and fluoxetine have been shown to provide limited improvement of ASD symptoms, probably by increasing synaptic concentrations of serotonin, which is thought to enhance synaptogenesis. Low-dose liquid fluoxetine has been shown to moderately reduce repetitive behaviors in childhood and adolescent ASDs in a small clinical trial; however, long-term trials are needed to replicate the results\textsuperscript{124} and the limited benefit may not be convincing enough to justify the risk of adverse side effects, such as disinhibition syndrome and agitation\textsuperscript{125,126}.

The better side-effect profile and promising preliminary data from trials on metabotropic glutamate receptor (mGluR) antagonists suggests that they may be promising pharmacological agents in the treatment of ASDs. In mouse studies, a mGluR antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), has been shown to enhance social behavior networks in an autistic Balb/c mouse model\textsuperscript{127}. At the same time, synaptic defects and LTD problems were ameliorated by a group II mGluR antagonist in a mouse model of Fragile X syndrome, which is related to the ASDs\textsuperscript{128}. In phase II trials, metabotropic glutamate receptor (mGluR) antagonists seem to improve socio-behavioral symptoms in ASDs and Fragile X syndrome\textsuperscript{129-131}. These agents thus signify substantial progress towards novel clinically applicable therapeutics.

Many convincing reports emphasize that antipsychotics such as haloperidol and risperidone can reduce the majority of ASD symptoms at relatively low doses. For instance, the first placebo-controlled trial of risperidone in autism, which involved 31 adults with ASDs, found that risperidone at a mean dose of 2.9 mg/d was significantly more efficacious than placebo. Fifty-seven per cent subjects on risperidone showed an improvement in the Clinical Global Impression–Improvement
scale, compared to none of the subjects on placebo. Specifically, risperidone appeared to reduce repetitive behavior as well as aggression toward the self, others, and property. However, although antipsychotics offer some promise, extrapyramidal side effects such as tardive dyskinesia with typical antipsychotics, as well as metabolic side effects such weight gain and dyslipidaemia with atypical antipsychotics, remain a major concern.\textsuperscript{123}

Other drugs being newly explored as potential therapeutic options include thiazolidinediones such as pioglitazone that are used in type 2 diabetes.\textsuperscript{1,132} Pioglitazone acts as an agonist to peroxisome proliferator activated receptor-gamma (PPAR-\(\gamma\)), and while it is often used to improve insulin sensitivity in diabetic patients, it has also demonstrated considerable benefit by suppressing symptoms associated with autistic behavior.\textsuperscript{1,133} Pioglitazone may achieve this therapeutic effect in ASDs by inducing apoptosis in activated T-lymphocytes and exerting anti-inflammatory effects in glial cells. These functions counteract the immune dysfunction that is thought to underlie ASD pathophysiology. In addition, studies have reported benefits of vitamin B6 therapy in ASDs for about three decades. Vitamin B6 is thought to balance specific metabolic pathways in neuronal cells, such as the methionine cycle, and is required for innumerable enzymatic reactions, including neurotransmitter synthesis and the production of glutathione for detoxification. Magnesium supplementation with vitamin B6 may help prevent hyperactivity and vitamin B6-induced peripheral neuropathy. A Cochrane meta-analysis in 2005 was unable to find significant evidence supporting the use of vitamin B6-Mg in ASDs due to the methodological quality and small number of trails available.\textsuperscript{134} As such, more comprehensive trials are required to evaluate the beneficial and harmful effects of agents such as PPAR-\(\gamma\) agonists and vitamin B6 in treating ASDs.

**Nano-Pharmaceuticals: Expanding the Therapeutic Toolbox**

Given the limitations of currently available small molecule drugs, it seems that novel agents with unique mechanisms of action are necessary. Nanoparticles may provide a springboard for new pharmacological agents, particularly because they would facilitate efforts to address the dysregulated immune activity and oxidative stress that have been implicated in autism.
In this regard, gold nanoparticles (AuNPs) are an interesting pharmaceutical candidate. Ghanizadeh et al. suggest that the administration of AuNPs with lipoic acid may alleviate symptoms of autism through numerous anti-inflammatory and anti-oxidant mechanisms, such as increasing glutathione peroxidase (GPx) activity and increasing the scavenging of free radicals.\textsuperscript{135} This suggestion was corroborated by another study which found that AuNPs increased expression of anti-oxidant enzymes GPx, superoxide dismutase and catalase, with an associated increase in the plasma oxygen radical absorbance capacity of autistic mice.\textsuperscript{136}

Furthermore, Kannan et al. conducted a study in which the well-established anti-inflammatory drug N-acetyl-cysteine (NAC) was conjugated to polyamidoamide (PAMAM) dendrimers and injected into newborn rabbits with cerebral palsy.\textsuperscript{137} They noted a rapid decrease in neuroinflammation and oxidative damage within 5 days of administration, as shown in Figure 7\textsuperscript{137}; given that autism is characterized by a similar neuroinflammatory process, they propose that NAC-conjugated dendrimers have the potential to be developed as a possible treatment for autism. Interestingly, it has been noted that there is normally no observable transplacental movement of G4-OH PAMAM dendrimers in humans.\textsuperscript{137} This means that prenatal therapies utilizing these dendrimers can be confined specifically to either the maternal or fetal compartment, allowing for separate regulation of maternal and fetal factors involved in autism.

It is worth emphasizing that although nanoparticles may be exquisitely suited to treat ASDs due to their unique structural and biochemical features, they may also offer an unprecedented sensitivity and resolution in in vivo biological imaging. For example, the superior optical properties and greater versatility in protein targeting of quantum dots, compared to conventional chemical labels, would be particularly useful in neuroimaging. Many studies have already deployed quantum dots to image neuronal proteins like the serotonin and dopamine transporters, as well areas of oxidative stress – and these are all parameters that are potentially altered in ASDs.\textsuperscript{138} This imaging method has notable advantages over traditional methods, in terms of minimizing ambiguities and discrepancies. As such, it should be remembered that nanoparticles are useful not just their direct therapeutic benefits, but also for their
potential role in visualizing anatomical or physiological variables within the brain, which in turn could inform other therapeutic interventions.

**Stem Cell Therapy: Hype or Hope?**

Currently, the approved therapies for ASDs alleviate the behavioral and physiological symptoms without targeting the underlying pathological causes. There is an urgent need for new and better targeted therapies, and stem cell therapy presents a particularly promising approach.\(^{139}\) Stem cell technology has expanded over time to target a wide range of neuropsychiatric disorders,\(^{140-143}\) including ASDs.\(^{141,144-148}\)

Very recently, an exciting study found that bone marrow transplantation could offer therapeutic promise in Rett syndrome and possibly other ASDs. Here, engraftment of brain parenchyma by bone marrow-derived myeloid cells of microglial phenotype occurred after transplantation of wild type bone marrow into irradiation-conditioned \textit{Mecp2}-knockout. These donor bone marrow-derived microglia were capable of restoring neural function in ASD-model \textit{Mecp2} knockout mice, potentially by counterbalancing dysregulated inflammatory pathways that occur in ASDs. This microglial engraftment was also associated with an arrest of disease development. Interestingly, the benefits mediated by wild type microglia were diminished with pharmacological inhibition of phagocytic activity. This study thus supports the notion that dysregulated immunity is an important aspect of the development and/or progression of ASDs, and that that bone marrow stem cell transplantation might alleviate this pathological factor by augmenting phagocytic activity in the brain.\(^{149}\)

While bone marrow transplantation may indeed by a feasible treatment for ASDs, studies in humans have focused instead on the targeted local delivery of stem cells within the CNS of autistic patients. It should be noted that stem cells, such as fetal and mesenchymal stem cells, have their own inherent immunomodulatory properties\(^ {150}\) and paracrine effects\(^ {151}\) that could be beneficial in treating the immune system dysregulation and deranged cytokine levels seen in ASDs. The use of hematopoietic stem cells (HSCs) to achieve these effects is illustrated in Figure 8.
A recent open label proof of concept study, involving autologous bone marrow mononuclear cells intrathecally transplanted into 32 patients with autism, successfully demonstrated improvements in various parameters associated with ASDs – including social and emotional responsiveness, communication and behavior. The therapy was also found to be safe, with three patients experiencing adverse side effects that were controlled with medication.\textsuperscript{152,153}

Ichim et al. have proposed that combination therapies using MSCs and cord blood cells could have a better outcome for autism treatment, as they could target both the immune dysregulation and neural hypoperfusion aspects of the molecular pathology in ASDs.\textsuperscript{144} This idea is based on the promising phase III results of using mesenchymal stem cells against immune dysregulation in Crohn’s disease, and positive effects of cord blood CD34\textsuperscript{+} cells as potent angiogenic stimulators in peripheral and cerebral ischemia. In fact, a non-randomized, open-label, single center phase I/II trial evaluated efficacy and safety of combined transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells (MSCs) in treatment of autistic children.\textsuperscript{154} The treatment was found to improve emotional and intellectual responses, adaptation to change, non-verbal communication, social withdrawal, stereotypic behavior, hyperactivity and inappropriate speech. The treatment did not demonstrate any safety concerns. The authors have suggested increased brain perfusion and/or immune system regulation as plausible mechanisms for the observed treatment effects. Some studies have also demonstrated potential therapeutic benefit by reducing the BBB problems in ASDs using HSCs.\textsuperscript{139}

**Conclusions and Future Directions**

In contemporary thought, neuropsychiatric disorders such as autism are often seen as the manifestation of dysfunctional genetic and brain development processes occurring during prenatal life. Yet evidence is increasingly pointing towards several non-genetic factors as having a crucial role during the prenatal period, and progress
is being made in linking these to genetic susceptibility, and ultimately to the neurodevelopmental abnormalities associated with autism. A better understanding of autism etiology could be attained by a more in-depth exploration of critical biochemical pathways/mechanisms - whilst a more detailed research focus on the prenatal environment may help resolve several outstanding diagnostic issues. Although there is much excitement surrounding the potential for pharmacological and stem cell intervention in the management of ASDs (Figure 9), such therapies remain in their infancy, and their current applications are largely experimental. However, with a greater fundamental understanding of ASDs and the neuropathological processes underlying them, such ideas could one day transform the ways in which we diagnose and manage autism in the clinical setting – and perhaps offer, for the first time, a definitive treatment.
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Captions

**Figure 1** represents the sequential discussion of etiology, diagnosis and treatment in this review, which aims to fit together these different pieces of the autism “puzzle” to obtain a more complete understanding of the condition.

**Figure 2** shows the positive and negative regulation of RORA by estrogen and testosterone respectively, as well as the downstream effect of RORA expression on aromatase (CYP19A1). The stimulatory effect of estrogen on RORA expression may explain the four-fold lower incidence of autism in females. Reproduced with permission from Sarachana et al. Copyright © 2011 PLOS

**Figures 3A and 3B** show the decreased expression of RORA and aromatase in the postmortem frontal cortices of autistic patients. Figure 3C shows the strong correlation between RORA and aromatase expression in frontal cortex neurons of autistic individuals in the same study, which is highly suggestive of a direct downstream effect of RORA on aromatase expression. Reproduced with permission from Sarachana et al 2011. Copyright © 2011 PLOS

**Figure 4.** Maternal IgG antibodies can cross the placenta and enter the developing brain of the fetus due to increased permeability of the immature BBB. These antibodies can trigger local immune destruction of brain matter, contributing to the pathophysiology of autism. Reproduced with permission from Braunschweig et al 2011. Copyright © 2011 AMA Publishing Group Journals.

**Figure 5.** Vasu et al. found that the levels of eight thirteen miRNAs were downregulated while five were upregulated in autistic children. The amount of miRNA present was quantified using normalized cycle threshold (Ct) values. Reproduced with permission from Vasu et al 2014. Copyright © 2014 BioMed Central Ltd.

**Figure 6** was obtained by fMRI and shows the brain areas that are activated above
the resting level when healthy and autistic subjects were exposed to an unpredictable tone in a sequence of otherwise predictable tones. There was markedly reduced cortical activation, especially in the frontal and temporal lobes, in autistic individuals, suggesting a compromised ability to process high-level auditory stimuli. This was accompanied by a compensatory increase in activation of the cerebellum, which has recently been implicated in sequence integration, time matching and error detection. This pattern of activation may provide a neural basis for resistance to unexpected changes in the environment in ASD children. Reproduced with permission from Hugdahl et al 2012. Copyright © 2012 Co-Action Publishing.

**Figure 7.** Panel A depicts brain sections from the periventricular white matter of a healthy rabbit kit (control) and rabbit kits with CP who were treated with PBS (acting as negative controls), 10mg/kg NAC (NAC_10), 100mg/kg NAC (NAC_100), 1mg/kg NAC-conjugated dendrimer and 10mg/kg NAC-conjugates dendrimer. Nuclei are stained with DAPI (blue), microglia are stained with lectin (red) while CD11b, which is a cell surface marker for the pro-inflammatory microglial phenotype, is stained with CD11b antibody (green). Merged areas appear yellow, corresponding to pro-inflammatory microglia which stain for both lectin and CD11b. Panel B shows the Western blot results for CD11b in the same subjects. Importantly, it can be seen that the use of dendrimers reduces the dose of NAC required, probably due to the selective accumulation of dendrimers in the brain. Reproduced with permission from Kannan et al 2012. Copyright © 2012 American Association for the Advancement of Science (AAAS).

**Figure 8.** illustrates the use of HSCs to reduce pro-inflammatory and increase anti-inflammatory cytokines, thereby suppressing the neuroinflammation that occurs in ASD. HSCs may also help recruit more tissue stem cells, positively reinforcing their therapeutic role. Reproduced with permission from Siniscalco et al 2013. Copyright © 2013 Frontiers Media S.A.

**Figure 9.** illustrates the general mechanism of action of some novel proposed therapies for ASDs, although these therapies are still in their infancy.
**Environment**
- Maternal exposures (e.g., drugs, toxins)
- Immune dysfunction, neuroinflammation

**Genes**
- Synaptic genes (e.g., neurexins)
- Genes for hormones/hormone receptors (e.g., estrogen, RORA)

**Treatment**
- Small molecule drugs
- Nanopharmaceuticals (e.g., drug-conjugated dendrimers)
- Stem cells

**Diagnosis**
- Hormone and miRNAs
- Immune and inflammatory markers
- Neuroimaging
1. Reduce inflammatory cytokines
2. Inhibit activation of microglia & astrocytes
3. Increase angiogenesis
Abbreviations

ADHD; attention deficit hyperactivity disorder
APA; American Psychiatric Association
AuNP; gold nanoparticle
ASD; autism spectrum disorder
BBB; blood-brain-barrier
CDD; childhood disintegrative disorder
CNS; central nervous system
CNV; copy number variation
CU; callous unemotional
DSM-5; Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
fMRI; functional magnetic resonance imaging
HSC; haematopoietic stem cell
MRI; magnetic resonance imaging
MSC; mesenchymal stem cell
MVPA; multi-voxel pattern analysis
OXTR; oxytocin receptor
RORA; retinoic acid related orphan receptor alpha
PAH; polynuclear aromatic hydrocarbon
PCB; polychlorinated biphenyls
RTT; Rett syndrome
TRP; tandem repeat polymorphism
Conflicts of Interest

The authors declare that no conflicts of interest exist.