

## Stem Cell Treatment of Autism Spectrum Disorders

Grace Gaston\* and Vincent S. Gallicchio

Department of Biological Sciences, College of Science, Clemson University, Clemson, SC, USA.

**\*Correspondence:**

Grace Gaston, Department of Biological Sciences, College of Science, Clemson University, Clemson, SC, USA.

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**ABSTRACT**

Autism is a spectrum of neurodevelopmental disorders. It appears differently in all individuals with ASDs and has recently become popular in research. There are many suspected genetic causes of autism, but no cure has been proven to work to treat the disorder. Stem cells have been studied for their potential role in treating ASDs. The most promising results have come from studies utilizing MSCs or hESCs. Experiments have shown that using these stem cells can reduce the severity of autism and increase the quality of life. Stem cells have also been used to model different forms of autism, including Rett Syndrome and Fragile X Syndrome. The most common stem cells used for modeling autism are iPSCs, which can retain the original genetics of the patient it was derived from. By studying autism precisely how it appears in the body, novel drug therapies and treatments can be designed. There are moral considerations that need to be taken into account when studying novel therapies for autism. The most common participants in these studies are children. This population needs to be protected, and researchers should keep their best interests in mind. Also, scientists should acknowledge their work's impact on the general population and hold each other accountable for the work they release.

**Keywords**

Autism, Stem Cells, Treatment, Modeling, Moral Considerations.

**List of Abbreviations**

ASD: Autism Spectrum Disorder, CHD8: Chromodomain Helicase DNA-binding 8, MSC: Mesenchymal Stem Cells, BMMNC: Bone Marrow Mononuclear Cell, CBMNC: Stored Cord Mononuclear Cell, CARS: Childhood Autism Rating Scale, BMAC: Bone Marrow Aspirate Concentrate, hASC: Human Adipose-derived Stem Cell, hESC: Human Embryonic Stem Cell, iPSC: Induced Pluripotent Stem Cell, FMRP: Fragile X Mental Retardation Protein.

**Introduction of Autism Spectrum Disorders**

Autism is a set of neurodevelopmental conditions that span from Kanner's syndrome to Asperger's syndrome [1]. The spectrum can be observed in many different variants and appears differently in individuals with ASD. Autism is defined in the DSM-5 as persistent deficits in social communication and social interaction, as well as restricted, repetitive patterns of behavior and interests in activities [1]. There has been rapid growth in studying ASDs in

the last 40 years, and there is a strong motivation in the research of autism genetics and treatments [2].

The disorder tends to appear differently in men and women. Studies have shown that autism affects males 4-5 times more than females, but there is also evidence that females are underrecognized [1]. There is no test for autism, as it appears differently in everyone. However, autism can be recognized early (typically as a toddler) due to atypical development [1]. It is suggested that parents could recognize the first signs of autism by 18 months of age [3]. These signs could include a lack of social smiles, ignoring people, lack of eye contact, impaired social interaction, and more [3]. As time progresses, the signs of autism could become more prevalent among parents. Between two to three of age, parents may notice impairments in language development, lack of interest in other children, or over-sensitivity to sounds or touch [3]. Once a child reaches school age, their teacher may notice signs of autism as well. These symptoms could include a lack of awareness of classroom "norms", unusual vocabulary for the child's age group, or failure to relate normally to adults [3]. Typically, parents would like to know the diagnosis of autism as soon as possible, as early

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intervention could lead to better outcomes.

There are numerous risk factors for autism have been identified. For example, complications during pregnancy and exposure to chemicals have been investigated for the risk of increasing autism [1]. There is no link between vaccinations and autism. There is a high prevalence of autism with concurrent conditions such as epilepsy and depression [1]. More severe conditions typically result in a higher autistic disability. This is suggested because of a possible shared pathophysiology or connection with the effects of growing up with autism [1].

The heritability of autism has been researched extensively, especially with twins. Study concordance rates were higher for monozygotic twins than dizygotic twins for ASDs and other developmental conditions [4]. This result has motivated researchers to uncover more of the genetic basis behind autism. Evidence has also been uncovered that *de novo* chromosomal rearrangements are higher in families where autism only affects one individual than in families where multiple individuals have autism [4]. This supports the theory that autism can be passed along in numerous ways.

There are many suspected genetic causes of ASD. Since 2016, there have been over 800 genes added to a database of genes implied to be involved in autism spectrum disorders [5]. Discoveries in the Wnt signaling pathway have revealed crucial discoveries in the progress of autism research. For example, a mutation of CHD8 has been linked to ASD risk genes. CHD8 is a transcriptional repressor that binds to  $\beta$ -catenin to decrease Wnt signaling [5]. Studies have looked into a connection between CHD8 and ASD or developmental delays. It was revealed that 15 different CHD8 mutations were found in an experiment of 3,730 children with ASD or developmental delays. It is estimated that 30% of all simplex and 45% of female diagnoses are attributed to disruptive *de novo* mutations in the network for chromatin remodeling, synaptic and Wnt/ $\beta$ -catenin signaling genes [6].

The Wnt/ $\beta$ -catenin signaling pathway is essential in the development of the central nervous system, and mutations in the pathway have led to the ubiquitination of  $\beta$ -catenin [6]. In previous studies, loss-of-function mutations in the Wnt/ $\beta$ -catenin signaling pathway have been connected to neurodegenerative diseases. In contrast, gain-of-function mutations have been linked to the onset and development of cancers [6]. Loss-of-function and gain-of-function mutations in the Wnt/ $\beta$ -catenin signaling pathway have been attributed to autism. In the loss-of-function mutation, there is abnormal myelination, severe intellectual disability, loss of function in interneurons, early differentiation, and microcephaly [6]. The gain-of-function mutation has dendritic spines defect, language delay, neuronal progenitor proliferation, and macrocephaly [6]. Overall, different genetic causes for autism and mutations in the Wnt/ $\beta$ -catenin signaling pathway could be explanatory for some cases. The relationship between genotype and phenotype has been investigated as well. It is essential to study the genetic and environmental causes behind ASD, as it greatly

impacts our society. It is estimated that about one percent of the population has autism [1]. It is usually diagnosed in childhood and evolves as a child continues to develop. Knowing the cause of ASDs can help drive treatments and help parents of children with autism further understand the disorder's causes [5].

### **Stem Cell Treatment of Autism**

As of where research is today, there is no cure for autism. There is a need for a treatment option for ASDs now more than ever. There has been an increase in the incidence rates of autism, possibly because of developments in the diagnosis of autism [7]. The prevalence of autism in 8-year-old children in the United States is 11.3 per 1000, or one in 88 [8]. Currently, research into stem cell treatment has favored using MSCs or hESCs. There have been promising results in both fields.

ASDs are associated with increased neuroinflammation and abnormal neuronal connections [9]. This has led to an interest in using MSCs to treat autism. MSCs are multipotent stem cells that can differentiate into various tissues and have high replicative capacity [8]. The uses of MSCs have been shown in neuroprotection, neurogenesis, and synaptogenesis [9]. MSCs can be derived from several tissue types, including bone marrow, adipose tissue, and birth tissues [10]. A typical source of MSCs is umbilical cord blood. A key advantage of MSCs is that they can be transplanted directly to patients without any genetic modification or pretreatment [11]. They will differentiate based on the tissues they are surrounded by, and they will not divide uncontrollably. Numerous studies have been conducted to discover the role that MSCs could have in autism treatment. A review of studies that utilized autologous BMMNC, autologous and allogeneic CBMNC, and CBMNC combined with umbilical cord MSCs to treat autism has shown promising results [9]. The findings were viewed in terms of imaging results and adverse events. The imaging results displayed an improvement in neuronal activity, hypermetabolic areas after treatment, and a highly predictive response to treatment [9]. There were no severe side effects reported. A similar study also utilized CBMNCs and umbilical cord derived MSCs to treat autism. The trial consisted of three groups: CBMNCs and rehabilitation therapy, both cell types and rehabilitation therapy, and only rehabilitation therapy [12]. While both groups receiving cell types demonstrated efficacy, the combination of CBMNCs and umbilical cord MSCs was more effective than CBMNCs alone [13]. Another study that utilized cord blood demonstrated improved communication skills in children without intellectual disabilities [10]. The children in this study also improved sustaining attention and had stronger brain waves when measured with an ECG. The use of autologous BMAC, which contains MSCs, has been shown to improve the quality of life of autistic participants [14]. It was hypothesized that MSCs would decrease inflammation, and the study revealed a reduction in the degree of impairment. In a study that investigated the use of adipose-derived MSCs with autism mice models, there was also recorded improvement in ASD [15]. The researchers specifically investigated the presence of repetitive behaviors, a common symptom of autism, in the mice by performing self-grooming tests. They found that self-grooming

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behaviors significantly decreased when treated with hASCs, which suggests the role that hASCs could have in reducing repetitive behaviors [15].

The use of hESCs to treat autism has also been studied. Embryonic stem cells are pluripotent cells that are obtained from the inner cell mass of a blastocyst-stage preimplantation embryo [16]. These cells can distinguish between all three germ layers. A case study of a three-year-old patient with ASD was conducted. The patient was reported to have a poor attention span, no eye contact, flapping of hands, lack of functional independence, and more [17]. This patient was treated with hESCs and therapy options for eight weeks. The hESC treatment involved phases, with the first phase (T1) involving three separate doses of hESCs. One dose, given once a day, was to “prime” the body. The second dose was given twice a week, and the third dose was given every seven days. The trial also used a nasal spray to enhance hESC absorption in the brain [17]. When the patient was treated with T1 for eight weeks, he showed improvement in eye contact, social behaviors, and following basic commands [17]. The second phase (T2) was conducted for four to six weeks following a four-month gap from T1. The patient's parents recorded tantrums and shorter eye contact spans during the gap between phases. However, following T2, the patient improved again [17]. The patient completed all three phases of the study and saw a great improvement in eye contact and attention span [17]. The results of this case study were like two more case studies conducted by researchers. The therapy showed promising results in improving the patients' social and behavioral skills [17]. Additionally, the researchers in this study also conducted brain scans to observe the patients' structural or functional changes in different brain regions. It has been shown that children with autism have impaired circulation in the central nervous system, which causes decreased perfusion in response to stimuli [18]. All three case studies showed improved brain blood perfusion [17]. The promising results of this study have also driven researchers toward the possibility of using hESCs to treat autism.

One of the biggest pitfalls of current research in stem cell treatment of autism is small sample sizes. This emerging field of research has demonstrated promising results, but there is still a need for larger studies to be conducted to validate the results of the smaller clinical trials. Also, another challenge in studying disorders in children are finding an effective way of tracking results accurately. For instance, it can be difficult to monitor the attentiveness or changes in communication skills in young children. Lastly, the correct dosages, timelines, ages, and how the stem cells should be administered still need to be researched thoroughly [7].

### **Stem Cell Modeling of Autism**

Disease modeling is a key tool scientists use to test and develop drugs for treating disorders. The lack of knowledge of the pathophysiology of ASD has led to a motivation to create a modeling system for autism [19]. The use of human-iPSCs to model autism has taken the forefront of this field. These could elucidate disease mechanisms and discover new therapies for treating autism [20]. The advantages of using iPSCs are similar to hESCs. For instance,

iPSCs can differentiate into all three germ layers (except cells in extraembryonic tissue), self-renew, and express stem cell markers [21]. However, iPSCs do not require embryos and can be patient-specific, unlike hESCs. iPSCs can be modeled to retain the original genetics from the patient it was derived from [22]. This ability to replicate autism in the same way it appears in the body will lead to more discoveries about autism in the future.

Rett syndrome is a neurodevelopmental disorder on the autism spectrum. It is caused by a mutation in methyl CpG-binding protein 2 [23]. From a patient with Rett syndrome, iPSCs were able to be generated. This allowed scientists to view and further understand the neuronal phenotype of this disorder. It was found that these cells had changes in voltage-gated Na<sup>+</sup> channels, impaired action potential generation, and more [23]. A similar experiment that utilized iPSCs to study Rett syndrome found two key findings. First, there was a down-regulation in genes responsible for neuronal development. Second, there was a deficit in neuronal markers directly tied to neuronal differentiation [24]. Understanding the mutations behind the syndrome and phenotypic results gives scientists a chance to test new therapies to find an effective treatment for Rett syndrome, hopefully.

Angelman Syndrome is a form of autism caused by the deletion of the maternal 15q11-q13 chromosomal region [25]. This causes a ubiquitin-protein ligase, UBE3A, not to be expressed normally. Modeling this syndrome with iPSCs has been useful. It was found that this syndrome results in impaired neuronal maturation, including a more depolarized resting membrane potential, as well as decreased excitatory synaptic activity [25]. As a result of studying Angelman Syndrome, researchers discovered the role of UBE3A on calcium- and voltage-dependent big potassium channels. This has driven researchers to develop drug targets against the big potassium channels to treat patients with this syndrome [25].

Fragile X Syndrome is due to the silencing or loss of the FMR1 gene [26]. It results in a lack of FMRP, which plays a key role in forming dendrites and synapses. iPSCs have been used to model Fragile X Syndrome and have been able to confirm the role that FMR1 plays in the phenotype. The phenotype of iPSC-derived neurons included reduced length, altered calcium influx, and fewer pre- and postsynaptic protein levels [26]. There have been advancements in developing drug therapies for Fragile X Syndrome. In a drug-screening study, six compounds were identified that increased FMR1 gene expression [26].

Another usage of iPSC modeling is in idiopathic autism. One of the biggest challenges scientists currently face is linking the phenotype to the genetics behind the disorder [27]. Studying forms of autism without a known gene association could lead scientists in the right direction of discovering more of the genetics behind autism and attempting to offer a treatment for these forms of autism. After growing iPSC from idiopathic ASD patients, it was found that there was a disproportionate ratio of inhibitory to excitatory neurons. It was also proven that the gene FOXG1 was overexpressed, and the degree of overexpression correlated

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with the severity of autism and head size [28]. An experiment examining non-syndromic autism found similar results. In the iPSC-derived neurons, there was a decrease in synaptic gene expression and a reduced spontaneous firing rate [29]. This experiment was able to tie the neuronal deficits to interleukin-6 and treat the patients by blocking interleukin-6 levels. These experiments demonstrate that even if the genetic origin of autism still isn't known, iPSC modeling could be a key to discovering more information about these ASDs.

### **Moral Considerations**

It is possible that an earlier diagnosis of autism in children can lead to more effective treatment, as a young child may be more susceptible to methods of treatment. This has led the field of autism research to be centered around children. With this, moral obligations must be considered when conducting research on ASD and stem cells. It is important to maintain a high level of research while also making it so the public can understand the results. For instance, if a paper regarding a clinical trial with vague results is published, it could cause the public to take the statements made in the paper as fact. As a result, clinical trials may push caregivers of those with autism to pursue direct-to-consumer treatments without proper regulation [30]. It is the moral responsibility of scientists, especially ones with a strong foundation in autism and stem cell research, to recognize the effects that research in this field will have on the general population. This should also motivate scientists to release the best, most accurate work they can avoid controversy and inaccurate reporting. Unclear or vague scientific findings should be avoided, and scientists should hold each other accountable for the work that they release.

While the state of current research is still in the clinical trial phases, individuals have voiced their concerns over the ethics behind these trials. For instance, a paper regarding bone marrow mononuclear cell transplantation for autism treatment has been scrutinized [31]. It is pointed out how the researchers in this trial failed on many counts, including a lack of proper stem cell preparation. The cited clinical trial also had reports of pain in almost half of the participants, and the study lacked a blinding procedure and a control group [31]. It is important to consider that autism is not a fatal condition, so invasive treatment of ASDs may need reconsidering [31]. While this is just referencing one trial in a wide field of stem cell treatment of autism, it is important to analyze the ethics behind this study. Children with autism are vulnerable and need to be protected from harmful experiments. While it is good to further scientific research in the field, it is necessary to take the proper precautions to protect this population from harm [31].

There is still a lot of progress to be made with stem cell research regarding treating ASDs. While promising studies have been published, much more research still needs to be done into the long-term effects of stem cells on the body. For instance, the ideal dosage or timeline of when treatment will be most effective is still unclear [32]. This new approach could be the future of autism treatment, but challenges still need to be overcome before it can be used as a valuable option [33].

### **Conclusion**

Autism is a set of neurodevelopmental conditions that has a wide impact on the world. The disorder appears differently and exists on a wide spectrum. While there isn't a screening process for autism, parents can typically recognize it in the first eighteen months of life. There are many suspected genetic causes of autism, including CHD8 mutations and mutations in the Wnt/ $\beta$ -catenin signaling pathway. The role of the environment and risk factors of autism have also been extensively researched. There is no current cure for autism, and there are few treatment options to help manage the disorder. This has led researchers to study stem cells as a treatment for ASDs. The two most used forms of stem cells that have been researched are MSCs and hESCs. MSCs are multipotent cells commonly derived from umbilical cord blood, while hESCs are pluripotent cells derived from the inner cell mass of embryos. Both stem cells have promising potential for treating autism. However, much more research still needs to be done before stem cells can be used as an effective treatment method for autism. Stem cells have also been researched in their role of modeling ASDs. Rett syndrome, Angelman Syndrome, Fragile X Syndrome, idiopathic autism, and more have been modeled with the use of iPSCs. By using stem cells to model autism, different drug therapies and treatments have already started to be developed. There are moral obligations that need to be considered in this field. As many of the patients being studied are children, it is the duty of scientists to ensure that they are being protected. Only accurate and proven evidence must be released to the public to avoid controversy.

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