BIOCELL
2022 46(4): 873-879

Tech Science Press

Future perspectives on cell therapy for autism spectrum disorder

Makoto NABETANI[1,*]; Takeo MUKAI[2]

[1] Department of Pediatrics, Yodogawa Christian Hospital, Osaka, 533-0024, Japan
[2] Department of Cell Processing and Transfusion, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan

Key words: Autism, ASD, Cell therapy, Umbilical cord blood cells, MSC, Mesenchymal stromal cells

Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by impairments in social communication, abnormal to absent verbal communication, the presence of repetitive stereotypic verbal and non-verbal behaviors and restricted interests, with onset early in life. We showed cognitive and behavioral characteristics of ASD by impairment of communication, cognition, perception, motor skills, executive function, theory of mind and emotion control. Recently, pathogenesis of immune pathology in the brains of individuals with ASD has been focused. New therapeutic approaches in the viewpoints of immune modulation and microglial function are logical for novel treatments for individuals with ASD. Cell therapies such as umbilical cord blood cells and mesenchymal stromal cells for ASD will be a challenging and encouraging field in the future clinical study with progress of biotechnological science.

Abbreviations

ASD:	Autism spectrum disorder
MSCs:	mesenchymal stromal cells
UCBs:	umbilical cord blood cells
EVs:	extracellular vesicles
HGF:	hepatocyte growth factor
GDNF:	glial cell line–derived neurotrophic factor
VEGF:	vascular endothelial growth factor
HIE:	hypoxic-ischemic-encephalopathy
CP:	cerebral palsy
IL-6:	interleukin 6
TNF-a:	tumor necrosis factor-alpha
BDNF:	brain derived neurotrophic factor
LPS:	lipopolysaccharide
GTPase:	guanosine triphosphatase

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by impairment in social communication, verbal communication, the presence of repetitive stereotypic verbal and non-verbal behavior and restricted interests, with an onset early in life. Prevalence of ASD has been increasing in the last two decades. In Japan, the prevalence of individuals with intellectual disabilities needing special educational program in school, including those with ASD, increased more than threefold from 21,000 in 2002 to 74,000 in 2013, similar to findings in the other countries (Ministry of Education, Culture, Sports, Science and Technology–Japan, 2013; Blumberg *et al.*, 2013; Guthrie *et al.*, 2019).

Cognitive and Behavioral Characteristics of ASD Due to Various Factors

The main cognitive and behavioral characteristics of ASD are impairment of communication, cognition, perception, motor skills, executive function, theory of mind and emotion control (Gabrielsen *et al.*, 2018; Robertson and Baron-Cohen, 2017; Mottron *et al.*, 2006; Ketcheson *et al.*, 2017; Demetriou *et al.*, 2018; Jones *et al.*, 2018; Haney *et al.*, 2018; Conner *et al.*, 2019; Baron-Cohen *et al.*, 1985) (Fig. 1) and various behavioral therapies based on neurophysiological viewpoints have been considered the foremost strategies for management of individuals on ASD (Sharma *et al.*, 2018b). Yet, there is currently no apparent effective therapy for ASD (Tachibana *et al.*, 2017).

Genetic factors (Sullivan and Geschwind, 2019; Ruzzo *et al.*, 2019; Parikshak *et al.*, 2018), chronic inflammation, impairment of the microglia, oxidative stress, hormones/neurotransmitters abnormality (Gao and Penzes, 2015; Volk *et al.*, 2015) and environmental factors (Oudin *et al.*, 2019; Rossignol *et al.*, 2014) are likely contributing to the development of ASD, however the precise pathophysiology is unknown.

More recently, pathogenesis of immune pathology in the brain of individuals with ASD has been a focus of research.

*Address correspondence to: Makoto Nabetani, a103111@ych.or.jp
Received: 02 July 2021; Accepted: 27 August 2021

Doi: 10.32604/biocell.2022.018218

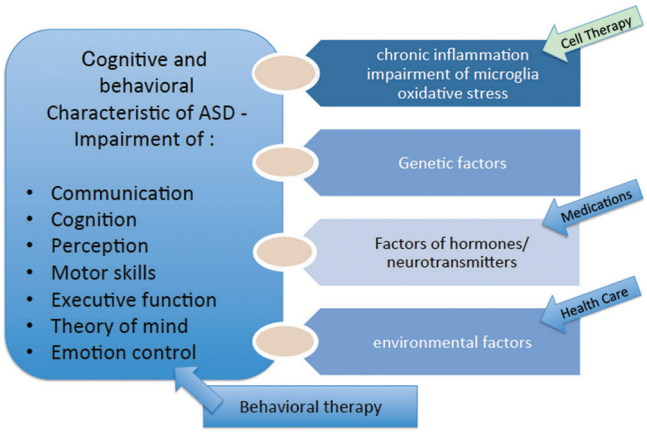

FIGURE 1. New strategy of ASD treatment.

Voineagu *et al.* (2011) reported over expression of immune-related gene networks (Voineagu *et al.*, 2011), Braunschweig *et al.* (2013) reported the presence of maternal antibodies in fetal brain tissue (Braunschweig *et al.*, 2013), Vargas *et al.* (2005) described atypical levels of pro-inflammatory cytokines (IL-6, TNF-a) in cerebrospinal fluid (CSF) of patients, and further reports suggested that excessive microglial activation leads to aberrant neural connections (Morgan *et al.*, 2010; Suzuki *et al.*, 2013).

From the recent basic research, abnormal activation of microglia by activation of TLR4 signaling pathway following maternal Lipopolysaccharide exposure, which in turn was involved in excessive synaptic pruning to decrease synaptic plasticity in the offspring may be one of the reasons for the autism-like behavior in the offspring mice (Xiao *et al.*, 2021). On the other hand, early postnatal allergic airway inflammation has been reported to induce dystrophic microglia that exhibit defective synaptic pruning upon short- and long-term allergen exposure resulting in excitatory postsynaptic surplus and ASD-like behavior (Saitoh *et al.*, 2021). These basic research suggest abnormal microglial activation which is responsible for synaptic pruning partially contributes to ASD pathology. These resulted in a novel therapeutic approach for an immunomodulation or repair of microglial function. Pre-clinical and clinical studies have shown that umbilical cord blood contains hematopoietic stem cells, endothelial progenitor cells, and mesenchymal stromal cells (MSCs) that, through paracrine signaling, alter brain connectivity and modulate inflammation (Meier *et al.*, 2006; Sun *et al.*, 2017). Infusion of autologous cord blood cells (UCBs) have been shown to be safe in individuals with cerebral palsy (CP), ASD, and other acquired brain injuries. Dawson *et al.* reported safe and feasible autologous UCBs infusions in young children with ASD and several promising outcomes were published (Dawson *et al.*, 2017; Dawson and Fletcher-Watson, 2021; Dawson *et al.*, 2020; Li *et al.*, 2015). Recently, MSCs for individuals with ASD had favorable outcomes (Lv *et al.*, 2013; Riordan *et al.*, 2019; Sun *et al.*, 2020) (Table 1).

Recent clinical trials in ASD were done by BM-MNC (n = 3), UCB (n = 2), UC-MSC (n = 2) and UCB + UC-MSC (n = 1). Frequency of administration are 1 times (n = 4), 2 times (n = 1), 3 times (n = 2) or 4 times (n = 1). Number of cells of administration are various (each dose 1×10^6–5×10^7 cells/kg). UCB and UC-MSC are performed mainly using IV, while BMMNC is performed using IT. IV injection is easier compared to IT, and UC-MSC and UCB are distributed to the central nervous system without being trapped in the lung and blood-brain-barrier. UC-MSCs therapy for ASD is performed considering neurotrophic effects of UC-MSCs in addition to immunomodulation against chronic inflammation. Few serious adverse events were observed after transplantation. Autologous BM-MNC transplantation is required in order to avoid transplant rejection, but the procedure to take BM-MNC might be harmful and administration of BM-MNC intrathecal injection in ASD could be harmful. On the other hand, most reports suggest that UC-MSCs has a therapeutic potential with relative safety and allogeneic MSCs can be ordered as a most suitable critical timing.

UCBs for individuals with ASD

UCBs deliver a protective effect from impairment of microglia, chronic inflammation and oxidative stress, as well as enhance neurological regeneration. Human CD34 positive cells have been shown to secrete various growth factors such as brain derived neurotrophic factor (BDNF), glial cell line–derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), and numerous angiogenic factors, including hepatocyte growth factor (HGF) and insulin-like growth factor-1 (Yoshihara *et al.*, 2008; Majka *et al.*, 2001). Considering that human CD34 cells have an effect on BDNF production and knowing that this neurotrophic factor is widely described as altered in ASD, this mechanism could be a potential therapeutic effect of UCBs" (Santos-Terra *et al.*, 2021; Kern *et al.*, 2013; Saghazadeh and Rezaei, 2017). Marchezan *et al.* (2018) reported that immunological and microglial changes are related to behavioral outcomes and that immunological and microglial changes in the TEA, as well as a behavioral improvement after managing these changes (Marchezan *et al.*, 2018).

Considering the angiogenic and vascular reparative capabilities of endothelial progenitor cells, there is an altered expression of genes associated with blood brain barrier integrity coupled with increased neuroinflammation and possibly impaired gut barrier integrity in the ASD brain (Fiorentino *et al.*, 2016). In 2004, Taguchi *et al.* (2004) reported that endothelial progenitor cells have angiogenic and vascular reparative capabilities that make them ideal for neurovascular repair (Taguchi *et al.*, 2004). With a rich vascular environment, along with the generation of other nurturing neuronal mediators from CD34 positive cells, such as VEGF, epidermal growth factor 2, and insulin-like growth factor 1, endothelial progenitor cells enhance subsequent neuronal regeneration (Nakatomi *et al.*, 2002). Some reports suggest that UCBs could suppress chronic inflammation in addition to paracrine and angiogenesis (Shahaduzzaman *et al.*, 2013). Impairment of microglia has been shown to have profound effect on neural development, possibly resulting in ASD (Takano, 2015). Therefore, modulation of the phenotype of the microglia may be a novel therapeutic strategy for the treatment of neurological disorders accompanied by inflammation. Li *et al.* (2016)

TABLE 1

Recent clinical review of cell therapy for Autism Spectrum Disorder

	Source	Number	Age (range)	Route of administration	Number of cells	Results	Severe adverse events
Sharma *et al.* (2012)	BM-MNC	1	8	IT	1×10^6/kg	Improvement	No
Nguyen Thanh *et al.* (2021)	BM-MNC	30	3–7	IT	1st: 4.2×10^{67}/kg 2nd: 4.1×10^7/kg	Significantly Improvement in the Childhood Autism Rating Scale (CARS), and the median Vineland Adaptive Behavior Scales	No
Lv *et al.* (2013)	UCB/UC-MSC	37	3–12	IV/IT + IV	UCB: 2×10^6/kg UC-MSC: 1×10^6/kg	Improvement in the Childhood Autism Rating Scale (CARS), Clinical Global Impression (CGI) scale and Aberrant Behavior Checklist (ABC)	No
Li *et al.* (2015)	UCB	14	3–12	IV/IT	$2 - 6 \times 10^6$ cells	Improvement in the Childhood autism rating scale (CARS) and increase of NGF levels in the CSF	No
Dawson *et al.* (2017)	UCB	25	2.26–5.97	IV	$1 - 5 \times 10^7$ cells	Significant improvements in children's behavior	No
Riordan *et al.* (2019)	UC-MSC	20	6–16	IV	3.6×10^7 cells $\times$ 4	The CARS and ATEC scores of eight subjects decreased	No
Sun *et al.* (2020)	UC-MSC	12	4–9	IV	2×106/kg $\times$ 3	6/12 participants demonstrated improvement in at least two ASD-specific measures	5 participants developed new class I anti-human leukocyte antigen (HLA) antibodies
Sharma *et al.* (2020)	BM-MNC	254	Under 5 –over 15	IT	1 time immediately after isolateon	Improvement of eye contact, attention and concentration, hyperactivity, sitting tolerance, social interaction, stereotypical behavior, aggressiveness, communication, speech, command following and self-stimulatory behavior	No

Note: UCB = umbilical cord blood; IT = intrathecal; IV = intravenous.

reported that UCBs administration at 12 h after hypoxic-ischemic event reduces white matter injury by affecting activated microglia (Li *et al.*, 2016).

In terms of glucose metabolism in ASD, Mitelman *et al.* (2018) reported that glucose metabolic rates were decreased in the parietal lobe, frontal premotor and eye-fields areas, and amygdala. Anil Kumar *et al.* (2017) reported that 4 out of the 10 patients with autism had abnormal PET scan findings, while none of the patients in the control group had abnormal PET scan. Findings of their study support the view of hypometabolism of glucose in subjects with ASD (Anil Kumar *et al.*, 2017). Sharma *et al.* (2018a) reported that [18F] 2-fluoro-2-deoxy-D-glucose PET scan was performed on 45 patients with ASD to study age-related developmental changes in the brain metabolism. Results showed that, in contrary to control data, the median standardized uptake values in patients with ASD decrease linearly with increase in age. As compared to controls, autism children below 5 years showed greater metabolism and older children showed lower metabolism. In ASD group, comparison of absolute standardized uptake values within different regions of the brain revealed relatively lower metabolism in amygdala, hippocampus, parahippocampal gyrus, caudate nucleus, cerebellum, mesial temporal lobe, thalamus, superior and middle temporal pole, and higher metabolic uptake in calcarine fissure and Heschl's gyrus. These results help in understanding the baseline metabolism and developmental changes of brain metabolism among different age groups in ASD (Sharma *et al.*, 2018a). Zhao *et al.* (2020) reported that characterized changes on glucose

metabolism, and also ASD-like behaviors in 1st and 2nd generations from 12- and 18-month-old male mice, respectively. Whole Genome Bisulfite Sequencing of sperm from advanced paternal age mice identified differentially methylated regions within the whole genome, and differentially methylated regions within promoter regions, suggesting that specific genes and relevant pathways might be associated with ASD and aberrant glucose metabolism in the offspring from advanced paternal age males. These results strongly suggest that epigenetic reprogramming induced by aging in male sperm may lead to high risks of aberrant glucose metabolism and the development of ASD behaviors in intergenerational and transgenerational offspring (Zhao *et al*., 2020). UCBs and peripheral blood mononuclear cells infusion therapy for patients with CP improved brain glucose metabolism by PET study (Min *et al*., 2013; Rah *et al*., 2017). Autologous bone marrow mononuclear cells therapies for patients with ASD also showed improvement of glucose metabolism and various cognitive and behavioral symptoms such as eye contact, attention and concentration, hyperactivity, sitting tolerance, social interaction, stereotypical behavior, aggressiveness, communication, speech, command following and self-stimulatory behavior as well as improvement of glucose metabolism and motor function in patients with CP (Sharma *et al*., 2015; Sharma *et al*., 2020). In 2020, Kikuchi-Taura *et al*. (2020) reported that angiogenesis is activated by bone marrow mononuclear cells via gap junction–mediated cell–cell interaction and that cell–cell interaction via gap junction is the prominent pathway for activation of angiogenesis at endothelial cells and improvement of glucose uptake. Transplanted BM-MNCs transferred small molecules to endothelial cells via gap junction followed by activated Hif-1α and suppressed autophagy at endothelial cells (Kikuchi-Taura *et al*., 2020). The mechanism of UCBs to improve glucose metabolism might lead to therapeutic potential for individuals with ASD.

MSCs for individuals with ASD

Sanagi *et al*. (2019) reported that microglia in patients and animals with ASD symptoms could frequently be in the apoptotic phase with high turnover rates of microglia found in some pathological conditions (Sanagi *et al*., 2019). MSCs have been reported to secrete heterogeneous lipid bilayer vesicles called extracellular vesicles (EVs), which act as mediators for inter-cell communication. These exosomes/EVs secreted from MSCs are known to improve neuronal function in neurologically injured models (Fuloria *et al*., 2021). Perets *et al*. (2020) reported long term beneficial effect of neurotrophic factors-secreting MSCs transplantation in the autism mouse model (Perets *et al*., 2020). Hadar *et al*. (2016) reported that transplantation of MSC resulted in a reduction of stereotypical behaviors, a decrease in cognitive rigidity and an improvement in social behavior. Tissue analysis revealed elevated BDNF protein levels in the hippocampus accompanied by increased hippocampal neurogenesis in the MSC-transplanted mice compared with sham treated mice. This result might indicate a possible mechanism underpinning the behavioral improvement (Segal-Gavish *et al*., 2016). Perets *et al*. (2017) reported that exosomes derived from MSC would have a direct beneficial effect on the behavioral autistic-like phenotype of the genetically modified Shank3B KO mouse model of ASD. They indicated that intranasal treatment with exosomes derived from MSC improves the core ASD-like deficits of this mouse model of ASD and therefore has the potential to treat ASD patients carrying the Shank3 mutation (Perets *et al*., 2017). Our group also demonstrated the amelioration of neuronal injury followed by functional improvement in MSC-administered mice models, which resulted from the secretion of trophic factors such as BDNF and HGF rather than neuronal differentiation and eternal cell replacement by MSCs (Mukai *et al*., 2017; 2018). Human CD34 positive cells have been shown to secrete various growth factors such as BDNF, GDNF, VEGF, and numerous angiogenic factors, including HGF and insulin-like growth factor-1.

We also demonstrated that secretomes from MSCs can change the phenotype of activated microglia. Umbilical cord derived MSCs immunomodulated microglia and changed the phenotype of LPS-activated microglia restoring actin dynamics and phagocytosis by increasing active Rho GTPase, in which microglia changed their amoeboid to a more ramified pattern (Mukai *et al*., 2021). This suppression and immunomodulation of activated microglia by MSCs may lead to therapeutic potential for individuals with ASD in which excessive microglial activation lead to aberrant neural connections. On the other hand, human UC-MSC therapies for individuals with CP showed improvement of motor function and much increase of glucose metabolism by PET-CT scan (Gu *et al*., 2020). The mechanism of MSCs to improve glucose metabolism might lead to therapeutic potential for individuals with ASD.

Future perspectives

Immune modulation and microglial function repair may serve as novel therapies for individuals with ASD and other neurological diseases. Multiple mechanisms of paracrine, angiogenesis, immunomodulation, glucose metabolism and repair of microglial function by cell therapies might lead to clinical therapeutic potential for individuals with ASD. These cell therapies for patients with ASD have been often directed at children who cannot consent clearly. We should consider cell therapy clinical trials in patients with ASD, once the ethical and diagnostic barriers can be resolved.

New strategy of ASD treatment

The main cognitive and behavioral characteristics of ASD are impairment of communication, cognition, perception, motor skills, executive function, theory of mind and emotion control. And various behavioral therapies based on neurophysiological viewpoints have been considered the foremost strategies for management of individuals on the Autism spectrum. Genetic factors, chronic inflammation, impairment of the microglia, oxidative stress, hormones/neurotransmitters abnormality and environmental factors are likely contributing to the development of ASD. These strategies such as behavioral therapies, medications to hormones/neurotransmitters abnormality and health care to prevent environmental factors show no apparent effectiveness for ASD. Cell therapies in the viewpoints of immune

modulation and microglial function could be new strategies of ASD treatments.

Acknowledgement: The authors thank Prof. Ran D. Goldman from the University of British Columbia for his valuable advice.

Author Contribution: The authors confirm contribution to the paper as follows: study conception and design: MN Author; data collection: TM Author; analysis and interpretation of results: TM Author; draft manuscript preparation: MN Author. All authors reviewed the results and approved the final version of the manuscript.

Funding Statement: We have no fund support regarding this study.

Conflicts of Interest: We have no conflict of interest to disclose regarding this study.

References

Anil Kumar BN, Malhotra S, Bhattacharya A, Grover S, Batra YK (2017). Regional cerebral glucose metabolism and its association with phenotype and cognitive functioning in patients with Autism. *Indian Journal of Psychological Medicine* **39**: 262–270. DOI 10.4103/0253-7176.207344.

Baron-Cohen S, Leslie AM, Frith U (1985). Does the autistic child have a "theory of mind"? *Cognition* **21**: 37–46. DOI 10.1016/0010-0277(85)90022-8.

Blumberg SJ, Bramlett MD, Kogan MD, Schieve LA, Jones JR et al. (2013). Changes in prevalence of parent-reported autism spectrum disorder in school-aged U.S. children: 2007 to 2011–2012. *National Health Statistics Reports,* 1–11.

Braunschweig D, Krakowiak P, Duncanson P, Boyce R, Hansen RL et al. (2013). Autism-specific maternal autoantibodies recognize critical proteins in developing brain. *Translational Psychiatry* **3**: e277. DOI 10.1038/tp.2013.50.

Conner CM, White SW, Beck KB, Golt J, Smith IC et al. (2019). Improving emotion regulation ability in autism: The Emotional Awareness and Skills Enhancement (EASE) program. *Autism* **23**: 1273–1287. DOI 10.1177/1362361318810709.

Dawson G, Sun JM, Baker J, Carpenter K, Compton S et al. (2020). A phase II randomized clinical trial of the safety and efficacy of intravenous umbilical cord blood infusion for treatment of children with autism spectrum disorder. *Journal of Pediatrics* **222**: 164–73.e5. DOI 10.1016/j.jpeds.2020.03.011.

Dawson G, Sun JM, Davlantis KS, Murias M, Franz L et al. (2017). Autologous cord blood infusions are safe and feasible in young children with autism spectrum disorder: Results of a single-center phase I open-label trial. *Stem Cells Translational Medicine* **6**: 1332–1339. DOI 10.1002/sctm.16-0474.

Dawson M, Fletcher-Watson S (2021). Commentary: What conflicts of interest tell us about autism intervention research–A commentary on Bottema-Beutel et al. 2020. *Journal of Child Psychology and Psychiatry* **62**: 16–18. DOI 10.1111/jcpp.13315.

Demetriou EA, Song CY, Park SH, Pepper KL, Naismith SL et al. (2018). Autism, Early Psychosis, and Social Anxiety Disorder: A transdiagnostic examination of executive function cognitive circuitry and contribution to disability. *Translational Psychiatry* **8**: 1606. DOI 10.1038/s41398-018-0193-8.

Fiorentino M, Sapone A, Senger S, Camhi SS, Kadzielski SM et al. (2016). Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Molecular Autism* **7**: 597. DOI 10.1186/s13229-016-0110-z.

Fuloria S, Subramaniyan V, Dahiya R, Dahiya S, Sudhakar K et al. (2021). Mesenchymal stem cell-derived extracellular vesicles: Regenerative potential and challenges'. *Biology* **10**: 172.

Gabrielsen TP, Anderson JS, Stephenson KG, Beck J, King JB et al. (2018). Functional MRI connectivity of children with autism and low verbal and cognitive performance. *Molecular Autism* **9**: 145. DOI 10.1186/s13229-018-0248-y.

Gao R, Penzes P (2015). Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders. *Current Molecular Medicine* **15**: 146–167. DOI 10.2174/1566524015666150303003028.

Gu J, Huang L, Zhang C, Wang Y, Zhang R et al. (2020). Therapeutic evidence of umbilical cord-derived mesenchymal stem cell transplantation for cerebral palsy: A randomized, controlled trial. *Stem Cell Research & Therapy* **11**: 1240. DOI 10.1186/s13287-019-1545-x.

Guthrie W, Wallis K, Bennett A, Brooks E, Dudley J et al. (2019). Accuracy of autism screening in a large pediatric network. *Pediatrics* **144**: e20183963. DOI 10.1542/peds.2018-3963.

Hadar SG, Golan K, Noy B, Ran B, Javier G, Liat E, Israel A, Daniel O, Tali K (2016). Mesenchymal stem cell transplantation promotes neurogenesis and ameliorates autism related behaviors in BTBR mice. *Autism Research* **9**: 17–32.

Haney JL, Houser L, Cullen JA (2018). Parental perceptions and child emotional and behavioral problems in Autism. *Journal of Autism and Developmental Disorders* **48**: 12–27. DOI 10.1007/s10803-017-3288-9.

Jones CRG, Simonoff E, Baird G, Pickles A, Marsden AJS et al. (2018). The association between theory of mind, executive function, and the symptoms of autism spectrum disorder. *Autism Research* **11**: 95–109. DOI 10.1002/aur.1873.

Kern JK, Geier DA, Sykes LK, Geier MR (2013). Evidence of neurodegeneration in autism spectrum disorder. *Translational Neurodegeneration* **2**: 193. DOI 10.1186/2047-9158-2-17.

Ketcheson L, Hauck J, Ulrich D (2017). The effects of an early motor skill intervention on motor skills, levels of physical activity, and socialization in young children with autism spectrum disorder: A pilot study. *Autism* **21**: 481–492. DOI 10.1177/1362361316650611.

Kikuchi-Taura A, Okinaka Y, Takeuchi Y, Ogawa Y, Maeda M et al. (2020). Bone marrow mononuclear cells activate angiogenesis via gap junction-mediated cell-cell interaction. *Stroke* **51**: 1279–1289. DOI 10.1161/STROKEAHA.119.028072.

Li J, Yawno T, Sutherland A, Loose J, Nitsos I et al. (2016). Preterm white matter brain injury is prevented by early administration of umbilical cord blood cells. *Experimental Neurology* **283**: 179–187. DOI 10.1016/j.expneurol.2016.06.017.

Li Q, Chen CF, Wang DY, Lü YT, Huan Y et al. (2015). Transplantation of umbilical cord blood mononuclear cells increases levels of nerve growth factor in the cerebrospinal fluid of patients with autism. *Genetics and Molecular Research* **14**: 8725–8732. DOI 10.4238/2015.July.31.21.

Lv YT, Zhang Y, Liu M, Qiuwaxi JN, Ashwood P et al. (2013). Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. *Journal of Translational Medicine* **11**: 2127. DOI 10.1186/1479-5876-11-196.

Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzkowski Z et al. (2001). Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and

regulate normal hematopoiesis in an autocrine/paracrine manner. *Blood* **97**: 3075–3085. DOI 10.1182/blood.V97.10.3075.

Marchezan J, Winkler Dos Santos EGA, Deckmann I, Riesgo RDS (2019). Immunological dysfunction in autism spectrum disorder: A potential target for therapy. *Neuroimmunomodulation* **25**: 300–319. DOI 10.1159/000492225.

Meier C, Middelanis J, Wasielewski B, Neuhoff S, Roth-Haerer A et al. (2006). Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatric Research* **59**: 244–249. DOI 10.1203/01.pdr.0000197309.08852.f5.

Min K, Song J, Kang JY, Ko J, Ryu JS et al. (2013). Umbilical cord blood therapy potentiated with erythropoietin for children with cerebral palsy: A double-blind, randomized, placebo-controlled trial. *Stem Cells* **31**: 581–591. DOI 10.1002/stem.1304.

Ministry of Education, Culture, Sports, Science and Technology–Japan (2013). Materials on how educational support should be after school-Data collection.

Mitelman SA, Bralet MC, Haznedar MMehmet, Hollander E, Shihabuddin L et al. (2018). Positron emission tomography assessment of cerebral glucose metabolic rates in autism spectrum disorder and schizophrenia. *Brain Imaging and Behavior* **12**: 532–546. DOI 10.1007/s11682-017-9721-z.

Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K et al. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological Psychiatry* **68**: 368–376. DOI 10.1016/j.biopsych.2010.05.024.

Mottron L, Dawson M, Soulières I, Hubert B, Burack J (2006). Enhanced perceptual functioning in autism: An update, and eight principles of autistic perception. *Journal of Autism and Developmental Disorders* **36**: 27–43. DOI 10.1007/s10803-005-0040-7.

Mukai T, Martino EDi, Tsuji S, Blomgren K, Nagamura-Inoue T et al. (2021). Umbilical cord-derived mesenchymal stromal cells immunomodulate and restore actin dynamics and phagocytosis of LPS-activated microglia via PI3K/Akt/Rho GTPase pathway. *Cell Death Discovery* **7**: 1314.

Mukai T, Mori Y, Shimazu T, Takahashi A, Tsunoda H et al. (2017). Intravenous injection of umbilical cord-derived mesenchymal stromal cells attenuates reactive gliosis and hypomyelination in a neonatal intraventricular hemorrhage model. *Neuroscience* **355**: 175–187. DOI 10.1016/j.neuroscience.2017.05.006.

Mukai T, Tojo A, Nagamura-Inoue T (2018). Umbilical cord-derived mesenchymal stromal cells contribute to neuroprotection in neonatal cortical neurons damaged by oxygen-glucose deprivation'. *Frontiers in Neurology* **9**: 281. DOI 10.3389/fneur.2018.00466.

Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O et al. (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* **110**: 429–441. DOI 10.1016/S0092-8674(02)00862-0.

Nguyen Thanh L, Nguyen HP, Ngo MD, Bui VA, Dam PTM, Bui HTP, Ngo DV, Tran KT, Dang TTT, Duong BD, Nguyen PAT, Forsyth N, Heke M (2021). 'Outcomes of bone marrow mononuclear cell transplantation combined with interventional education for autism spectrum disorder'. *Stem Cells Translational Medicine* **10**: 14–26.

Oudin A, Frondelius K, Haglund N, Källén K, Forsberg B et al. (2019). Prenatal exposure to air pollution as a potential risk factor for autism and ADHD. *Environment International* **133**: 105149. DOI 10.1016/j.envint.2019.105149.

Parikshak NN, Swarup V, Belgard TG, Irimia M, Ramaswami G et al. (2018). Author Correction: Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. *Nature* **560**: E30. DOI 10.1038/s41586-018-0295-8.

Perets N, Oron O, Herman S, Elliott E, Offen D (2020). Exosomes derived from mesenchymal stem cells improved core symptoms of genetically modified mouse model of autism Shank3B. *Molecular Autism* **11**: 409.

Perets N, Segal-Gavish H, Gothelf Y, Barzilay R, Barhum Y et al. (2017). Long term beneficial effect of neurotrophic factors-secreting mesenchymal stem cells transplantation in the BTBR mouse model of autism. *Behavioural Brain Research* **331**: 254–260. DOI 10.1016/j.bbr.2017.03.047.

Rah WJ, Lee YH, Moon JH, Jun HJ, Kang HR et al. (2017). Neuroregenerative potential of intravenous G-CSF and autologous peripheral blood stem cells in children with cerebral palsy: A randomized, double-blind, cross-over study. *Journal of Translational Medicine* **15**: 304. DOI 10.1186/s12967-017-1120-0.

Riordan NH, Hincapié ML, Morales I, Fernández G, Allen N et al. (2019). Allogeneic human umbilical cord mesenchymal stem cells for the treatment of autism spectrum disorder in children: Safety profile and effect on cytokine levels. *Stem Cells Translational Medicine* **8**: 1008–1016. DOI 10.1002/sctm.19-0010.

Robertson CE, Baron-Cohen S (2017). Sensory perception in autism. *Nature Reviews Neuroscience* **18**: 671–684. DOI 10.1038/nrn.2017.112.

Rossignol DA, Genuis SJ, Frye RE (2014). Environmental toxicants and autism spectrum disorders: A systematic review. *Translational Psychiatry* **4**: e360. DOI 10.1038/tp.2014.4.

Ruzzo EK, Pérez-Cano L, Jung JY, Wang LK, Kashef-Haghighi D et al. (2019). Inherited and *de novo* genetic risk for autism impacts shared networks. *Cell* **178**: 850–866.e26. DOI 10.1016/j.cell.2019.07.015.

Saghazadeh A, Rezaei N (2017). Brain-derived neurotrophic factor levels in autism: A systematic review and meta-analysis. *Journal of Autism and Developmental Disorders* **47**: 1018–1029. DOI 10.1007/s10803-016-3024-x.

Saitoh BY, Tanaka E, Yamamoto N, Kruining DV, Iinuma K et al. (2021). Early postnatal allergic airway inflammation induces dystrophic microglia leading to excitatory postsynaptic surplus and autism-like behavior. *Brain Behavior & Immunity* **95**: 362–380. DOI 10.1016/j.bbi.2021.04.008.

Sanagi T, Sasaki T, Nakagaki K, Minamimoto T, Kohsaka S et al. (2019). Segmented Iba1-positive processes of microglia in autism model marmosets. *Frontiers in Cellular Neuroscience* **13**: 151. DOI 10.3389/fncel.2019.00344.

Santos-Terra J, Deckmann I, Schwingel GBrum, Vinicius Contri Paz A, Gama CS et al. (2021). Resveratrol prevents long-term structural hippocampal alterations and modulates interneuron organization in an animal model of ASD. *Brain Research* **147593**.

Segal-Gavish H, Karvat G, Barak N, Barzilay R, Ganz J et al. (2016). Mesenchymal stem cell transplantation promotes neurogenesis and ameliorates autism related behaviors in BTBR mice. *Autism Research* **9**: 17–32. DOI 10.1002/aur.1530.

Shahaduzzaman M, Golden JE, Green S, Gronda AE, Adrien E et al. (2013). A single administration of human umbilical cord blood T cells produces long-lasting effects in the aging hippocampus. *Age* **35**: 2071–2087. DOI 10.1007/s11357-012-9496-5.

Sharma A, Gokulchandran N, Chopra G, Kulkarni P, Lohia M, Badhe P, Jacob VC (2012). Administration of autologous bone

marrow-derived mononuclear cells in children with incurable neurological disorders and injury is safe and improves their quality of life. *Cell Transplant* **21**: S79–S90.

Sharma A, Gokulchandran N, Sane H, Nivins S, Paranjape A, Badhe P (2018a). The baseline pattern and age-related developmental metabolic changes in the brain of children with autism as measured on positron emission tomography/computed tomography scan. *World Journal of Nuclear Medicine* **17**: 94–101.

Sharma AK, Gokulchandran N, Kulkarni PP, Sane HM, Sharma R et al. (2020). Cell transplantation as a novel therapeutic strategy for autism spectrum disorders: A clinical study. *Americal Journal of Stem Cells* **9**: 89–100.

Sharma A, Sane H, Gokulchandran N, Kulkarni P, Gandhi S et al. (2015). A clinical study of autologous bone marrow mononuclear cells for cerebral palsy patients: A new frontier. *Stem Cells International* **2015**: 1–11. DOI 10.1155/2015/905874.

Sharma SR, Gonda X, Tarazi FI (2018b). Autism Spectrum Disorder: Classification, diagnosis and therapy. *Pharmacology and Therapeutics* **190**: 91–104. DOI 10.1016/j.pharmthera.2018.05.007.

Sullivan PF, Geschwind DH (2019). Defining the genetic, genomic, cellular, and diagnostic architectures of psychiatric disorders. *Cell* **177**: 162–183. DOI 10.1016/j.cell.2019.01.015.

Sun JM, Dawson G, Franz L, Howard J, McLaughlin C et al. (2020). Infusion of human umbilical cord tissue mesenchymal stromal cells in children with autism spectrum disorder. *Stem Cells Translational Medicine* **9**: 1137–1146. DOI 10.1002/sctm.19-0434.

Sun JM, Song AW, Case LE, Mikati MA, Gustafson KE et al. (2017). Effect of autologous cord blood infusion on motor function and brain connectivity in young children with cerebral palsy: A randomized, placebo-controlled trial. *Stem Cells Translational Medicine* **6**: 2071–2078. DOI 10.1002/sctm.17-0102.

Suzuki K, Sugihara G, Ouchi Y, Nakamura K, Futatsubashi M et al. (2013). Microglial activation in young adults with autism spectrum disorder. *JAMA Psychiatry* **70**: 49–58. DOI 10.1001/jamapsychiatry.2013.272.

Tachibana Y, Miyazaki C, Ota E, Mori R, Hwang Y et al. (2017). A systematic review and meta-analysis of comprehensive interventions for pre-school children with autism spectrum disorder (ASD). *PLoS One* **12**: e0186502. DOI 10.1371/journal.pone.0186502.

Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H et al. (2004). Administration of $CD34^+$ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *Journal of Clinical Investigation* **114**: 330–338. DOI 10.1172/JCI200420622.

Takano T (2015). Role of microglia in autism: Recent advances. *Developmental Neuroscience* **37**: 195–202. DOI 10.1159/000398791.

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology* **57**: 67–81. DOI 10.1002/(ISSN)1531-8249.

Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y et al. (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* **474**: 380–384. DOI 10.1038/nature10110.

Volk L, Chiu SL, Sharma K, Huganir RL (2015). Glutamate synapses in human cognitive disorders. *Annual Review of Neuroscience* **38**: 127–149. DOI 10.1146/annurev-neuro-071714-033821.

Xiao L, Yan J, Feng D, Ye S, Yang T et al. (2021). Critical role of TLR4 on the microglia activation induced by maternal LPS exposure leading to ASD-like behavior of offspring. *Frontiers in Cell and Developmental Biology* **9**: 99. DOI 10.3389/fcell.2021.634837.

Yoshihara T, Taguchi A, Matsuyama T, Shimizu Y, Kikuchi-Taura A et al. (2008). Increase in circulating CD34-positive cells in patients with angiographic evidence of moyamoya-like vessels. *Journal of Cerebral Blood Flow & Metabolism* **28**: 1086–1089. DOI 10.1038/jcbfm.2008.1.

Zhao WL, Gu NH, Li ZZ, Wang GS, Cheng CY, Sun F (2020). Autism-like behaviors and abnormality of glucose metabolism in offspring derived from aging males with epigenetically modified sperm. *Aging* **12**: 19766–19784. DOI 10.18632/aging.104061.