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REVIEW ARTICLE

Social Skills Deficits in Autism Spectrum Disorder: Potential Biological Origins and Progress in Developing Therapeutic Agents

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Abstract

Autism spectrum disorder is defined by two core symptoms: a deficit in social communication and the presence of repetitive behaviors and/or restricted interests. Currently, there is no US Food and Drug Administration-approved drug for these core symptoms. This article reviews the biological origins of the social function deficit associated with autism spectrum disorder and the drug therapies with the potential to treat this deficit. A review of the history of autism demonstrates that a deficit in social interaction has been the defining feature of the concept of autism from its conception. Abnormalities identified in early social skill development and an overview of the pathophysiology abnormalities associated with autism spectrum disorder are discussed as are the abnormalities in brain circuits associated with the social function deficit. Previous and ongoing clinical trials examining agents that have the potential to improve social deficits associated with autism spectrum disorder are discussed in detail. This discussion reveals that agents such as oxytocin and propranolol are particularly promising and undergoing active investigation, while other agents such as vasopressin agonists and antagonists are being activity investigated but have limited published evidence at this time. In addition, agents such as bumetanide and manipulation of the enteric microbiome using microbiota transfer therapy appear to have promising effects on core autism spectrum disorder symptoms including social function. Other pertinent issues associated with developing treatments in autism spectrum disorder, such as disease heterogeneity, high placebo response rates, trial design, and the most appropriate way of assessing effects on social skills (outcome measures), are also discussed.

Key Points

Currently, there is no US Food and Drug Administration-approved drug for the core symptoms of autism spectrum disorder.

Oxytocin, propranolol, and vasopressin agonists and antagonists are particularly promising agents for treating social function defects for individuals with autism, which are currently undergoing clinical investigations.

Bumetanide and microbiota transfer therapy have promising effects on core autism symptoms including social function.

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1 Introduction

Autism spectrum disorder (ASD) is a behaviorally defined neurodevelopmental disorder now affecting almost 2% of children in USA [1]. Although ASD is a very heterogeneous disorder with many associated psychiatric and medical co-morbidities, specific core symptoms define its diagnosis. ASD is defined by a deficit in social communication along with the presence of repetitive and/or restricted interests and/or behaviors. Until recently, the deficits in social function and communication were considered two separate core symptoms. One consistency in the definition of autism as it has evolved over the past century is that a deficit in social function has always been included in defining autism.

This article reviews the history of autism, the development of social skills in typically developing (TD) children and how they develop abnormally in autism, the general pathophysiology of autism, outcome measures used to assess social function, and unique challenges in studying autism. Promising therapies that may address social deficits as well as the biological mechanisms that they target are discussed in detail. Overall, this article provides insights into

the promising therapies for treating abnormalities in social function in individuals with ASD as well as the challenges faced in this area of research.

2 Evolution of the Diagnosis of Autism Spectrum Disorder

Bleuler introduced the term 'autism' in 1911 to describe adults with schizophrenia. Some believed that the term 'autism' was derived from the Freudian term 'autoerotism', a term used to describe a self-soothing, very immature pre-infantile stage of hallucinatory thinking that preceded engagement with external reality [2]. Other accounts suggested that it originated from the Latin 'Autismus', which is derived from the Greek 'autos' meaning self, thus describing conditions where patients are morbidly self-absorbed. Piaget first applied the term to children to describe a pre-verbal stage of infant development [3]. Through the first half of the twentieth century, psychologists and psychiatrists in Britain and USA recognized a type of schizophrenia of childhood that is believed to be similar to what we know as autism today [2].

In the 1940s, two practitioners independently first used the term autism to describe a syndrome of childhood psychopathology. In 1943, Leo Kanner used the word autism to describe a unique syndrome in a series of children with obsessiveness, stereotypy, echolalia, and almost a complete lack of interaction with people [4]. In parallel, Hans Asperger, a Viennese child psychologist, described a disorder characterized by a lack of empathy, little ability to form friendships, one-sided conversation, intense absorption in a special interest, and clumsy movements [5]. This syndrome was later known as Asperger's syndrome (AS).

Psychiatry considered autism to be part of childhood schizophrenia until 1980 when it was described as its own entity in the *Diagnostic Statistical Manual of Mental Disorders*, 3rd Edition (DSM-III). In 1987, the DSM-III-Revised introduced the category of Pervasive Developmental Disorder, Not Otherwise Specified to capture children who did not meet the full criteria for autism and, in 1994, the DSM-IV first officially recognized AS [6]. AS was defined in the DSM-IV as having a deficit in social function along with repetitive and/or restrictive interests and/or behaviors but without early language impairment.

The most recent version of the DSM (DSM-V) has come full circle, dropping the subcategories of Pervasive Developmental Disorder, Not Otherwise Specified, AS, and autistic disorder, now describing autism as a true spectrum depending on levels of impairment. Previous to the DSM-V, autism was diagnosed by impairment of both social interactions and communication along with the presence of repetitive movements and/or restrictive interests. Currently, the DSM-V posits that social interactions and communication are so inter-related that they are hard to separate. Thus, the DSM-V has combined these two impairments to define one of the core impairments as one significant abnormality in social communication. The DSM-V now only recognizes ASD as a diagnosis. Thus, ASD will be used throughout the remainder of the article.

3 Social Development and Autism Spectrum Disorder

The study of children who develop ASD has informed our knowledge of early social development. Early social behavior has been studied by reviewing videotapes of first birthday parties of children eventually diagnosed with ASD. Osterling and Dawson [7] demonstrated that children eventually diagnosed with ASD demonstrated significant fewer pointing and showing behaviors and less looking at people and orienting to their name as compared with TD peers. Likewise, Osterling et al. [8] found that children diagnosed with ASD or intellectual disability used fewer gestures and showed more repetitive motor movements than TD peers but only children with ASD looked at people and oriented to their name less than TD peers. Other studies have used retrospective questionnaires to investigate specific deficits in children that develop ASD as compared to TD peers or those who proceed to have developmental delays but not ASD [9, 10] (see Table 1).

One of the most consistent social deficits in children who develop ASD is a lack of non-verbal social gestures such as pointing, showing, and giving. Pointing starts to develop around 8 months of age and should make up the majority of gestures by 12 months of age [11]. Two types of pointing develop during childhood. Protoimperative pointing, a gesture that indicates what a child wants, is developmentally absent in young children with ASD although it sometimes develops in older children with ASD. Protodeclarative pointing is a joint attention gesture that is used to share experiences. Other important protodeclarative gestures that develop in early childhood include 'showing' and 'giving.' In 'showing' gestures, a child brings an object of interest

Table 1 Early developmental deficits in children diagnosed with autism as compared with typically developing children and children with other neurodevelopmental disorders

Imitation	Eye contact
Pointing at objects	Orienting to name
Playing 'peek-a-boo'	Joint attention
Seeking and enjoying cuddling	Responding to name
Checking for parents	Following someone's point
Interest in other children	Social smiling
Waving bye-bye without prompting	Demanding attention

to someone and extends their arms out holding the object toward a person's face to share their interest. In 'giving' gestures, a child places an object in someone's hand to share the object of interest with the person. These protodeclarative gestures are characteristically absent in ASD.

Quantitative measures of early behavior have concentrated on visual attention. In one of the first reports, children later diagnosed with ASD demonstrated different patterns of visual attention to point-light animation that simulated human movements at 15 months of age as compared to TD children [12]. Eye movement studies then demonstrated that children that went on to develop ASD looked at the mouth rather than the eyes at 15 months of age when looking at faces [13]. More recently, Jones and Klin [14] demonstrated that children later diagnosed with ASD lose the normal preference of attendance to the eyes when looking at faces in the first year of life.

The social skills deficits in individuals with high-functioning ASD (HFASD) have been attributed to deficits in several cognitive components, including the theory of mind and pragmatic competence [15], cognitive processing speed [16, 17], and metacognitive processes such as initiation and planning [18]. Deficits identified in AS provide some insight into the subtleties of the cognitive deficits in social function [19]. For example, although language is not overtly abnormal in AS, particular components of language competence may be deficient. Individuals with AS have adequate vocabulary and grammar skill but poor inference and comprehension of narrative. Although those with AS are thought to have a poor sense of humor, they actually have a deficit in gelotophilia (i.e., laughing at themselves) but show intact katagelasticism (i.e., laughing at others).

Despite research defining the basic cognitive deficits that underlie ASD, it is important to remember that the key neurophysiological deficit is probably at a cellular level, suggesting a systems-level dysfunction of the nervous system rather than a specific neural pathway or region of the brain that is affected. Thus, the systems-level dysfunction in neural systems may express itself in a slightly different manner depending on other intrinsic and extrinsic factors, leading to the heterogeneity that we find in the ASD population.

4 General Pathophysiology of Autism

4.1 Neuropathological Abnormalities

Abnormalities have been reported in almost every brain region, from the lower brainstem to the cortex, in individuals with ASD. First reported were abnormalities in the cerebellum and brain stem, including changes in cerebellar volume [20, 21], vermis agenesis [22], and loss of Purkinje and granule cells and changes in the inferior olive [23]. Reductions in neuronal size and neuronal density are found in the limbic system [23, 24], particularly in CA1 and CA4, and hippocampal dendritic branching is reduced [25]. The trajectory of amygdala growth is unique in ASD with atypical age-dependent changes in dendritic spine density leading to amygdala dysfunction [26].

Brain growth in some individuals with ASD appears accelerated during the first years of life [20] followed by being prematurely diminished by early childhood [27, 28]. The early acceleration in head circumference is linked to increased brain volume [29, 30], non-neural tissue [31], and extra-axial fluid [32]. Increased white [21, 29] and gray [20, 21] matter volume has been reported in ASD. Increased white matter volume has been attributed to more short association fibers in the frontal and temporal lobes [33]. This is proposed to cause an imbalance between local and distant cortical communication and change the whole brain network architecture [34], leading to a deficit in large-scale cortical integration that is required for language, behavioral regulation, and social interactions. Other gray matter cortical abnormalities such as smaller, more compact, and numerous cortical minicolumns, particularly in the frontotemporal areas [35, 36] are reported in ASD. This is associated with a reduction in peripheral neuropil space [37], a space that contains gamma aminobutyric acid (GABA) inhibitory interneurons [36–42].

The association of tics and repetitive movement and response to anti-psychotic medications implicate basal ganglia circuits in ASD. The basal ganglia is also essential in eye movement, coordination, sensory modulation, and inhibition control, all neurological functions that are impaired in ASD [43]. Interestingly, the cerebellum, another structure implicated in ASD, and basal ganglia are connected through a short disynaptic pathway, highlighting their functional co-dependence [43]. The cerebellum is recognized for its role in cognition and affect, given the rich connection with the cerebral cortex [44].

4.2 Neurotransmitter Abnormalities

Autism spectrum disorder is associated with multiple neurotransmitter abnormalities, most notably abnormalities in monoamine (i.e., dopamine, norepinephrine, serotonin) [45–47] and amino acid (i.e., glutamate, GABA) [48–50] neurotransmitters. Excitation-to-inhibition imbalance [51] is believed to result in cortical hyperexcitability, which is associated with such ASD symptomatology as auditory-tactile hypersensitivity and seizures, in some cases [52], and abnormal gamma oscillations [53]. Several medications that target GABA (inhibitory) and glutamate (excitatory) neurotransmission have been developed using the Fragile X mouse model of ASD [54, 55].

$GABA_A$ neurotransmitters can be either excitatory or inhibitory, depending on the intracellular chloride level,

which is regulated by the balance between the cation–chloride importer NKCC1 and exporter KCC2 [50]. Normally, early in life, KCC2 expression significantly increases, thereby decreasing intracellular chloride, causing $GABA_A$ channels to be inhibitory by hyperpolarizing neurons. It is believed that some children with ASD have a failure in this developmental change in KCC2 expression resulting in $GABA_A$ remaining excitatory [56].

There are multiple etiologies for neurotransmitter abnormalities including genetic mutations [55, 57] and metabolic disturbances. Abnormalities in redox metabolism found in the brain of children with ASD [58, 59] can disrupt glutamate metabolism [60, 61] and metabolic disorders associated with ASD, such as succinic semialdehyde dehydrogenase deficiency [62] and mitochondrial disorders [63], can influence GABAergic neurotransmission [64]. Monoamine neurotransmitter production can be disrupted by known metabolic abnormalities associated with disturbances in folate [65, 66] or tetrahydrobiopterin [45–47] metabolism. One neurotransmitter that is becoming increasing recognized to have a role in ASD, particularly in social impairment, is oxytocin [67]. Oxytocin is being found to modulate the activity of key brain regions associated with social cognition during socially relevant tasks [68].

4.3 Metabolic Disorders

Several metabolic disorders associated with ASD have associated treatments [65]. Folate is essential for many critical metabolic processes, including redox metabolism and methylation [69] and ASD is associated with polymorphisms in folate-related genes [70–81] and impaired folate transport across the blood–brain barrier as a result of folate receptor-α dysfunction [66, 82, 83]. Lower cobalamin levels have been reported in post-mortem ASD brain [84] and polymorphisms in cobalamin-associated genes [79] and enzymes [84] are associated with ASD. Tetrahydrobiopterin (BH_4) is critical for brain function, including the production of monoamine neurotransmitters, the breakdown of phenylalanine, and the production of nitric oxide [47]. The central (i.e., cerebrospinal fluid) level of BH_4 is depressed in some individuals with ASD, particularly early in life [45, 47]. Carnitine deficiency may be common in ASD, based on adult reference ranges [85], and a defect in the gene that codes for the first enzyme in the carnitine biosynthesis pathway (TMLHE) is a risk factor for ASD [86]. In addition, some individuals with ASD have abnormal redox metabolism [59, 61, 71] and mitochondrial dysfunction [87]. It should be noted that metabolic systems are very interconnected and influenced by multiple factors, making the evaluation, treatment, and significance of metabolic disturbance very heterogeneous and complex.

4.4 Immune Abnormalities

ASD is associated with autoantibodies to neural tissue, including neuron-axon filament proteins, cerebellar neurofilaments, myelin basic protein, caudate, and serotonin receptors, just to name a few [88]. Recent studies implicate maternal antibodies that are believed to bind to and disrupt the development of the fetal brain prenatally [89]. Elevated cytokine levels in the cerebrospinal fluid and blood have been identified, particularly in cytokines associated with the innate immune system [90]. ASD has been associated with prenatal and postnatal infections, familial autoimmunity, and gastrointestinal inflammation, further suggesting a role for the immune system [88].

4.5 Microbiome Imbalance

There is a growing body of evidence that the trillions of microbes that inhabit the human digestive tract, known as the enteric microbiome, play a role in brain development and behavior [91]. The enteric microbiome may be atypical in ASD [92–97] with a decrease in diversity and an overrepresentation of disruptive species, including Clostridial species [98, 99]. Microbiome disruption may be associated with children who have gastrointestinal symptoms at the time of, or prior to, the onset of ASD symptoms and in those with regressive-type ASD [100, 101]. An intriguing mouse model showed that altering the enteric microbiome using a probiotic ameliorated ASD-like behaviors [102]. Because most microbiome studies are correlational, atypical diet, altered metabolic and immune function, stress and/or inflammation could be driving microbiota alterations.

5 Brain Circuits Specifically Involved in Social Interactions

Social behavior involves a wide range of cognitive processes, including perception, attention, memory, motivation, and emotion [103]. The medial prefrontal cortex, temporoparietal junction, and posterior temporal sulcus are developmentally involved in the theory of mind skills while limbic structures including the amygdala, insula, and ventral striatum are intimately involved in emotional perception, expression, and regulation [104]. Structural and functional connectivity studies have found differences between TD individuals and individuals with ASD in these areas involved in social cognition [105]. Multiple studies have demonstrated changes in resting-state activity associated with ASD but heterogeneous methodology has prevented solid conclusions in this area of imaging research [106].

6 Outcome Measures for Assessing Social Function

A large majority of clinical trials use parent-reported outcomes. The Aberrant Behavior Checklist (ABC), a measure of disruptive behaviors, is most common, while trials focusing on social symptoms, commonly used the Social Responsiveness Scale (SRS). Despite some trials demonstrating a large placebo effect with the SRS [107], a meta-analysis of outcomes for the controlled group, social skills intervention trials found that the SRS demonstrated a large effect size [108]. Given the large heterogeneity in symptoms in children with ASD as well as the fact that some symptoms are more important to parents than other symptoms, an innovative approach is to individualize outcomes by rating targeted behaviors selected by parents [109].

Psychiatric studies widely use the clinician-rated Clinical Global Impression (CGI) scale. A scale based on the CGI that is specific for ASD, known as the Ohio Autism Clinical Impression Scale, has been developed and validated [110] and found to be reliable across cultures [111], yet it has only been used in a handful of studies [82, 112, 113]. Many clinical trials have measured change in ASD symptoms using the Childhood Autism Rating Scale (CARS) or the Autism Diagnostic Observation Schedule (ADOS), although these are instruments designed for diagnosis. The Calibrated Severity Score has been developed to measure change in ASD symptoms over time using the ADOS [114–116]. The Brief Observation of Social Communication Change has been developed to be more sensitive than the Calibrated Severity Score [117] and others have piloted alternative measures such as the General Social Outcome Measure [118].

Many studies use standard diagnostic instruments to measure changes in language, cognition, or intelligence. One of the issues with this approach is that they have a floor to the range in which they will measure ability. Because many individuals with ASD will be below the floor of the instrument, the instrument may not be sensitive to any change in ability as even the repeat measurement may be below the floor of the measurement range. In our recent clinical trial, we developed an algorithm to select the most ability-appropriate language test with an adequate dynamic range for optimally measuring change over time [82].

Beyond standard traditional outcome measures, investigators have utilized technology to develop more objective outcome measures. For example, actigraphy, which has been pioneered in monitoring sleep, has also been utilized to monitor behavior and activity level during the daytime [119]. Image analysis of movements with instruments such as the Kinect camera has been used to quantify repetitive movements [120]. Social visual stimuli such as faces with various emotions are used in conjunction with eye-tracking devices to monitor changes in visual attention [121]. Last, clinical trials are utilizing functional imaging in individuals with ASD to determine changes in activation and connectivity of key brain regions to validate treatment effectiveness.

Still, one must consider the face validity of the instruments used. Social function is a very complex cognitive construct with many components. Many outcome measures merely measure specific cognitive or behavioral components that may be necessary but not sufficient for successful social function. Some instruments represent very contrived social situations that may not represent real-world situations, particularly for the spontaneity and dynamics necessary for social competency. Clearly, better outcome measures using more natural social interactions need to be developed to better predict outcomes.

7 Drug Treatments for Improving Social Interaction in Autism Spectrum Disorder

The standard-of-care treatment for core ASD symptoms, particularly social interactions, is behavioral therapy such as applied behavioral analysis. However, such therapy requires full-time engagement with a one-on-one therapist over several years starting early in life [122, 123], is inconsistently covered by medical insurance, is not uniformly available in the education system [124, 125], typically results in incomplete recovery [122], and does not address the underlying pathophysiology of ASD. Indeed, despite standard-of-care behavioral therapy, optimal outcomes occur in only a minority of individuals with ASD and many individuals with ASD require life-long supportive care into adulthood [126]. Thus, safe and effective treatments that can augment available behavioral and educational interventions could accelerate achievement of optimal outcomes for a greater proportion of children with ASD.

Development of drug treatments for ASD uses several approaches. One approach targets neural pathways involved in social cognition by manipulating specific neurotransmitters, such as OT. The other targets brain-specific physiological abnormalities or non-brain systematic abnormalities to improve overall brain function. Table 2 outlines the controlled clinical trials discussed in this section that have at least one measure of social function. Secondary outcome measure(s) pertaining to social function are outlined if the primary outcome measure does not reflect a measure of social function. For outcomes that are significant, either the effect of the treatment and placebo are reported or the overall effect size. In some cases, neither was reported, thus the outcome is just noted as significantly different between the two groups. If there is no significant (NS) difference that is noted also noted. All the secondary measures used in the study are also listed. Table 3 outlines the significant adverse effects reported in controlled trials for the treatments discussed.

Table 2 Summary of the results of controlled studies of promising pharmacological agents

Study	Primary/social outcome	Treatment effect	Placebo effect	Effect size/sig	Secondary outcomes
Risperidone					
Scahill et al. [127]					
Study 1 [128]	ABC-SW (2)			0.42	ABC
Study 2 [129]	ABC-SW (2)			0.65	ABC
Naharaj et al. [130]	CARS/CGAS (1)			Sig	GIR
	Social responsiveness item of parent questionnaire (2)			Sig	
Arbaclofen					
Delahunty et al. [139]	ABC-SW (1)			NS	ABC, CGI-I, CGI-S, VABS
Bumetanide					
Lemonnier et al. [140]	CARS (1)	−5.6 ± 4.0	−1.8 ± 5.1		CGI-TI, ADOS
	ADOS: reciprocity (2)			NS	
Lemonnier et al. [141]	CARS (1)	0.5 mg: −5.0 ± 4.3 1.0 mg: −3.1 ± 3.3 2.0 mg: −3.2 ± 4.0	−1.6 ± 2.3		SRS, CGI-I
	SRS (2)	0.5 mg: −12.4 ± 23.6 1.0 mg: −13.2 ± 20.5 2.0 mg: −21.8 ± 19.8	−1.6 ± 20.4		
Memantine					
Ghaleiha et al. [148]	ABC-SW (2)			NS	ABC
Aman et al. [107]	SRS (1)			NS	CATS, CAASTS, CCC-2, CGI, ABC
Propanolol					
Zamzow et al. [151]	GSOM (1)			0.40	SCAS, BAI, ECG, SC
Zamzow et al. [152]	Facial scanning				
	Mouth (1)			0.59	HR, BP
	Eyes/nose (1)			NS	
Oxytocin (single)					
Hollander et al. [159]	Affective speech Comprehension (1)			0.66	None
Guastella et al. [160]	RMET (1)	49 ± 15%	45 ± 18%		
Auyeung et al. [161]	Facial scanning (no. of fixations)				
	Eyes (1)			0.86	Total fixation time
	Mouth/other (1)			NS	Subgroup analysis
Kanat et al. [162]	House–face dot-probe Paradigm (100 ms/500 ms)				Correlation with social anxiety
	Reaction time (1)			NS	
	Attentional bias (1)			NS	
	Adherence (1)			NS	
	Allocation (500 ms) (1)			Sig	
Oxytocin (prolonged)					
Anagnostou et al. [163]	RBS-R (1)			0.64	RMET, SRS, YBOCS, WHOQOL–E
	DANVA/CGI (1)			NS	
Watanabe et al. [165]	ADOS (1)				
	Reciprocity	−8.8 ± 15.2	12.2 ± 24.1	0.78	rsFC, AQ, SRS, RBS-R, STAI, CESD, QOL, CGI-E, GAF
	Communication/repetitive			NS	
	CARS (1)			NS	
Yatawara et al. [166]	SRS (1)	13.5 ± 17.2	5.2 ± 17.3		ADOS, DBC, CGI-I, CSQ
	RBS-R (1)			NS	

Table 2 (continued)

Study	Primary/social outcome	Treatment effect	Placebo effect	Effect size/sig	Secondary outcomes
Dadds et al. [113]	Family interaction task (1)			NS	Global ratings of social interaction, SSRS, SRS-AM, OACIS, CARS, FERT, DISCAP-ASD
Guastella et al. [167]	SRS/CGI-I (1)			NS	DBC, RBS-R, RMET, DANVA, Biological motion
Kosaka et al. [168]	CGI-I overall (1)			NS	ABC, ZSRDS, STAI, TAS
	ABC-SW (2)			NS	
Vasopressin					
Umbricht et al. [185]	Biological motion				ABC, ASR, CGI-I, RMET, STAI, SCIT, OI
	Orienting preference			0.80	
	Composite			NS	
Bolognani et al. [186]	SRS			NS	VABS, ABC, RBS-R, CGI-I, STAI, ADAMS
Leucovorin calcium					
Frye et al. [82]	Verbal communication (1)			0.70	VABS, ABC, OACIS, ASQ, SRS, AIM
	ABC-SW (2)			Sig	
	SRS (2)			NS	
Cobalamin					
Hendren et al. [194]	CGI-I (1)			0.70	ABC, SRS
	ABC-SW (2)			NS	
	SRS social motivation (2)			0.73	
	SRS other (2)			NS	
***N-acetyl*-L-cysteine**					
Hardan et al. [196]	ABC-I (1)			0.96	ABC, RBS-R, SRS, CGI-I
	ABC-SW (2)			NS	
	SRS social cognition (2)			0.99	
	SRS other (2)			NS	
Ghanizadeh et al. [197]	ABC-I (1)			0.14	ABC
	ABC-SW (2)			NS	
Nikoo et al. [198]	ABC-I (1)	9.3 ± 4.1	5.4 ± 3.2		ABC
	ABC-SW (2)			NS	
Dean et al. [199]	SRS, CCC-2, RBS-R (1)			NS	DBC-P, PGI-I, CGI-I, CGI-S
Wink et al. [200]	CGI-I (1)			NS	CGI-S, ABC, SRS, VABS
	ABC-SW/SRS (2)			NS	
Tetrahydrobiopterin					
Danfors et al. [201]	CARS: overall			NS	CARS: communication and stereotyped behavior
	CARS: social interactions	1.6 ± 1.1	0.3 ± 1.4		
Klaiman et al. [202]	CGI-I/CGI-S			NS	PLS, SRS, ABC, VABS
	SRS social awareness			Sig	
	SRS autism mannerism			Sig	
	ABC-SW			NS	
Carnitine					
Geier et al. [203]	CARS	1.9 ± 2.5	0.1 ± 1.4		
	CGI	−0.5 ± 0.6	0.1 ± 0.7		
	ATEC total			NS	
	ATEC sociability			NS	
	Hand strength			NS	
Fahmy et al. [204]	CARS			Sig	

Table 2 (continued)

Study	Primary/social outcome	Treatment effect	Placebo effect	Effect size/sig	Secondary outcomes
Sulforaphane					
Singh et al. [205]	ABC total	21.4 ± 4.5	2.0 ± 3.5		None
	SRS total	20.4 ± 4.5	2.0 ± 3.5		
	OACIS: improved				
	Social	46%	0%		
	Aberrant behavior	54%	9%		
	Verbal	42%	0%		
	Other scales			NS	

The primary outcome for all studies is given. If the primary outcome is not a measure of social function, then secondary outcomes that reflect social function are listed. The number in parenthesis after the outcome measures indicates whether it is a primary or secondary outcome measure. Studies that did not have any social outcome measures were not included in this table

ABC Aberrant Behavior Checklist, *ABC-I* ABC-Irritability Subscale, *ABC-SW* ABC-Social Withdrawal Subscale, *ADAMS* Anxiety, Depression and Mood Scale, *ADOS* Autism Diagnostic Observation Schedule, *AIM* Autism Impact Measure, *AQ* Autism Spectrum Quotient, *ASR* affective speech recognition, *ASQ* Autism Symptoms Questionnaire, *ATEC* Autism Treatment Evaluation Checklist, *BAI* Beck Anxiety Inventory, *BP* blood pressure, *CAASTS* Core and Associated Autism Symptom Treatment Scale, *CARS* Childhood Autism Rating Scale, *CATS* Core Autism Treatment Scale, *CATS-I* Core Autism Treatment Scale-Improvement, *CCC-2* Children's Communication Checklist-2, *CESD* Centre for Epidemiologic Studies Depression Scale, *CGAS* Children's Global Assessment Scale, *CGI* Clinical Global Impression Scale, *CGI-E* CGI-Efficacy, *CGI-I* CGI-Improvement, *CGI-S* CGI-Severity Scale, *CGI-TI* CGI-Therapeutic Index, *CSQ* Caregiver Strain Questionnaire, *DANVA* Diagnostic Analysis of Nonverbal Accuracy, *DBC* Developmental Behavior Checklist, *DBC-P* Developmental Behavioural Checklist-Primary Caregiver Version, *DISCAP-ASD* Diagnostic Interview Schedule for Children, Adolescents, and Parents, *ECG* electrocardiography, *FERT* facial emotion recognition task, *GAF* Global Assessment of Functioning, *GIR* General Improvement Rating, *GSOM* Conversational Reciprocity task of the General Social Outcome Measure, *HR* heart rate, *NS* no significant differences between treatment and placebo group, *PGI-I* Parent Global Impression-Improvement, *PLS* Preschool Language Scales, *QOL* quality-of-life questionnaire, *OACIS* Ohio Autism Clinical Impression Scale, *OI* olfactory identification, *RBS-R* Repetitive Behaviour Scale-Revised, *RMET* Reading Mind in the Eyes Test, *rsFC* resting-state functional connectivity, *SC* skin conductance, *SCAS* Spence Children's Anxiety Scale, *SCIT* Scripted Communication and Interaction Test, *Sig* significant differences between treatment and placebo group, *SRS* Social Responsiveness Scale, *SRS-AM* Social Reciprocity Scale Autistic Mannerisms, *SSRS* Social Skills Rating Scale, *STAI* State and Trait Anxiety Inventory, *TAS* Toronto Alexithymia Scale, *VABS* Vineland Adaptive Behavior Scale, *WHOQOL-E* World Health Organization Quality of Life Questionnaire - emotional/social subscales, *YBOCS* Yale Brown Obsessive Compulsive Scale, *ZSRDS* Zung Self-Rating Depression Scale

7.1 Pharmacological Agents

7.1.1 Antipsychotics

The only US Food and Drug Administration (FDA)-approved drugs for ASD are atypical antipsychotics, which are indicated for irritability, a serious behavioral problem associated with ASD. Repeated reports document improvements in disruptive behaviors with risperidone and aripiprazole, but other reports are mixed on whether these medications improve social function as a secondary benefit.

In 2013, Scahill et al. [127] performed a secondary analysis of two previously published, large risperidone clinical trials, one double-blind placebo-controlled (DBPC) ($n = 101$) [128] and one comparative efficacy trial examining risperidone treatment with and without parent training ($n = 124$) [129]. Significant improvement on the ABC-Social Withdrawal (ABC-SW) subscale was found with a medium effect size. In a medium-sized ($n = 39$) DBPC trial of risperidone, significant improvements in "social responsiveness" were reported for children taking risperidone on a parent questionnaire [130]. However, two uncontrolled follow-up studies demonstrated mixed effects of social symptoms with long-term risperidone treatment. In a 21-month follow-up study after a DBPC study, improvements in both ABC-SW ($n = 83$) and social skills on the Vineland Adaptive Behavior Scale (VABS) ($n = 76$) showed a positive relationship with risperidone exposure [131]. In contrast, in a 10-year longitudinal study, the 33 children who reportedly improved with risperidone, showed improvement in focus and aggression but no significant change on the SRS [132].

Although atypical antipsychotics are beneficial for some ASD symptoms, the detrimental effects on lipid and glucose metabolism and body weight [133–135], and the increased risk of cardiovascular disease and type 2 diabetes mellitus [136] and tardive dyskinesia [137] are concerning. Thus, alternative safe medications that affect target pathophysiological processes and treat core ASD symptoms are needed.

Table 3 Adverse effects (AEs) reported in controlled studies of promising pharmacological agents

Treatment	Reported AEs
Risperidone	Weight gain, appetite increase, fatigue, drowsiness, drooling, dizziness
Arbaclofen	No significant AEs
Bumetanide	Hypokalemia, diuresis, appetite loss, dehydration, asthenia
Memantine	Irritability, aggression
Propanolol	No significant AEs
Oxytocin (single-dose administration)	No significant AEs
Oxytocin (prolonged administration)	Thirst, urination, constipation
Vasopressin	Dizziness, attention disturbance, anxiety, infusion-site rash
Leucovorin calcium	No significant AEs
Cobalamin	No significant AEs
N-acetyl-L-cysteine	Drowsiness, fatigue, constipation, increased appetite, nervousness
Tetrahydrobiopterin	No significant AEs
Carnitine	No significant AEs
Sulforaphane	Weight gain

7.1.2 GABAergic Modulation

7.1.2.1 Arbaclofen Baclofen is a $GABA_B$ agonist used to treat spasticity. Because the S-isomer is believed to account for adverse effects such as somnolence, STX-209 (i.e., Arbacofen), the active R-enantiomer was developed [55]. An 8-week open-label trial enrolled 32 children and adolescents with ASD and high irritability [138]. Improvement occurred in most outcomes measured, including the SRS. A follow-up 12-week DBPC trial of 150 children, adolescents, and young adults with ASD and reduced social function did not show improvements in the primary outcome measure, the ABC-SW [139]. However, a per-protocol analysis demonstrated a significant improvement in the VABS Socialization subscale.

7.1.2.2 Bumetanide The diuretic bumetanide, a chloride-importer antagonist, targets abnormalities in intracellular $GABA_A$ chloride levels [56]. In the first single-center trial, 60 children with ASD received 1 mg of bumetanide daily for 3 months. Bumetanide improved scores on the CARS but the ADOS Reciprocity subscale did not improve [140]. In a large ($n=80$), six-center 3-month DBPC with three doses (0.5 mg, 1.0 mg, or 2.0 mg twice daily), bumetanide improved scores on the CARS and SRS [141]. The treatment was well tolerated in these trials with some patients showing mild hypokalemia requiring potassium supplementation.

Two studies measured biomarkers of social cognition during open-label bumetanide treatment. Ten months of bumetanide treatment in adolescents and young adults ($n=7$) improved emotion recognition and enhanced activation in social and emotional perception areas of the brain during the viewing of emotional faces [142]. In a study on the same population plus two additional participants ($n=9$), bumetanide normalized amygdala activation during a constrained eye contact task and increased the time spent looking at the eyes in face stimuli [143].

7.1.3 Glutamate Neurotransmission

Memantine, an *N*-methyl-D-aspartate receptor antagonist, has been investigated for its ability to improve social function in both open-label and controlled studies [48]. Two small ($n=30$; $n=18$) open-label studies reported improvements in eye contract [144] and social withdrawal [145] in children with ASD. A large ($n=151$) open-label study reported improved social behavior in children and young adults with ASD [146]. Memantine significantly improved SRS scores in a small ($n=18$) 12-week open-label trial of adults with HFASD [147].

Two DBPC studies have examined the effect of memantine on social function. A medium-sized ($n=40$) DBPC add-on to risperidone study of children with ASD reported improvements in ABC Irritability, Stereotypy, and Hyperactivity, but not Social Withdrawal, subscales [148]. In a large ($n=121$) DBPC 12-week study, once-daily extended-release memantine did not significantly affect the SRS owing to a large improvement in the placebo group [107]. Thus, DBPC studies do not show improvement in social symptoms with memantine, although they do suggest an excellent safety profile.

7.1.4 Propranolol

Propranolol is a blocker of the beta-adrenergic receptor that acts both peripherally and centrally. Peripheral effects primarily target the autonomic nervous system where propranolol affects primarily heart rate and blood pressure. Central

targets include the widespread norepinephrine circuits, including targets in the brainstem, cortex, and, notably, the amygdala.

Propranolol was first reported to improve social behaviors and speech in a small ($n = 8$), open-label, 4- to 19-month trial of adults with ASD [149]. A recent case study demonstrated that prolonged (several months) treatment with propranolol improves hypersexual behaviors in individuals with ASD [150].

Two small DBPC trials have examined the effects of single-dose propranolol on social function. In a DBPC ($n = 20$) crossover study, individuals with ASD demonstrated improved conversational reciprocity with a blinded researcher after a single dose of propranolol [151]. Simultaneous measures of autonomic activity and anxiety did not account for this effect. In another DBPC crossover study ($n = 14$), a single dose of propranolol decreased time spent looking at the mouth during face scanning in individuals with ASD as compared with TD controls [152]. Currently, the Thompson Center for Autism and Neurodevelopmental Disorder at the University of Missouri is conducting DBPC trials aimed at better understanding the effect of propranolol on social interaction in adults and adolescents with HFASD (NCT 02871349) and in young children with ASD combined with early intensive behavioral interventions (NCT 02428205).

Studies have examined the cognitive and behavioral benefits of propranolol as well as its effect on brain circuitry. Three controlled studies found that single-dose propranolol improves language. In a small ($n = 20$) DBPC crossover study of adults and adolescents with ASD, propranolol treatment improved the speed of solving verbal anagram problems with the improvement related to autonomic activity and anxiety [153]. Still, in another small ($n = 14$ individuals with ASD, 14 control individuals) DPBC crossover study on adults and adolescents with HFASD, propranolol improved category fluency, but not letter fluency [154]. Another small DBPC study ($n = 14$ adults with HFASD, 13 TD controls) demonstrated that propranolol improves working memory but not inhibitory control on a continuous performance task [155].

The effect of propranolol on functional brain activity has been investigated in relation to the default mode resting-state network connectivity in ASD. The first study of this type ($n = 10$ adults with ASD) demonstrated that single-dose nadolol, a beta-antagonistic that does not cross the blood–brain barrier, did not influence functional connectivity like single-dose propranolol, thereby demonstrating that changes in brain function were not the result of peripheral changes in autonomic function [156]. In a more recent study of 15 adolescents and young adults with HFASD, single-dose propranolol modulated the default mode network by decreasing connectivity between the default mode resting-state network and the dorsal medial prefrontal cortex and increasing connectivity between the default mode resting-state network and medial temporal lobe [157].

7.1.5 Oxytocin/Vasopressin Pathways

7.1.5.1 Oxytocin Treatment with OT is one of the most active areas of drug treatment research in ASD. At least a dozen clinical trials have investigated the use of OT in ASD with over a dozen other registered trials currently underway. Clinical trials have been conducted in several ways: examining the effect of a one-time OT dose or examining the effect of prolonged OT treatment.

7.1.5.1.1 Single Oxytocin Dose Studies Many studies have examined the effect of an 'oxytocin challenge' by examining changes in specific measurements of social function resulting from a single dose of OT. In a small ($n = 15$) DBPC study of patients with ASD, intravenous OT reduced repetitive behaviors [158]. In a second small ($n = 15$) study of adults with ASD, intravenous OT improved the comprehension of affective speech [159].

The remainder of the 'oxytocin challenge' trials has used an intranasal (IN) OT spray. In a small ($n = 16$) DBPC crossover study of adolescents with ASD, 18 or 24 IU of OT improved performance on the Reading the Mind in the Eyes Task, particularly in those that were younger and received a lower dose [160]. In a medium-sized ($n = 32$ individuals with ASD; 34 control individuals) DBPC crossover study, 24 IU enhanced gaze time to the eyes in a real-time interaction with a researcher, especially in those with the most impaired levels of eye contact at baseline [161]. In a medium sized ($n = 29$ individuals with ASD; 30 control individuals) DPBC crossover trial, 24 IU increased the allocation of attention to faces, as compared to houses, in individuals with ASD for a 500-ms stimuli presentation but not the 100-ms stimuli presentation. Oxytocin also decreased the effect of baseline social anxiety on modulating attention to faces [162].

Thus, OT seems to improve the performance of individuals with ASD on social tasks, particularly those with high social dysfunction at baseline. However, most of these studies have been performed on adults with HFASD. In addition, the combination of the treatment with a specific task may represent a somewhat contrived social situation and does not explicitly translate to real-time situations where the need for social engagement is not necessarily planned. Thus, other studies have examined prolonged treatment with OT on ASD.

7.1.5.1.2 Prolonged Oxytocin Treatment In a small ($n = 19$) DBPC study, 24 IU of IN OT twice a day for 6 weeks improved the Repetitive Behaviour Scale-Revised but not the Diagnostic Analysis of Nonverbal Accuracy or the CGI

in adults with ASD [163]. In another early study, eight adolescent male individuals with ASD underwent an open-label dose escalation from 8 IU to 24 IU, increasing the dose by 8 IU every 2 months. The majority showed improvements on the reciprocity scale of the ADOS [164]. These promising trials led to further controlled studies.

Several controlled studies demonstrated positive findings. In a small ($n=20$) DBPC of male adults with HFASD, 6 weeks of OT significantly improved social reciprocity as measured by the ADOS [165]. In a medium-sized ($n=31$) DBPC crossover trial, twice-daily (12 IU AM, 24 IU PM) OT for 5 weeks significantly improved SRS scores [166].

Other studies failed to find positive results. In a medium-sized ($n=38$) 5-day DPBC trial of young male individuals with ASD, 12 IU or 24 IU of IN OT administered during parent-child interaction training sessions did not improve emotion recognition, social interaction skills, or general behavioral adjustment [113]. In another medium-sized ($n=50$) DBPC trial of adolescent male individuals, twice daily 18 IU or 24 IU of IN OT for 8 weeks did not improve SRS or CGI-I [167].

In perhaps the largest ($n=60$) clinical trial on OT, young adults with HFASD received low-dose (16 IU) OT, high-dose (32 IU) OT, or placebo during a 12-week DBPC trial. Although there was no overall improvement in the CGI-I, male individuals in the high-dose group demonstrated significant improvement in CGI-I as compared with placebo and OT receptor polymorphisms were related to improvement in the low-dose groups (see below) [168]. This study demonstrates the complexity of OT clinical trials and highlights the fact that subject characteristics, including genetic background, as well as OT dosage, may contribute to OT effectiveness.

7.1.5.1.3 Effect of Oxytocin on Neural Circuits In a small placebo-controlled crossover study of adults ($n=14$ individuals with ASD; $n=14$ control individuals), 24 IU of IN OT increased right amygdala activity to socially relevant facial stimuli in the individuals with ASD [169]. In a medium-sized ($n=40$) DBPC crossover trial of male adults with HFASD, 24 IU of IN OT increased the activity and neuronal metabolism of and connectivity with the medial prefrontal cortex as well as improved the ability of the participants to use non-verbal information to make judgments about conflicting social information [170, 171]. In a small ($n=20$) DBPC of male individuals with ASD, 24 IU of IN OT improved performance on the Sally-Anne Task, a well-known first-order false-belief task that measures the ability to infer another person's social emotions, and increased activity in the right anterior insula, an area found to be diminished in activity during this task in male individuals with ASD [172]. One small ($n=20$) DBPC crossover clinic trial of prolonged (6 weeks) treatment with OT resulted in an enhancement of task-independent resting-state functional connectivity between the anterior cingulate cortex and dorso-medial prefrontal cortex as well as task-dependent activity in these regions [165]. Thus, in several functional brain imaging studies, OT has been shown to enhance activity, connectivity, and metabolism of key brain regions involved in social cognition.

7.1.5.1.4 Predictors of Oxytocin Response Several studies have examined the effect of specific polymorphisms on the response to OT treatment. In one study of 56 healthy young men, the rs3796863 polymorphism of the transmembrane protein involved in OT secretion (CD38 gene) modulated activation of the left fusiform gyrus during processing of visual social stimuli such that individuals with this polymorphism had more marked neural responses to OT [173]. In another study of 20 young adults with HFASD, the rs6791619 polymorphism of the OT receptor gene (OXTR) predicted improvement in male participants with ASD to lower OT doses (<21 IU) [168].

In an interesting DBPC crossover study (31 individuals with ASD, 30 control individuals) that measured the cardiac evoked and cortical evoked long-latency parietal positivity responses resulting from viewing pictures selected from the International Affective Picture System during placebo and OT treatment conditions in TD male individuals and male individuals with ASD, several factors were found to predict physiological responses. Higher baseline plasma OT levels predicted a greater treatment response in individuals with ASD [174]. Male individuals with ASD who were easily distressed when seeing others in stressful situations and male individuals with TD who were highly sensitive to anticipated punishment and criticism or had a low drive for goal achievement were found to have a higher effect [175].

7.1.5.1.5 Ongoing Clinic Trials of Oxytocin There are 11 ongoing clinical trials examining of IN OT for individuals with ASD. Five studies are enrolling only children or adolescents with ASD, four studies are enrolling only adults with ASD (all but one only enrolling male individuals), and one study is enrolling both children and adults with ASD. One unique study (NCT02302209) is examining adult family members of individuals with ASD. Several studies are examining whether OT can improve standard behavior interventions or psychotherapy. For example, the quadruple-blind placebo-controlled trial (NCT01914939) is determining whether 24 IU of IN OT can enhance 20 weekly 60-min cognitive-behavioral psychotherapy sessions concentrating on either social skills or stress management in male adults with ASD. Likewise, other studies are examining the effect of IN OT in children prior to pivotal response therapy (NCT02918864) and social cognitive skills group or facilitated play therapy (NCT03370510). One study

(NCT0337035) is examining the addition of OT to probiotic therapy. While most studies are using a dose of 24 IU, other studies (NCT02985749, NCT01931033) are using higher doses (48 IU) while one study (NCT03466671) is directly comparing low- vs. high-dose and once- vs. twice-daily dosing. Several other studies are examining the effect of a single dose or several doses on changes in brain activity (NCT02940574, NCT03033784) or cognitive function and eye movements (NCT03183674).

Studies examining dose and frequency are particularly important as there are few data on the pharmacokinetics of IN OT as well as factors that influence the intra-subject variation in response. Indeed, nasal anatomy can influence nasal airflow dynamics, which in turn influences OT delivery and deposition on the mucosal membranes, while nasal vascularization can influence OT absorption [176]. A small study of eight healthy men suggest that 26 IU of IN OT results in a substantial rise in OT plasma levels in 30 min with levels returning to baseline 90 min after administration in most individuals [177]. Another study of 14 healthy ecstasy users found that 40 IU but not 20 IU of IN OT resulted in a significant increase in plasma levels within 30–60 min [178]. However, in both non-human primates [179] and rodents [180], IN OT results in a greater increase in cerebrospinal fluid OT levels as compared with plasma levels. Thus, the use of plasma OT measurements may be misleading in regard to the level of OT in the target organ.

7.1.5.2 Vasopressin Interest has developed in vasopressin, a neuropeptide that is closely associated with OT. Variants of the vasopressin receptor 1A gene (AVPR1A) in humans have also been associated with ASD [181] and IN vasopressin has been shown to modulate brain regions involved in processing emotional information, such as the medial prefrontal cortex–amygdala [182] and temporal-parietal junction [183]. In addition, knockout, rescue, and overexpression of AVPR1A in rodents modulates social function [184]. Curiously, both vasopressin agonists and antagonists are being studied in clinical trials.

In a multicenter DBPC crossover study of 19 male adults with HFASD, 20 mg of the potent and highly selective V1a receptor antagonist, RG7713, was infused intravenously. Several exploratory measures were conducted but only one, a preference for orienting to biological motion, was statistically significant [185]. As a follow-up to this initial small pilot study, the Vasopressin Antagonist to Improve Social Communication in Autism (VANILLA) study examined another V1a receptor antagonist with good oral availability known as RG7714 (or by its trade name Balovaptan). The VANILLA study was a large ($n=223$) multi-center DBPC dose-escalation study using three doses (1.5 mg, 4 mg, 10 mg), which assessed for safety and efficacy in adults with ASD. In an unpublished presentation, the drug was reported to be safe and, although the SRS did not show a significant effect, the VABS Communication and Socialization subscales, secondary outcome measures, improved for the 4- and 10-mg doses [186]. With these preliminary results, Roche was been granted FDA Breakthrough Therapy Designation for Balovaptan.

Currently, there are two clinical trials, both being conducted at Stanford University, examining the effect of IN vasopressin in ASD. One clinical trial (NCT01962870) will treat with either 12 IU or 16 IU twice a day for 4 weeks while another (NCT03204786) will examine the effect of 4 or 8 weeks of 16 IU in children. Both will provide a comprehensive assessment of social cognition as well as standard measures of change in ASD symptoms. Preliminary unpublished data suggest that the IN vasopressin improves SRS scores and that response is dependent on baseline vasopressin blood levels as well as OXTR and AVPR1A gene expression [187].

7.2 Metabolic Agents

7.2.1 Folate Metabolism

Treating central folate abnormalities in ASD has demonstrated promising results. Both small and large uncontrolled case series show that the treatment of children with ASD who are positive for FRα autoantibodies with leucovorin calcium improves communication, social interaction, attention, and stereotypical behavior [66, 188–191] with some patients demonstrating complete recovery of core ASD symptoms [191, 192]. An open-label study of children with ASD positive for FRα autoantibodies ($n=44$) and a DBPC study of a general ASD population ($n=48$) demonstrated that leucovorin calcium primarily improves verbal communication, language, and behavior [66, 82]. The DPBC study demonstrated a significant improvement in ABC-SW that was greater than the minimal clinical important difference but did not show a significant effect on the SRS [82].

7.2.2 Cobalamin Metabolism

In a prospective open-label study ($n=37$) of methylcobalamin (75 µg/kg) injected subcutaneously every 3 days combined with oral folinic acid (400 µg twice a day) for 3 months in children with ASD who were preselected to have abnormal redox and methylation metabolism, significant improvements were found on the Social subscale of the VABS [193]. In an 8-week DBPC study of 50 children from the general ASD population, social motivation as measured by the SRS, along with the CGI-I, significantly improved with methylcobalamin subcutaneously injected (75 µg/kg every 3 days) without additional oral folinic acid [194].

7.2.3 *N*-acetyl-L-cysteine

N-acetyl-L-cysteine (NAC), which provides the precursor to glutathione and reduces brain glutamate, is useful for a wide range of psychiatric and neurological disorders [60] and magnetic resonance imaging studies have confirmed that it modulates brain glutamate [195]. A small ($n = 33$) 12-week study of children with ASD showed that 900 mg of NAC three times per day improved ABC Irritability and SRS Social Motivation and CGI-I [196]. In a medium-sized ($n = 40$) DBPC add-on study to risperidone, 600 mg of NAC twice a day significantly decreased ABC Irritability but did not change ABC-SW [197]. In a similar medium-sized ($n = 40$) risperidone add-on study, children with ASD were treated with up to 900 mg NAC daily. *N*-acetyl-L-cysteine reduced the ABC Irritability and Hyperactivity subscales but not the ABC-SW subscale [198]. The SRS was not improved in two DBPC studies, one medium sized ($n = 48$) and one small sized ($n = 16$) [199, 200]. Thus, these data suggest that NAC may help with behavioral symptoms but not social symptoms in children with ASD.

7.2.4 Tetrahydrobiopterin Metabolism

Three controlled ($n = 83$, 12, 46) [201–203] and several open-label trials (total $n = 280$) have documented improvements in communication, cognitive ability, adaptability, social abilities, and verbal expression with treatment with sapropterin, a synthetic form of BH_4, in children with ASD [47]. In the two DPBC studies that measured social skills specifically, the social interaction cluster of the CARS [201] and the Social Awareness and Autism Mannerism subscales of the SRS [202] significantly improved with BH_4. Interestingly, an open-label biomarker study ($n = 10$) suggested that response to sapropterin involves nitric oxide metabolism [46]. Thus, sapropterin remains an interesting potential treatment for children with ASD but given its orphan drug status for type IV hyperphenylalaninemia and no FDA-approved indication for ASD, it remains exceedingly challenging to access for routine use in the treatment of ASD.

7.2.5 Carnitine Metabolism

Core and associated ASD behaviors have been shown to improve with L-carnitine in two DBPC studies ($n = 30$, 30) [204, 205]. Both studies used the CARS as the primary outcome measure and one study used an additional measures, the modified CGI. This is of interest because limited evidence suggests that L-carnitine is depressed in children with ASD [85] and because of a recently described genetic disorder that causes abnormal carnitine metabolism in ASD [86].

7.2.6 Sulforaphane

The phytochemical sulforaphane, a broccoli sprout extract, has been widely evaluated in clinical medicine, particular cancer, for its ability to upregulate intrinsic, cellular physiological protective mechanisms. In a DBPC 18-week trial of 44 young men with ASD, those receiving 50–150 μmol of daily sulforaphane had a significantly greater improvement on the ABC and SRS as well as a greater number of individuals had improvement on the Ohio Autism Clinical Impression Scale Social subscale. Four weeks of discontinuation of the treatment resulted in a relapse of scores toward pretreatment levels [112]. In an open-label study of children and young adults in a school for ASD, 12 weeks of sulforaphane significantly improved the SRS [206]. Urinary metabolites associated with oxidative stress, amino acid, gut microbiome, neurotransmitters, hormones, and sphingomyelin metabolism correlated with clinical improvement.

7.3 Microbiome Treatments

Treatments targeting microbiome imbalances improve social behavior [207]. In a DBPC crossover study, a probiotic containing lactobacillus improved ASD behaviors, including socialization, as well as stool consistency and reduced *Clostridium* species [208]. However, it should be noted that this study had significant limitations including high inter-individual variability and an extremely high dropout rate; of the 62 children enrolled, only 17 completed the trial. In an open-label study of children with ASD and gastrointestinal symptoms treated for 21 days with a commercial product Delpro® that contains five probiotic strains and the immunomodulator Del-Immune V®, 88% of caretakers reported an improvement in ASD as measured by the Autism Treatment Evaluation Checklist [209].

A recent small ($n = 18$) open-label study replaced the gut microbiota of children, adolescents, and adults with ASD using microbiota transfer therapy. Following 2 weeks of vancomycin treatment and a bowel cleanse, gut microbiota from healthy individuals was replaced in the participants with ASD using fecal microbiota transplant for about 8 weeks. Aside from a significant reduction in gastrointestinal symptoms, improvement was also found on the CARS, ABC, and SRS scales suggesting an improvement in ASD symptoms, particularly social symptoms [210]. As an extension of this, a DBPC trial of microbiota transfer therapy in adults with ASD is ongoing (NCT03408886).

The enteric microbiome produces short-chain fatty acids known to affect metabolism and behavior. Studies from our laboratory have demonstrated that both propionic acid and butyrate, two of the most abundant short-chain fatty acids, affect mitochondrial metabolism uniquely in ASD cell lines [211, 212] and other studies demonstrate that a subset of

children with ASD have biomarkers consistent with the propionic acid-induced ASD animal model [213]. Interestingly, butyrate may be therapeutic in normalizing social behavior in ASD as, in animal models of ASD, butyrate rescued ASD behavior in the prenatal valproic acid exposure model [214] and increased social behavior in the BTBR mouse model [215]. Despite this interesting evidence of a potential connection between the enteric microbiome and ASD symptoms, it is important to appreciate that the mechanisms by which the microbiome influences behavior are still just beginning to be understood and potentially include immune modulation, metabolic regulation, as well as altering the availability of essential nutritional substrates.

8 Challenges in Clinical Trial Design for Studying Autism

The heterogeneous nature of ASD presents unique challenges when conducting clinical trials [207]. Individuals with ASD have large variation in intellectual development and many have common medical co-morbidities [207]. Heterogeneity can be decreased by carefully selecting participants. Some studies target specific genetic syndromes that overlap with ASD as an extension of animal models, such as Fragile X [55, 216]. Other studies select participants to match their outcome measure. Antipsychotic trials have targeted highly irritable individuals with ASD because their primary outcome measure was ABC irritability [128]. This is also an area where biomarkers can be designed to select participants predisposed to respond to targeted treatments and can help drive a precision medicine approach to drug treatment [217].

Open-label trials need to be considered cautiously especially if the trial does not have a control group. Blinded controlled trials are particularly important given the complex response to placebo. One very compelling study demonstrated that baseline factors including disruptive behavior, mood, and caregiver strain were significant negative predictors of placebo response [218]. Another factor being recognized is the nocebo response where individuals demonstrate adverse effects to placebo. Open-label trials, particularly longitudinal trials are also very vulnerable to bias depending on how drop-outs are handled, resulting in potential evaluation of a select population. Despite these limitations, open-label studies can provide useful information such as determining which outcome measure or subpopulation might be most sensitive to the effect of the treatment. Such information can be very helpful for designing better DPBC trials.

Selection of an outcome measure is important. Some studies focus on a specific outcome while other studies use a multitude of primary and/or secondary outcome measures. This is problematic and can lead to a type I error. Few studies reviewed corrected for multiple comparisons: out of the controlled studies reviewed in Table 1, only three studies corrected for multiple comparisons: one study used the Bonferroni correction [152] while two studies used the false discovery rate [82, 165].

Many individuals may be taking concurrent treatments and/or may be at risk for active change in their medical regime. For example, many trials exclude patients with concurring epilepsy as many antiepileptic drugs have drug–drug interactions and such individuals are at high risk for having a seizure during the trial, requiring such an event to be reported as an adverse event. Educational and behavioral therapies commonly change throughout the year and may be absent during the summer break. In addition to receiving inconsistent therapy, individuals with ASD commonly have difficulty with changes in routine.

9 Summary

Social deficits define ASD and result in significant disability. Overall, social function is a complex outcome to study and individuals with ASD require special considerations in clinical trials. Although there are currently no FDA-approved drugs for social deficits in individuals with ASD, several agents demonstrate promise for the treatment of this core symptom. Oxytocin and propranolol are particularly promising agents undergoing active investigation. Significant excitement has also surrounded vasopressin with both agonists and antagonists showing promise but evidence for these compounds is not peer reviewed at this time. They appear to be safe and Balobaptan, a novel compound developed by Roche, has been granted FDA Breakthrough Therapy Designation. Agents that address core ASD symptoms such as bumetanide and microbiota transfer therapy also have the potential to significantly improve social function.

Compliance with Ethical Standards

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