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AUTISM SPECTRUM DISORDERS IN ADULTS:

CLINICAL DIAGNOSIS AND POTENTIAL SERUM BIOMARKER

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Synthesis

My doctoral course has focused on Autism Spectrum Disorders (ASD) in adulthood. The research reported in this thesis has been conducted at the Laboratorio Autismo, a research center belonging to the Department of Brain and Behavioral Sciences (University of Pavia). Particularly, our group focused on the diagnosis of this condition in adulthood and the research of potential biomarkers. My research is in line with this research mission. My thesis is mainly composed of four parts. Whenever appropriate I reported the abstract of the published (1-3) - or submitted for publication - research to which I have contributed during my doctoral course that were tangent to the main topic and could not be extensively discussed in this report. In the summary those sections have been reported in bold font.

Firstly, I have provided a general outlook of the ASD discussing epidemiologic aspects, element of pathophysiological theory, and psycho-pathological comorbidity. In this section I have also reported few notions of the possible interventions and outcome assessment. The extensive explanation of the diagnostic procedure and the challenges connected with the diagnostic assessment of adult people conclude the first chapter, to provide the detailed framework of the three following research chapters.

Chapter two focus on a broad systematic review including all clinical controlled trials (CCTs) in ASD published from 1980. The findings reported here highlight the limit of the diagnostic methodology adopted in most CCTs but support the agreement towards the use of standardized assessment tools for ASD assessment. Adult Diagnostic Observation Schedule (ADOS) and Adult Diagnostic Interview (ADI) are confirmed by these results as the most trusted of the available standardized tools.

In chapter three, the diagnostic reliability of ADOS and ADI have been tested in a large sample of adults without intellectual disability referring to the Laboratorio Autismo for a diagnostic assessment. The results confirmed the accuracy of those tools and highlighted some of their limitation for their use in specific subgroups of patients. Predictors of the agreement between diagnostic instruments and clinical diagnosis have also be examined. These data have been published by our group in a peer-reviewed publication (4).

Finally, the fourth chapter focused on the evaluation of a potential serum biomarker (ciliary neurotrophic factor, CNTF) that was tested for the first time in adults with ASD and ID. The data support the putative role of CNTF in this specific population as an additional tool during the diagnostic assessment. These data have been published by our group in another peer-reviewed paper (5) and with cautious optimism further research and independent replication are awaited.

1. Autism Spectrum Disorders (ASD): background

1.1. Epidemiology

Autism spectrum disorders (ASD) are defined within the fifth edition of the Diagnostic and Statistic Manual (6) as neurodevelopmental disorders defined by pervasive deficits in communication and social interaction, and by the presence of restricted, stereotypic, and repetitive behaviors, insistence on sameness or hypo- or hyper-sensitivity, that must be present from the early development and cause significant functional impairment.

Epidemiological estimates have recently reported that about 1 every 68 youngster in the United States could be in the autism spectrum (7). In UK, Brugha, McManus (8) reported a prevalence of 9.8 every 1000 people older than 16. The picture depicted, suggested a significant increase if compared with the early estimates of the prevalence of this condition. In 1966 in fact, Lotter (9) estimated a prevalence of 5/10000. This dramatic increase of ASD diagnoses has been recently labeled as “autism epidemic” from the media, and the scientific community is debating about the possible reasons subsiding this phenomenon. However, there is a general agreement about the changes in the diagnostic criteria (10) and to the increased awareness towards the condition (11) that appeared to be the most significant factors of the prevalence increase. However, a real growth of the condition associated with multiple risk factors cannot be completely excluded (12). The change in the epidemiological estimates is associated with a crucial clinical and sociological consequences: considering almost stable the real incidence of the condition, many children, now adults, could have been unrecognized as having an ADS, or worst, mislabeled with other possible psychiatric conditions and now presenting to the clinical services with a burden of unmet needs. This concept has been named as the “lost generation” of adults with ASD (13). This acknowledgement emphasizes the

need to develop and validate practical and reliable diagnostic instruments to evaluate the presence of ASD in adulthood and adequately plan and provide welfare interventions.

Despite the changes in the prevalence estimates, the gender distribution of this condition appears to be almost stable: ASD are more frequent in males, with a ratio of about 1 female every 4 males diagnosed (7), although the difference slightly decreases in individuals with intellectual disability (ID) (11). Intellectual ability of children diagnosed with ASD has recently been estimated as deficient in 31% of the overall sample (Intellective Quotient, IQ <70), borderline in 25% (70≤ IQ ≤85), normal or above average in 44% of the sample (IQ >85) (7). A significant difference between genders has been found in the CDC children sample diagnosed with ASD: girls appeared more likely diagnosed with intellectual disability (ID) and male being more likely to be without ID.

1.2. Etiology and pathophysiology

Despite the extensive research and the huge investment in the field, the etiology and pathogenesis of ASD remain unexplained. Nevertheless, it is now widely acknowledged that ASD has multifactorial basis (12, 14). Most scientists agree that genes are one of the risk factors that can make a person more likely to develop ASD (15). Apparently, heritability of ASD in twins ranged from 64% to 91% (16). However, except for few genetic and chromosomic syndromes associated with ASD such as down syndrome, fragile X syndrome and tuberous sclerosis (17), genetics of ASD is characterized by significant heterogeneity (18). Hereditable genetic and chromosomal abnormalities have been identified only in a small subgroup of individuals with ASD, however, *de novo* abnormalities are surely involved in the pathogenesis of ASD (19).

A variety of potential environmental risk factors appear to be associated with ASD (20) both during the gestational and perinatal period (21). A recent review suggested that specific events such as perinatal hypoxia could have a link to ASD. However, also non-specific factors such as advanced parental age have been strongly associated with the risk of ASD (22). Among the most robust risk factor, Valproate administration during pregnancy has been associated with the development of ASD (23). On the other hand, other factors, such as maternal obesity and gestational diabetes, showed a less defined association with risk of ASD (24).

In summary, the general framework suggested that the genes-environment interactions may cause alterations in brain structures and functioning that could be responsible for the specific behavioral pattern (12). Neuropathological research has largely focused on the study of ASD brain using different approaches. Neuroanatomical data suggested an increased rate of brain growth in early childhood. Specifically, the temporal, frontal, and parietal lobes, and some cerebellar lobules seemed affected. This is followed by slow cerebral and cerebellar development in childhood and adolescence. Some of the ASD symptoms appear early during the development and have been correlated with cerebellar abnormalities already present during the intrauterine growth. The cerebellar abnormalities could result in abnormal functioning and development of the neocortical systems that intensively grow further during the childhood (25). Some Authors suggested that the aforementioned cerebellar abnormalities could thus result in altered cerebello-thalamo-cortical circuits, involved in several superior cognitive functions (26, 27). Structural alterations of the corpus callosum, hippocampus, and amygdala have also been reported (28).

The reasons for the neuroanatomical abnormalities are not clear. However, significant alteration in neurotrophic factors (NTF) have been associated with ASD (29). NTF are proteins that regulate cellular proliferation, migration, differentiation and integrity, thus contributing to the normal brain development and maintenance, critically influencing the formation and elimination of neuronal connections. Several proteins are now considered NTFs (brain derived neurotrophic factor, BDNF; nerve growth factor; neurotrophins). Other proteins also exerting immunological activities are considered part of this group and called neruokines (ciliary neurotrophic factor, CNTF; leukemia inhibitory factor; insulin-like growth factors; transforming growth factor-β). This is relevant, considering that there is substantial evidence implicating chronic inflammation and immune imbalance leading to high levels of inflammatory cytokines in the brain (30).

Several observation studies found BDNF peripheral levels alteration in subjects diagnosed with ASD as compared with healthy comparisons (31). BDNF levels resulted higher in ASD children but the effect disappeared when adult samples was considered (31). However, recent studies found contrasting evidences. Francis, Dougali (32) conducted a prospective observational study and their results suggested lower levels of peripheral BDNF as compared with healthy controls. However, the BDNF levels were not correlated with any behavioral features and did not predict adaptive behaviors at 3 years follow-up (32). However, further studies are needed. Our research group contributed to this topic and during my doctoral course we published a study investigating the correlation between BDNF peripheral levels and autistic traits in the general population (1). The abstract of the paper is reported below.

• BDNF levels are associated with autistic traits in the general population (1)

"Evidence supports the notion that autistic symptoms and behaviors should be regarded as dimensional traits. The present study aimed to investigate the role of vasopressin (AVP), brain-derived neurotrophic factor (BDNF) and oxytocin (OXT) as potential biochemical correlates of subclinical autistic traits in a cohort of healthy young adults. One hundred and fifty-three subjects (80 males, 73 females) were recruited. Participants completed the Autism Spectrum Quotient (AQ), a widely used measure for the identification of autistic traits in the general population. Additionally, blood samples were obtained from all participants at the same time of the day to control for circadian variation. We conducted a multiple regression analysis using the AQ score as the dependent variable and age, sex, AVP, BDNF and OXT levels as the independent variables. The model explained approximately the 22% of the variance of the AQ score. Among the parameters included in the analysis, only BDNF levels were independent predictors of AQ score."

At the histological level, the cerebral cortex of ASD subjects appear to be abnormal. Alterations at the level of the cortical minicolumns (33) appeared to be related with an excitation-inhibition imbalance (34) that could be partly responsible for the behavioral symptoms and the frequent comorbid neurological manifestations such as epilepsy (35).

The neurochemical aspects that have been linked to this theoretical framework are extremely complex. The excitation-inhibition imbalance could also be related to excitotoxic processes, resulting in oxidative stress, which have been linked with

cognitive and behavioral feature of ASD (36). Furthermore, several studies have focused on neuromodulators. Serotoninergic system alteration seems strongly linked to autism (37). A recent meta-analysis also addressed as potential risk factor for ASD the prenatal exposure to selective serotonin reuptake inhibitors (SSRI) (38). However, the Authors acknowledged some limitations regarding the possible confounding effect of underestimated maternal depression or psychiatric comorbidities and the possible influence of polypharmacy prescriptions, thus recognizing the need for further research.

Opposite to the serotoninergic system, there is little evidence supporting alterations or dysfunctions of the norepinephrine or endogenous opioids systems. Also, the findings regarding the role of dopaminergic system are conflicting. Other areas of research included the cholinergic system, oxytocin, and amino acid neurotransmitters (39). During my doctoral course I contributed to this topic with a metanalysis of the published literature studying the possible role of peripheral oxytocin and vasopressin as potential biomarkers of psychiatric conditions. It emerged that there was not enough evidence to support the use of neither of these as peripheral biomarkers in ASD (3). The abstract of the publication has been reported below.

• ***Peripheral oxytocin and vasopressin: Biomarkers of psychiatric disorders? A comprehensive systematic review and preliminary meta-analysis (3)***

"A large array of studies has investigated peripheral oxytocin (OT) and vasopressin (ADH) as potential biomarkers of psychiatric disorders, with highly conflicting and heterogenous findings. We searched Web of Knowledge℠ and Scopus® for English original articles investigating OT and/or ADH levels in different biological fluids

(plasma/serum, saliva, urine and cerebrospinal fluid) across several psychiatric disorders. Sixty-four studies were included. We conducted 19 preliminary meta-analyses addressing OT alterations in plasma/serum, saliva, urine and cerebrospinal fluid of 7 psychiatric disorders and ADH alterations in plasma/serum, saliva, urine and cerebrospinal fluid of 6 psychiatric disorders compared to controls. Hedge's g was used as effect size measure, together with heterogeneity analyses, test of publication biases and quality control. None of them (except serum OT in anorexia nervosa) revealed significant differences. There is no convincing evidence that peripheral ADH or OT might be reliable biomarkers in psychiatric disorders. However, the lack of significant results was associated with high methodological heterogeneity, low quality of the studies, small sample size, and scarce reliability of the methods used in previous studies, which need to be validated and standardized."

Apart from biological models, some cognitive hypothesis of ASD have also been formulated to explain the differences in functioning as compared with healthy controls. Difficulties in theory of mind, the ability to comprehend the mental states in self and others, have been suggested (40). Additionally, people with ASD showed executive functions deficits (41) and present a cognitive style prioritizing non-significant details over the global picture (42).

1.3. Comorbidities

Apart from ID, that has already been discussed in the paragraph focusing on the epidemiology of ASD, language disorders are among the most frequent comorbidities in

ASD. Language delay was needed for a DSM-IV diagnosis of autism, but it has no longer been included in DSM-5. Furthermore, attention-deficit hyperactivity disorder (ADHD) and tic disorders are often associated with ASD. Around 80% of ASD people present motor abnormalities (12).

Considering medical comorbidities, a diagnosis of epilepsy has been formulated in ASD subjects with a prevalence ranging from 6% to 37%. Specifically, a greater risk for this comorbidity involved those with ID or associated genetic syndromes (43). This aspect have already been discussed in the pathophysiology paragraph regarding the excitation-inhibition imbalance which appears to be one of the main theoretical link between the observed clinical manifestations and the anatomo-functional findings (34). Gastrointestinal problems appeared significantly more frequent in children with ASD as compared to typically developing individuals, with a symptomatology that may include abdominal pain, constipation, chronic diarrhea, and gastro-esophageal reflux (44). Another clinically relevant aspect, that could be more properly ascribed among behavioral symptoms, is food selectivity. This behavior is of particular concern as it could lead to malnutrition (45).

Both clinical practice and epidemiological research suggest that psychological and psychiatric comorbidities are very common in ASD (12). Sleep disorder, particularly insomnia, have a high prevalence (50%) among people with ASD. Poor sleep hygiene appears to be associated with nocturnal agitation, co-sleeping behavior, and early awakening (46). This appears to be particularly related with anxiety disorders and mood disorders, conditions regarded to be significantly higher in adolescents and adults with ASD without ID as compared to neurotypical adults (47). The prevalence of anxiety disorders in high-functioning people with ASD is around 50% (48, 49). In the same

population, a recent systematic review suggested depression rates varying from 1% to 47% (50). In adulthood psychotic disorders could be diagnosed. Oppositional behaviors have been reported. These are often a behavioral correlate of anxiety, persevering belief in the righteousness of own point of view, and poor consequences evaluations. For these reasons there are not considered specific symptoms or comorbidity but instead the result of them (12). In a similar fashion, aggressive and self-injurious behaviors are relatively common and more typical of patients with lower IQ. They could be the result of frustration feelings in individuals with reduced communication abilities, as well as sensory overload or perturbation of sameness (51).

1.4. Interventions

Several interventions strategies are available for individuals with ASD and their families (52). However, despite the huge effort of the scientific community, to date there is no specific pharmacological intervention that has been approved for the treatment of the core symptoms. Most interventions are in fact educational or psychosocial. It is worthy to summarize some comprehensive approaches that are frequently applied in young children but could be useful also with adults suffering from ASD. The Applied Behavior Analysis (ABA; (53)) aims at the reduction of aggression and problem behavior through the mean of functional behavioral assessment to teach alternative behaviors. More recently, the Early Intensive Behavioral Intervention (EIBI; (54)) and the Early Start Denver Model (ESDM; (55)) have been proposed for younger children. Several educational approaches have focused mainly on the communicative deficient aspect of ASD. Among them, the Treatment and Education of Autism and related Communication-handicapped Children (TEACCH; (56)) can be used for any age and provides structured environment and activities that can be shared by the patients. The Picture Exchange

Communication System (PECS; (57) also showed efficacy in teaching social-communication skills using of pictures or symbols. Early interventions frequently involve parents and teachers in order to apply intervention strategies with continuity in the home environment or in community settings (58). For high functioning ASD subjects, cognitive behavioral therapy (CBT), specifically tailored for the peculiarities and needs of people with ASD, could help in reducing anxiety and teaching adaptive strategies. Social-skills training, such as PEERS® program (59), could also be useful for teaching social behavior.

Pharmacological treatments are often necessary given the frequent presence of psychiatric comorbidities and oppositional aggressive behaviors (60). However, these medications are also frequently over-prescribed (61). Antipsychotic drugs are among the most prescribed medication, even without any guidelines recommendations, even if they may present a high rate of adverse events. On the other hand, the SSRI are frequently used for to treat of comorbid anxiety and depression.

Given the lack of specific pharmacological treatment, several studies have explored potential complementary and alternative therapies for ASD, including drugs acting on the GABAergic system. However, the results are scarce, and more research is needed (62, 63).

1.5. Outcome

As a recent meta-analysis reported, the prognosis of ASD is critical. This life-lasting condition appear to be associated with remarkable impairment in several outcome measures. Steinhausen, Mohr Jensen (64) reported that, across the studies about 20% demonstrated a good outcome, the outcome was fair in 30%, and was poor or even very poor in almost 50% of the adults. However, according with other Authors, the outcome

could be heterogeneous (65). Adaptive functioning appeared to improve in most studies while social functioning, cognitive ability and language skills remained relatively stable or even deterioration over time. Diagnosis of ASD was generally stable, although severity of behavioral symptoms was often reported to improve (65). It is not infrequent that people with ASD need life-lasting support (65-67) and this is in line with the little impact on global outcome. However, early intervention is expected to be associated with larger effect on core symptoms and better outcome (54, 68). This is the main reason for promoting early detection and diagnosis.

The needs of adults with autism have been neglected by society for a long time. During the last century, given the scarce knowledge of this conditions and the paucity of effective interventions it was frequent to institutionalize subjects with severe forms of ASD. Nowadays, there are more possibilities of effective behavioral and environmental techniques of treatment that can be applied both in the home environment and within residential facilities. These approaches are still important to treat the most severe condition associated with ID, where the other approaches for people with ID without ASD would be inadequate. Among the diverse residential facilities, the farm-community represents a recognized approach, with a focus on social role valorization, building of capacity and deinstitutionalization (69). The adaptive outcome of a cohort of 22 subjects living in a farm-community specifically designed for autistic people in Pavia has been recently described (70).

Overall, the discussion about the outcome measurement in ASD is still controversial. During my doctoral course I have contributed to an extensive literature review to understand which are in literature the most adopted outcome measures. The paper,

which abstract has been reported below, has been submitted for publication and under review. A brief report has been reported in chapter 2.

• ***What are we targeting when we treat autism spectrum disorder? A systematic review of 406 clinical trials***

Prevalence of autism spectrum disorder (ASD) has been increasing progressively, together with the number of trials aimed at evaluating treatments for this condition. However, it is not clear which outcome measures should be used to assess their efficacy, especially for treatments which target core symptoms. The present review aimed to provide a comprehensive overview regarding the outcome measures used in clinical trials for people with ASD. We systematically searched the Web of Knowledge^{SM} database between 1980 and 2016 to identify published controlled trials investigating the efficacy of interventions in ASD. We included 406 trials in the final database, from which a total of 327 outcome measures were identified. Only seven scales were used in more than 5% of the studies, among which only three measures core symptoms (ADOS, CARS, SRS). Of note, 69% of the tools were used in literature only once. Our systematic review has shown that the evaluation of efficacy in intervention trials for ASD relies on heterogeneous and often non-specific tools for this condition. The fragmentation of tools may significantly hamper the comparisons between studies and thus the discovery of effective treatments for ASD. Greater consensus regarding the choice of these measure should be reached.

Aside from the common view of outcome as “lack from normality”, one of the most fascinating aspects of ASD is probably related to their putative talents (71-73). Interestingly, these peculiar areas of giftedness, could be positively exploited in rehabilitation programs to promote positive reward, social interactions, and communicative behavior (71). The case report of a woman affected by severe ASD with special musical talent has been the focused of a pubblication (74).

1.6. Diagnosis

Given the lack of consensus on biomarkers for ASD, the diagnosis remains essentially clinical and based on behaviors, observed by the assessor or described by patients or by caregivers (75). Since the first reports, the clinical definition of autism has changed. Initially, autism was considered a disorder characterized by extreme aloofness associated with repetitive and sensorimotor behaviors. The conceptualization evolved and now the diagnosis of ASD encompass a group of heterogeneous conditions and much more importance is given to the socio-communication deficits (75). As already said, ASD can be associated with a broad range of intellectual skills. As for other psychiatric conditions, symptoms could vary across individuals and though the life span. Thus, a correct identification of behaviors for an accurate diagnosis is a complex task (76).

Leo Kanner was the first to publish a detailed clinical description of few autistic children (77). The Author highlighted some core elements of the phenotype: the absence of a communicative language, the profound lack of affective contact with others, a repetition of verbal and motor behaviors, and the need for sameness. This was true despite the variety of observed cognitive abilities and without any clear congenital abnormality (77).

Almost simultaneously in Austria, Hans Asperger noticed similar features in a group of children. These subjects showed higher intellectual capabilities but, at the same time, presenting impairing difficulties in socio-communication. According to Kanner's description, their interests were very circumscribed and they showed repetitive behaviors. Another remarkable aspect observed by Asperger was that the children presented unusual sensory responses (78). It is not clear if the two Authors have known each other, probably not, given the limited diffusion of Asperger's original publication which was written in German (78). Other Influential approaches were those developed by Rutter (79), and by the National Society for Autistic Children (80). Officially, the first inclusion of these condition by the American Psychiatric Association in the Diagnostic and Statistical Manual (DSM) arrived in 1980 (81). DSM-III represented a crucial point in psychiatric nosography with the adoption of an untheoretical approach, focused on clinical descriptions (82). Included as a childhood disorder, autism took place among the Pervasive Developmental Disorders (PDD), together with other disorders. The core characteristics of the new diagnostic category were the lack of responsiveness to others, deficits in language development, peculiar speech features, and bizarre behaviors, including resistance to change, with an onset before 30 months of age. All criteria for the diagnosis had to be present and recognizable in a complete developmental history. Additionally, for the first time, the distinction between autism and schizophrenia was clear as psychotic symptoms could not be present in autism. Seven years later, the revised version of the manual (83) categorized the already described symptoms in three domains: impairment in reciprocal communication, social interaction, and restricted or repetitive behaviors. Age of onset was not any more an essential feature. Additionally, individuals with autism could have a co-occurring diagnosis of schizophrenia (84). The

fourth edition of DSM (85) was related to the International Classification of Diseases (ICD-10) (86), with the attempt to have consensus on a robust definition of autism (87). However, only minor changes were introduced in respect of the DSM-III-R. During these years the scientific community increases the research in the field of ASD. In this period in fact, Lord, Corsello (76) developed two new dimensional assessment instruments specifically anchored to DSM-IV. PDD included other three disorders new to DSM: childhood disintegrative disorder, Rett disorder, and Asperger disorder, along with the Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) category, already included in the previous versions. As already mentioned, the converging definitions of DSM-IV and ICD-10 facilitated the scientific agreement among different cultural framework. In this context, dimensional approaches enhanced further research (84). The last edition of DSM (81) significant changed to the overall structure of the diagnostic criteria. The major change regards the introduction of dimensional term "autism spectrum disorder": symptoms of ASD should be conceptualized on a continuum, with individuals showing milder symptoms, while others having severe symptoms and requiring extensive support. Apart from specific genetic condition that now deserved a specific diagnosis (ex. Rett Syndrome), all the conditions that were previously differentiated through minor developmental aspect (ex. the timing of language development in Asperger's syndrome vs autism) were now included in the same dimensional diagnosis. Furthermore, another significant change is that the core symptoms have been re-organized into two groups: (A) deficit of social communication and social interaction and (B) repetitive behavior, restricted interests, or stereotyped activities. Both the aspects are required for the diagnosis of ASD.

Again, according with DSM-5 criteria, individuals with ASD must present symptoms from the young age. However, it is specified that symptoms can be recognized later in life, especially with the increase of social demands. These criteria change supports earlier diagnoses of ASD but also allows later diagnosis in those whose coping abilities allowed to compensate the aberrant behaviors in early life situations.

Finally, the severity of functioning impairment is required by the DSM-5. This should be rated by clinicians in a three point scale for each of the two diagnostic domains and should reflect the level of needed support. Further specifiers could be also added (82). The box below reported DSM-5 diagnostic criteria and specifier for ASD.

***DSM-5* Diagnostic Criteria for Autism Spectrum Disorder 299.00 (F84.0)**

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history. *Specify* current severity.

1. Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interest, emotions, or affect; to failure to initiate or respond to social interactions.
2. Deficits in nonverbal communicative behaviors used to social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expressions and nonverbal communication.
3. Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behavior to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.

B. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history. *Specify* current severity.

1. Stereotyped or repetitive motor movements, use of objects, or speech (e.g., simple motor stereotypies, lining up toys or flipping objects, echolalia, idiosyncratic phrases).
2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior (e.g., extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat
same food every day).
3. Highly restricted, fixated interests that are abnormal in intensity or focus (e.g., strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interests).
4. Hyper- or hyporeactivity to sensory input or unusual interest in sensory aspects of the environment (e.g., apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement).

C. Symptoms must be present in the early developmental period (but may not become fully manifested until social demands exceed limited capacities, or may be masked by learned strategies in later life).

D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

E. These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum disorder and intellectual disability, social communication should be below that expected of general developmental level.

Note: Individuals with a well-established *DSM-IV* diagnosis of autistic disorder, Asperger's disorder, or pervasive developmental disorder not otherwise specified should be given the diagnosis of autism spectrum disorder. Individuals who have marked deficits in social communication, but whose symptoms do not otherwise need criteria for autism spectrum disorder, should be evaluated for social (pragmatic) communication disorder.

Specify if:

- **With or without accompanying intellectual impairment**
- **With or without accompanying language impairment**
- **Associated with a known medical or genetic condition or environmental factor**
- **Associated with another neurological, mental, or behavioral disorder**
- **With catatonia**

1.7.1 Diagnosis of ASD in adulthood

Given the developmental nature of ASD, researchers have mainly focused on the recognition and treatment of ASD in early childhood (12). However, as already anticipated with the concept of the "lost generation", interest in recognition and evolution of the ASD in adulthood is growing (88, 89). In fact, if the clinical picture is usually clearer in individuals with severe symptoms (e.g. extreme social aloneness, deficient eye contact, mannerism) and concurrent developmental difficulties (e.g. language delay), however both milder behavioral phenotype or the comorbidity with profound ID could let the ASD unrecognized for many years. Additionally, as already explicated in DSM-5 criteria, some forms of ASD might not be diagnosed until adulthood. Considering the recent sensitization of the clinicians towards this diagnosis, we should avoid the over-simplistic hypothesis that we are now facing "new needs". We are now instead trying to provide adequate support to unmet needs that were already present (13, 90).

There is a general consensus that the diagnosis of ASD in adulthood requires a multistep and multidisciplinary assessment (91, 92). According with the diagnostic criteria the evaluation process needs to investigate the developmental history alongside with the actual behavior assessment. This process should be undertaken by trained and competent professionals (87, 91, 93). For more complex assessments, it is suggested to support the clinical judgment with standardized instruments, which could improve the reliability of diagnosis (81, 91). However, this aspect could be controversial and the usefulness of standardized instrument in adults' assessment has been part of my doctoral course research. In general, diagnosing ASD in adults is difficult, also considering the scarce number of diagnostic instruments specifically conceptualized for

this age group (94). Furthermore, if direct observation and clinical interview can be easily conducted, clinicians could experience difficulties in collecting information about the patient's early development (13).

1.7.2 Differential diagnosis

Differential diagnosis could be particularly challenging for psychiatrists who did not receive a specific training on neurodevelopmental disorders (95). Furthermore, comorbid psychiatric disorders are frequent, and the behaviors described by patients and caregivers are frequently the combination of several factors. It is thus essential to involve into the multidisciplinary assessment a professional with expertise in ASD in order to make a differential diagnosis (92).

The description of some anxiety disorders may resemble sometimes some aspects of ASD: social phobia, generalized anxiety disorder (GAD) could significantly affect the social functioning (96). However, it is usually easy to determine the onset of the symptoms and differentiate them from developmental conditions. Also, obsessive-compulsive disorder (OCD) could shares some features with ASD, such as the presence of pervasive thoughts, rituals, and repetitive behaviors. However, in OCD the presence of unpleasant, anxious or obsessive thoughts is frequently the core of the disease, with the rituals frequently associated. On the other hand, in ASD, repetitive behaviors and thoughts are frequently present as part of a sameness, and the anxiety, if present, usually appears when the sameness is interrupted by unexpected events(97).

Depression may also determine a social deficit. However, depressive behavior is frequently described as withdrawal, associated with the lack of interest in social activity

and an accurate evaluation of social skills, together with a psychiatric interview focused on depressive symptoms, should allow to determine the presence of a depressed mood (92). In ASD, the social deficit is more frequently described as a struggle to stay with other people, associated with the sense of frustration and failure due to intrinsic difficulty in social communication. It can be associated with a depress mood (that represent the first major psychiatric comorbidity) and for this reason it is particularly relevant to investigate the behavior in a developmental perspective.

Another frequent comorbidity which is difficult to distinguish from ASD is attention deficit hyperactivity disorder (ADHD). In this condition subjects often have impairments in executive functioning, appears easily distractible and sometimes appear restless, such as autistic subjects; additionally, socially inappropriate behavior could sometimes lead to scarce socialization that could be mistaken for ASD. Nevertheless is clear that, people with ADHD do not show communication deficits or restricted interests and behaviors (98). The presence of this comorbidity in ASD is particularly relevant and deserve special effort to be adequately assessed as several medications are available to improve ADHD, thus facilitating cognitive and behavioral intervention.

Psychotic disorders could also be associated with social isolation and socially inappropriate behaviors. Additionally, tangentiality, circumstantiality, and neologisms are common to both conditions. It is thus fundamental to collect a detailed clinical history to determine the onset of symptoms. In fact, while ASD onset is typically recognized during early childhood, psychotic conditions become frequently manifest during adolescence or early adulthood. It could be almost impossible to distinguish some form of chronic schizophrenia from ASD only on the basis of the clinical examination.

However, some symptoms, such as delusions or hallucinations, are not common in ASD and, when observed, they need to be carefully evaluated. The comorbidity of the two conditions could be present even if not so common (99).

Many personality disorders, particularly those belonging to cluster A or C of DSM-IV-TR, share several features with ASD. As for psychosis disorders, determination of symptom onset is critical: personality disorders usually appear later in life (92). Particularly complex is the differential with schizotypal personality disorder. It is detrimental to carefully assess the presence of the behavior described as criterion B in DSM chapter of ASD, even though the social impairment is usually milder in schizotypal personality disorder. Even though the presence of ideas of reference and paranoid ideation, described as schizophrenia spectrum phenomena, could be reported in ASD, the phenomenological description of the symptoms are usually associated with rigid logical thinking instead of frankly delusion and bizarre interpretation. Finally, abnormal perceptual experiences are very uncommon in people with ASD (92) and not rare in schizotypal personality.

People suffering from schizoid personality disorder have explicit disinterest in social relationships and exhibit flattened affect. At the opposite, in ASD individual there is frequently the desire of meaningful social relationships, but they do not have sufficient skills to build them. Finally, schizoid personalities do not present ASD criterion B behaviors (92).

Probably one of the most challenging differential diagnoses is about the presence of ASD in people with severe and profound ID. As already reported more than 30% of the ASD

subject receive a diagnosis of ID which is per se very common in the general population. Individuals with ID could present stereotypies and language limitations analogously to ASD even when ASD features are not present. However, the two diagnosis when not overlapping could be discriminated by social interaction descriptions: ID patients without ASD do not usually present abnormal eye contact or difficulties in shared enjoyment that are instead frequent in ASD individuals. Nonverbal communication tent to be more compromised in ASD. It is also important to perform a careful cognitive evaluation with the use of standardized tests whenever possible. Usually, an heterogeneous cognitive profile, characterized by areas of strengths ("island of abilities") and weaknesses is suggestive for the presence of ASD (100). The difficult diagnosis of ASD when associated with severe ID in adulthood is probably the stronger claim for reliable ASD biomarkers, as the lack of standardized diagnostic instrument appear critical in this population.

1.7.3 Standardised diagnostic instruments

Initially developed for research purposes diagnostic instruments for ASD have grown in number during the last 30 years, and the use of these tools in clinical practice was associated with important changes. Nowadays, a number of screening questionnaires for ASD in adults are available. Some examples are the Autism-Spectrum Quotient (AQ; (101)), the Social Communication Questionnaire (SCQ; (102)), the Ritvo Autism Asperger Diagnostic Scale-Revised (RAADS-R; (103)), and the Social responsiveness Scale (SRS; (104)) . However, the clinical diagnosis is crucial and cannot rely solely on screening tools as, by definition, they lack specificity (76). Furthermore, because some individuals with ASD may have insight and metacognitive difficulties (105, 106), self-report ratings could not accurately estimate autistic symptoms in ASD subjects. Significant incongruence

between self- and parent-reported questionnaires were described (107-109). In fact, subjects with ASD could have difficulties in estimating their own behavior and feelings. A possible reason for this finding could be looked for in the problems of verbal and non-verbal communication. In addition, screening tools for ASD have been developed and validated in the general population, thus they might not be accurate in clinical samples, for possible overlapping psychiatric symptoms of other conditions. Another limitation of these instruments is that they are frequently designed for the high end of the autistic spectrum. During my doctoral course I collaborated with my research group to a multicenter study for the development of a new screening tool for subtle expression of autism, including for the validation also psychiatric comparison groups. The abstract of the publication focusing on this topic has been reported below.

- ***Adult Autism Subthreshold Spectrum (AdAS Spectrum): Validation of a questionnaire investigating subthreshold autism spectrum (2)***

"AIM: Increasing literature has shown the usefulness of a dimensional approach to autism. The present study aimed to determine the psychometric properties of the Adult Autism Subthreshold Spectrum (AdAS Spectrum), a new questionnaire specifically tailored to assess subthreshold forms of autism spectrum disorder (ASD) in adulthood. METHODS: 102 adults endorsing at least one DSM-5 symptom criterion for ASD (ASDc), 143 adults diagnosed with a feeding and eating disorder (FED), and 160 subjects with no mental disorders (CTL), were recruited from 7 Italian University Departments of Psychiatry and administered the following: SCID-5, Autism-Spectrum Quotient (AQ), Ritvo Autism and Asperger Diagnostic Scale 14-

*item version (RAADS-14), and AdAS Spectrum. RESULTS: The AdAS Spectrum demonstrated excellent internal consistency for the total score (Kuder-Richardson's coefficient=.964) as well as for five out of seven domains (all coefficients>.80) and sound test-retest reliability (ICC=.976). The total and domain AdAS Spectrum scores showed a moderate to strong (>.50) positive correlation with one another and with the AQ and RAADS-14 total scores. ASDc subjects reported significantly higher AdAS Spectrum total scores than both FED (p<.001) and CTL (p<.001), and significantly higher scores on the Childhood/adolescence, Verbal communication, Empathy, Inflexibility and adherence to routine, and Restricted interests and rumination domains (all p<.001) than FED, while on all domains compared to CTL. CTL displayed significantly lower total and domain scores than FED (all p<.001). A significant effect of gender emerged for the Hyper- and hyporeactivity to sensory input domain, with women showing higher scores than men (p=.003). A Diagnosis*Gender interaction was also found for the Verbal communication (p=.019) and Empathy (p=.023) domains. When splitting the ASDc in subjects with one symptom criterion (ASD1) and those with a ASD, and the FED in subjects with no ASD symptom criteria (FED0) and those with one ASD symptom criterion (FED1), a gradient of severity in AdAS Spectrum scores from CTL subjects to ASD patients, across FED0, ASD1, FED1 was shown. CONCLUSIONS: The AdAS Spectrum showed excellent internal consistency and test-retest reliability and strong convergent validity with alternative dimensional measures of ASD. The questionnaire performed differently among the three diagnostic groups and enlightened some significant effects of gender in the expression of autistic traits."*

Better specificity has been recognized to several standardized instruments, employed in more systematic assessment of ASD. However, many of these tools have been developed for children and their accuracy has been limitedly study in adulthood (94). Again, it is worth emphasize the need of a multistep assessment as the test per se could be insufficient for the ASD diagnosis (110).

Diagnostic instruments might be divided in observational assessment tools and interviews that could be administered to parents or caregivers. The first group include the Autism Diagnostic Observation Schedule (ADOS; (111)) and the Childhood Autism Rating Scale (CARS; (112)). Among the caregiver interviews, the most widely used are the Autism Diagnostic Interview-Revised (ADI-R; (113)), the Diagnostic Interview for Social and Communication Disorders (DISCO; (114)), and the Developmental, Dimensional, and Diagnostic Interview (3Di; (115)). The ADOS-2 and the ADI-R have been translated in many languages and are currently used worldwide and considered the "gold standard" tools for the diagnosis of ASD given their strong validity (116, 117). However, result interpretation require caution as some evidences showed that these standardized tools could be less reliable in specific groups of individuals. The ADOS-2, in fact, was validated mainly on male characteristics and could not be able to completely capture the female phenotype (118, 119). Furthermore, a recent study (120) showed that gender could affect diagnostic evaluation in a sample of adults with suspected ASD. Within the ASD population in fact, females could show better adaptive skills and social (121), and tend to show less frequently externalizing behaviors (122, 123). Some authors

attributed the slightly low proportion of ASD females with high cognitive abilities to an under-identification of this particular subsample (124).

1.7.3.1. The Autism Diagnostic Observation Schedule-2 (ADOS-2)

The ADOS-2 is a semi-structured observation for the diagnosis of ASD (111). It is composed by five different domains: Communication, Reciprocal Social Interaction, The sum of the two, Creativity, and Stereotyped Behaviors. The ADOS-2 consists of five modules, each specific for different age and language level. Adolescents or adults without ID and with good verbal fluency could be evaluated by means of Module 4. The major limitation of this tool is that it cannot be used to evaluate adult subjects without fluent language use, as frequently observed in ID.

The assessment duration should be about 45 minutes and is composed by some tasks (i.e. puzzle, storytelling with objects provided by the interviewer), and conversation (for example evaluating social relationships, daily life, school, job). Each item could be use comprehensively evaluate several factors. The notes could be subsequently scored according to the symptom's domains previously exposed. The sum of the items provides the scores needed to evaluate the possible inclusion in the ASD.

According to the original algorithm (111), Module 4 score is considered suggestive of a diagnosis of ASD if the score met the threshold values for the "autism spectrum" in the Communication domain (2 or above), Social domain (4 or above), as well as in the combined Communication/Social domain (7 or above). Since the instrument was developed before the DSM-5, the scores for creativity and repetitive behaviors are not considered for final classification. However, it has be proposed a revised version of

ADOS-2 Module 4 algorithm (125) in which a score of 8 or above in the combintion of social affect (SA) and restricted and repetitive behaviors (RRB) domains is suggestive of a diagnosis of ASD.

ADOS has proven reliability and validity for the assessment of ASD in children and adolescents (126-130). However, psychometric properties evaluation of Module 4, supposed to be administered in adolescents and adults with fluent language skills, are less extensive. Bastiaansen, Meffert (94) studied the ADOS-2 Module 4 in a sample of adults diagnosed with ASD without ID as compared to other clinical and non-clinical groups. The ADOS-2 showed adequate discriminant ability to distinguish ASD from psychopathy and from typically developed adults; discrimination from schizophrenia was less accurate. More recently, de Bildt, Sytema (131) found an better sensitivity using the revised algorithm proposed by Hus and Lord. The revised algorithm appeared to be slightly better in term of sensitivity and specificity also according to the study of Langmann, Becker (128). However, discriminating ASD from other severe psychiatric condition appears to be difficult using the ADOS-2, as suggested by the high frequent false positives, particularly with psychotic patients have been reported in the study of Maddox, Brodkin (132).

1.7.3.2. The Autism Diagnostic Interview-Revised (ADI-R)

The ADI-R is a parent semi-structured interview developed on the basis of DSM-IV that investigate three behavioral domain of the children: the quality of reciprocal social interaction; the quality and quantity of communication; the repetitive, restricted, and stereotyped patterns of behavior (113). It is mainly focused on the childhood (between

the ages of 4 and 5), a period that is supposed to be critical for evaluating behavioral differences among individuals with various levels of functioning.

The ADI-R score is considered indicative of a diagnosis of ASD if the scores exceed the cut-off values in the three domains. The cut-off score is 8 for the communication and language domain for verbal subjects. For all subjects, the cut-off is 10 for the social interaction domain. Finally, the cut-off is 3 for restricted and repetitive behaviors. Additionally, some abnormalities should be present by 36 months of age in at least one area.

The ADI-R, have been considered a valid instrument independently from age and level of functioning (126). Some studies investigated the diagnostic stability of the ADI-R over lifetime in non-ID samples (133-135). However, to our knowledge, its utility in adulthood has been studied and reported in two pubblications. Sappok, Diefenbacher (136) studied the validity of both ADOS-2 and ADI-R in a sample of adults with ID. Th ADI-R showed a good specificity (80%) and sensitivity (88%). More recently, Talari, Balaji, & Stansfield (2017) found that ADI-R had a high sensitivity (100%), but an extremely low specificity (37%). Furthermore, specificity was lower in male than females, and in people with ID than without ID.

2. Systematic review of ASD diagnostic tools in clinical trials

2.1. Aims

During my doctoral course I participated to a large review project at the Laboratorio Autismo of the University of Pavia. The literature review aimed at including all controlled clinical trials (CCT) on ASD, both with randomized (RCT) and non-randomized design (CT), published from 1980 to present. The main purpose of this study was to summarize the methodological and clinical variable in order to understand the trends and agreement of the scientific community over this complex and heterogeneous field.

Particularly the final database should allow to:

- review the number and type of instruments used to assess the clinical outcome and treatment effect in CCTs published from 1980 (as already mentioned in paragraph 1.5. these data have recently been submitted for publication);
- review the number and type of instruments used to diagnose ASD in CCTs and trace a temporal trend in diagnostic tools use;
- perform meta-analytic summaries of the intervention studies.

In this chapter, I provided the preliminary summary of the systematic review of the tools used for the diagnosis of ASD in CCT. The data have also been analyzed in correlation to some relevant clinical variables (age and IQ of included population) to support the discussion of the original data reported in chapter 3.

2.2. Methods

2.2.1. Search strategies

A comprehensive two-step search has been conducted following the guidelines outlined in the PRISMA Statement (137). Firstly, we performed an electronic search using The Web of Science™ database by Thomson Reuters® (including several databases) from

1980 until Dec 2016. The search was restricted to English language, adopting the following search string:

(autis OR (developm* AND disorder) OR asperger OR Kanner OR ASD OR PDD) AND (RCT OR trial OR observational OR 'open label' OR prospective OR longitudinal OR randomized OR cohort)*

This procedure was followed by hand searching of reference lists of the included review to identify any missed potential publication.

2.2.2. Selection criteria

All abstracts were extracted to EndNote reference management software. After duplicate removal, abstracts were screened to identify potentially relevant studies and full texts were inspected for selection. Each item was double checked by at least two researchers and any doubt was solved through consultation among the researchers.

To be selected each study must fulfill the following inclusion criteria:

(a) original peer reviewed article;

(b) including subjects diagnosed with PDD or ASD;

(c) clinical controlled trial against placebo (or head-to-head treatment), both with randomized or observational longitudinal design, directed to people with ASD;

(d) reporting at least one clinical outcome.

Consequently, we excluded:

(a) congress abstract, review, meta-analysis, case report;

(b) studies with retrospective design or lacking a comparison group;

(c) studies investigating the effect of an indirect treatment (i.e. intervention directed to parents);

(d) studies failing to report a clinical outcome measure (i.e. biomarkers and imaging were not considered clinical outcome measures).

2.2.3. Data Extraction

A standardized methodology was applied to extract data from the included studies. In a similar way, we standardized the assessment of study quality and quality of reporting. The following variables were extracted: study name; year of publication; study design; active treatment; comparison; duration of the study; sample size; diagnostic tools; primary and secondary outcome measures; presence of any psychiatric comorbidity (excluding ID); sample age; sample IQ; IQ evaluation tools; female proportion; study location. As RCT were the most frequent study design we also decided to adopt the Cochrane Risk of Bias assessment tool (138) for quality assessment. For this thesis, only the following variable have been reported: study name; year of publication; type of intervention; diagnostic tools; age; IQ.

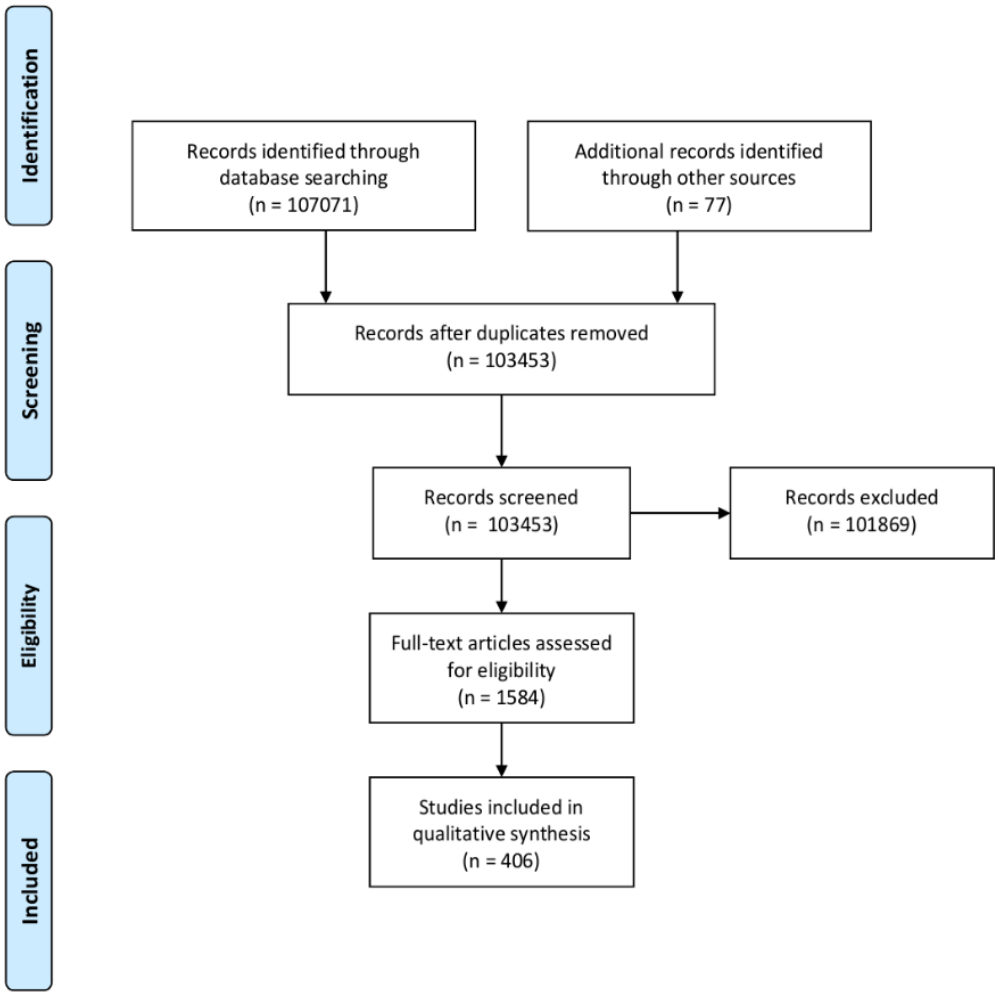
2.2.4. Statistics

Data are reported as count or percentages as appropriate. For descriptive purposes the clinical variables have been categorized and χ^2 statistics was used to evaluate the relationship between age and diagnostic tools, and between IQ and diagnostic tools. Results were considered statistically significant at the two-tailed $p \leq 0.05$ level. Statistical analysis was performed using SPSS 24.0 software packages (SPSS, Chicago, IL).

2.3. Results

We identified a total of 107148 records (77 studies from hand-searching). Our research included 406 studies, reported in 402 publications. A Prisma flow chart of the study selection process is reported in Figure 2.1.

Figure 2.1. PRISMA flow chart



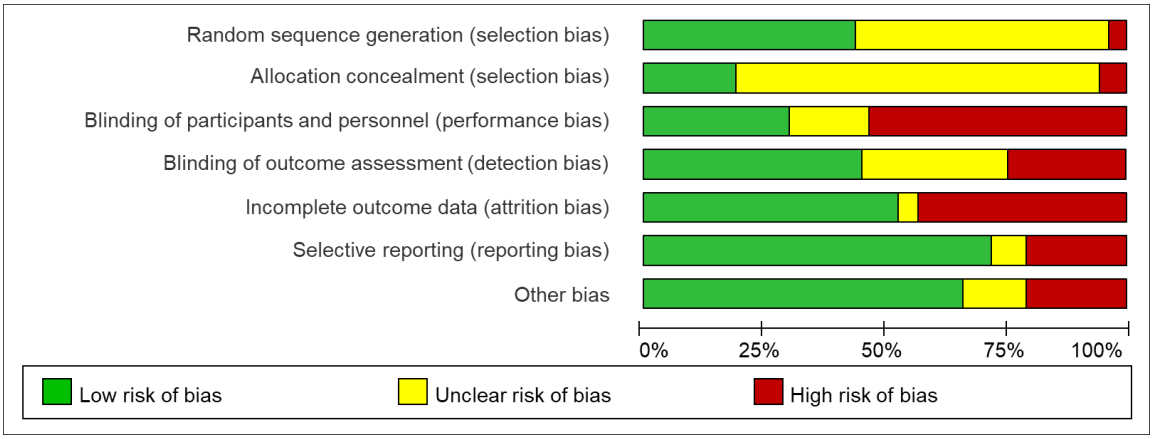
The reviewed studies included 354 RCT (77.2%) and 52 non-randomized trials (12.8%) with a mean follow-up length of 17.4 weeks, varied from a single administration (one

day) to 208 weeks. The active intervention was categorized as educational in 137 studies (34%), pharmacological in 132 studies (33%), nutraceutical in 50 (12%). Psychotherapy was the evaluated treatment in 30 studies (7%). Overall, miscellaneous interventions that did not fell in the previous categories were studied in 57 studies (14%). Most of the researches were conducted in the United States (54% of the included studies). Appendix A report a detailed list of the included trials.

Our database included 17240 participants. Samples sizes in each study ranged from 4 to 308. The average female proportion was 17.7% (range 0 - 51%, unclear in 30 studies). The sample included only children in 315 studies, while 19 studies included only adults, and 39 studies included both children and adults (unclear in 33 studies). Psychiatric comorbidities (excluding ID) were excluded in 56 studies and acknowledged in 52 studies. Surprisingly, 298 studies did not mention any information about psychiatric comorbidities. Maybe more surprisingly, IQ characteristics of the sample were unclear and not reported in 227 studies. Only 25 studies focused on samples with ID, while 81 studies included only individuals without ID and 73 studies recruited both ASD people with and without ID.

According to the Cochrane's collaboration tool (138), only 11 (3%) studies obtained good quality evaluation, 107 (30%) scored as fair, 235 (66%) had poor quality of reporting. A summary of the quality of reporting studies is depicted in Figure 2.2.

Figure 2.2. Quality assessment.



2.3.1. Diagnostic instruments

Several different approaches were adopted among diagnostic instruments: diagnostic manuals or guidelines, interviews to caregivers, questionnaires, and direct observations. In 44 studies (11%) no tool neither diagnostic manuals or guidelines were specified. A single diagnostic instrument was used in 158 studies, while in 204 studies at least two instruments were used (maximum 5 instruments). Excluding DSM and ICD varous versions, 25 diagnostic instruments were used (Table 4.1).

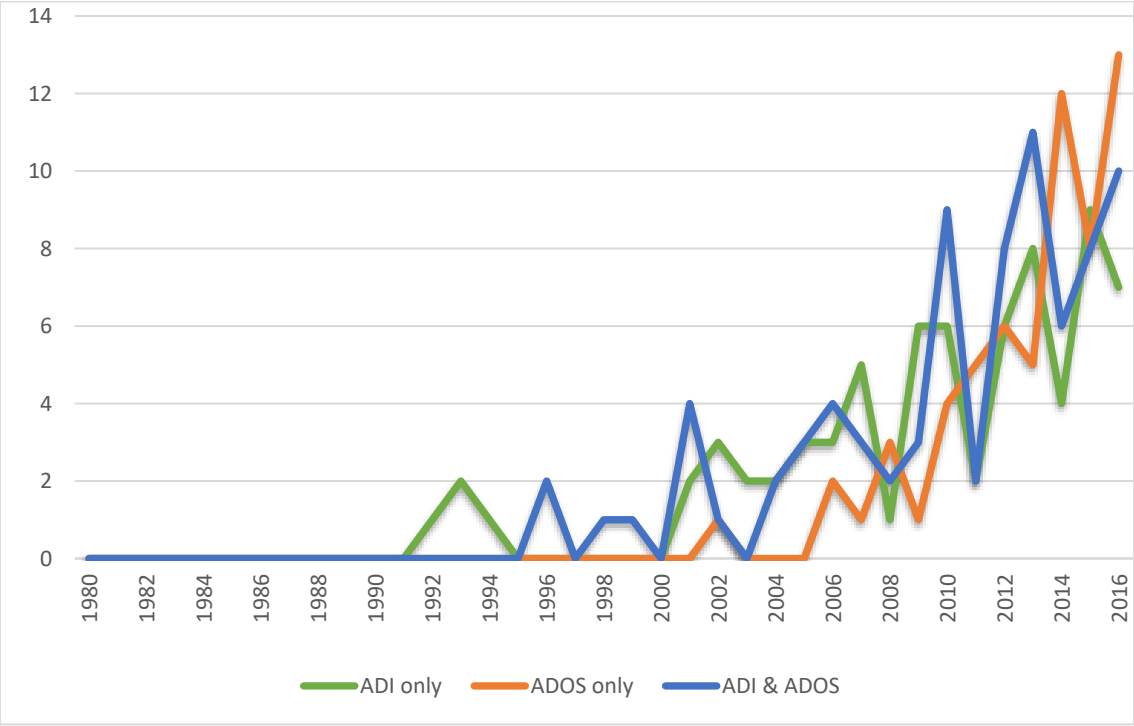
Table 4.1. Diagnostic instruments sorted by frequency of use.

Acronym	Diagnostic Instrument	Times used
ADI	Autism Diagnostic Interview	153
ADOS	Autism Diagnostic Observation Schedule	141
DSM-IV-TR	DSM - 4th Ed TR	105
DSM-IV	DSM - 4th Ed	94
	Unspecified diagnostic instrument	44
DSM-III-R	DSM - 3rd Ed Revised	31
CARS	Childhood Autism Rating Scale	27
ICD-10	International Classification of Diseases - 10th Ed	26
DSM-III	DSM - 3rd Ed	23
SCQ	Social Communication Questionnaire	16
CAST	Childhood Asperger Spectrum Test	5
DSM-5	DSM - 5th Ed	4
SRS	Social Responsiveness Scale	4
DISCO	Diagnostic Interview for Social and Communication Disorders	3
AQ	Autism-Spectrum Quotient	2
ASDI	Asperger Syndrome Diagnostic Interview	2
ASSQ	Autism Spectrum Screening Questionnaire	2
AUBC	Autism Behavior Checklist	2
NSAC	National Society for Autistic Children Diagnostic Criteria	2
RDEC	Rimland Diagnostic E-2 Checklist	2
3Di	Developmental, Dimensional and Diagnostic Interview	1
ABC	Aberrant Behavior Checklist	1
ASASC	Australian Scale for Autism Spectrum Conditions	1
BSE	Behavioral Summarized Evaluation	1
DBC	Developmental Behavior Checklist	1
DIPAB	Diagnosis of Psychotic Behavior in Children	1
DISCAP	Diagnostic Interview Schedule for Children, Adolescents and Parents	1
M-CHAT	Modified Checklist for Autism in Toddlers	1
OAGIS	OSU Autism Global Impression Scale	1
OARS	OSU Autism Rating Scale DSM-IV	1
PARS	PDD Autism Society Japan Rating Scale	1
PDDBI	PDD Behavior Inventory	1

Clinical diagnostic criteria (i.e. DSM, ICD, or NSAC) were the only diagnostic approach in 26% of the studies. About half of the studies reported at least one of the two "gold standard" diagnostic instruments (ADOS and ADI), or both (9%). These instruments were the most used, respectively in 141 and 153 studies. In 10% of the studies, other diagnostic tools were used, alone or in combination with diagnostic manuals. Finally, as already mentioned, in 11% of the studies no diagnostic tools nor diagnostic system were reported.

Analyzing the use of the two "gold standard" instruments (ADOS and ADI) across time, we find an increasing trend which resemble the number of CCT published. Figure 2.3 shows the raw number of studies adopting ADOS or ADI alone or combined.

Figure 2.3. CCT adopting ADOS or ADI from 1980.



2.3.2. Correlation of diagnostic instrument with age and ID

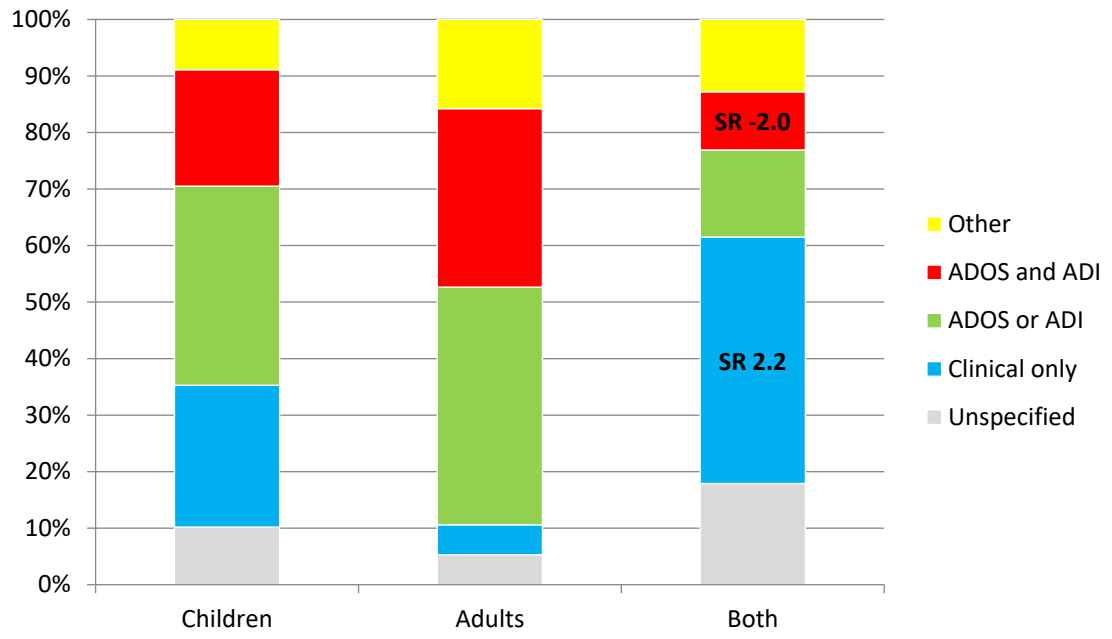
As already reported, most of the studies included only children (315). Among them 35.3% of the studies did not adopt any specific diagnostic tool, whereas 176 trials (55.8%) used at least one gold standard measure or both ADI and ADOS. In 28 studies (8.9%) other tools were adopted. Of the 19 CCTs conducted only on adult subjects, 14 used ADI, ADOS or both (73.7%). In this subsample, the diagnosis was unspecified in 2 studies, and other tools were used in 3 studies.

39 studies were conducted in samples of mixed age, including both children and adults. This sub-group showed the most inaccurate reporting of the diagnostic approach as only ten confirmed the diagnosis of participants with at least ADOS or ADI. The diagnosis was unspecified in seven cases, and relied only on clinical criteria in 17 cases. Other tools were used in five studies.

The correlation between age of participants and diagnostic instruments was tested with the χ^2 test of independence. A significant correlation emerged ($\chi^2(8, n=373)=19.43$, $p=0.013$). Specifically, in trials including both children and adults, ADOS or ADI alone were used less than expected (standardized residual, SR=-2.0), while clinical criteria alone were used more than expected (SR=2.2) as reported in figure 2.4.

Unfortunately, data regarding ID were reported only in 179 studies (44.08%). Among the trials reporting IQ, 25 studies (14%) involved only people with ID. In 81 studies (45.3%) the sample was composed only by people without ID. Finally, in 73 cases (40.8%) the sample included both subjects with and without ID.

Figure 2.4. Type of diagnosis according to the age of the sample.



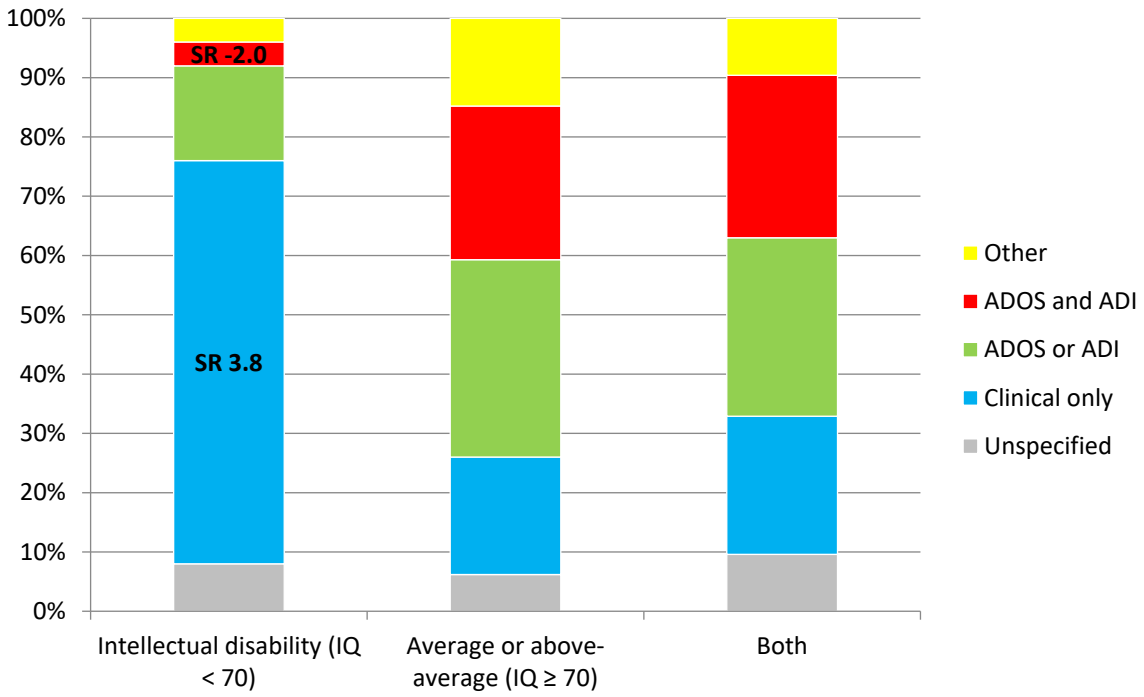
In the subset of studies including ID subjects, participants were mainly diagnosed with clinical criteria only (68%), while in 20%, the diagnosis was validated by the use of ADOS, ADI or both. Other tools were selected for diagnostic purposes in one study, and diagnostic criteria were unspecified in two studies. Finally, gold standard tools were used in 48 of the studies including only people without ID (59.2%). Other tools were employed in 12 trials (14.8%) and diagnostic classifications though clinical criteria was the only diagnostic instrument in 16 CCTs (19.8%). Finally, 5 studies did not specify any diagnostic methodology.

Considering the third subset of studies who recruited both high- and low-functioning people, 57.5% (42 trials) used ADOS, ADI or both, while 17 studies (23.3%) applied only

DSM or ICD criteria. In seven studies (9.6%) other tools were used, and in other 7 the diagnostic approach was unclear.

The χ^2 test of independence revealed a significant correlation among the use of diagnostic instrument and the ID categorization of the samples ($\chi^2(8, n=179)=26.40$, $p=0.001$). In particular, in trials including individuals with ID only, combination of the two gold standard tools, ADOS and ADI, were used less than expected (SR=-2.0), while DSM and ICD criteria were used more than expected in this group of CCTs (SR=3.8).

Figure 2.5. Type of diagnosis according to sample cognitive abilities.



2.4. Discussion

This systematic review is part of a large project aimed at reviewing the characteristics of clinical trials conducted on individuals diagnosed with ASD and published from 1980 to present. I have reported here a brief synthesis of a preliminary analysis regarding the tools used for the diagnosis in all CCTs published from 1980 to 2016. In the last years the

number of clinical trials in ASD has grown exponentially, testifying not merely the interest for ASD among the scientific community but also the need for effective intervention from a clinical perspective. However, as already reported in paragraph 1.5, the targets of the interventions are multiple, heterogeneous and there is no consensus on the outcome measures adopted. Since most of the recent research efforts have also been directed toward the diagnosis of ASD, we expected more consistency across the diagnostic instruments adopted in CCTs. As expected, our data confirmed that ADOS and ADI are the most widely used diagnostic tools. Of 406 studies, we found that 153 used the ADI (37.7%) and 141 (34.7%) used the ADOS for diagnosis confirmation, while 37 CCTs adopted both tools (9.1%). It is noteworthy that the number of CCTs using the ADI was superior to those which used the ADOS. One possible argumentation could rely on the fact that while both were developed in the same year, ADI became available earlier than ADOS. Furthermore, as most of the studies have been conducted on children, it is possible that, given the chance to involve parents in the diagnostic assessment, ADI could have been the interview of choice to minimize the stress on young patients. Our results also highlighted an increasing trend toward the use of standardized assessment tools over the last hear. Since 1994 (year of publication of the ADI-R), as reported, the proportion of CCTs which used ADOS, ADI or both, have been progressively increasing. Nevertheless, the use of clinical application of standardized diagnostic criteria have been the only assessment procedure in 26% of the retrieved CCTs and 11% of the studies did not clarify how the diagnosis was made. The global picture appears complex, and age and ID have been significantly associated with different diagnostic procedures. In fact, from the retrieved studies it emerged that individuals with ID tent to be less frequently assessed with standardized tools, while being more frequently diagnosed by means of

clinical criteria only. This is an important issue, since it could highlight the lack of trust or worst, the perceived lack of need to use diagnostic tools specifically designed for the diagnosis of ASD in people with ID. In fact, even if it possible, ADOS is rarely used in people with ID, particularly adults, since Modules 3 and 4, which are directed to adolescents or adults require good verbal fluency. In adults with severe language impairment (frequently associated with ID) the use of ADOS has not been validated and it is not recommended. It is also worth mentioning that ASD symptomatology might be more severe and consequently more evident in this last subgroup, probably reducing the perceived need of standardized tools. Also, standardized tools were used less than expected in trials including a mixed sample of both adults and children, and in samples including adults only. This could be associated with the difficulty in administrating the ADI on older caregiver and is also confirmed by the more frequent use of ADOS or ADI in study conducted on children. This finding is crucial and support the need of reliable biomarkers to support the diagnosis, especially in the group with comorbid ASD and ID.

It is worth discussing the tendency toward the adoption of standardised tools different from ADOS and ADI in the studies recruiting participants with an IQ ≥ 70. One possible explanation could be the simplicity of some paper and pencil questionnaires, directed to the parents or to the patient herself. Some examples are the AQ, the CAST (139), the SCQ (102), the SRS (140), the ASASC (141). In other rare cases, standardized interviews or direct observations, such as the DISCAP (142), the ASDI (143), the DISCO (144), or the 3Di (115, 142) were used.

It is important to stress the preliminary approach of our analyses regarding the relationship between diagnostic instruments use and clinical and demographic

characteristics of the sample. For this reason, it should be considered carefully. In fact, several studies were unclear about the age of participants (8.13%) and, more worrying, the presence of ID was not assessed in almost 66% of the CCTs. These data highlight the lack of well-designed trials and the poor reporting in papers for ASD, as underlined by the poor quality of the included RCTs. Especially considering the heterogeneous nature of ASD, a long-life condition with a spectrum of phenotypical presentations, it is important to better characterize and describe the subjects recruited in CCTs. Subjects, interventions and outcomes could be extremely different in samples with different clinical pictures. There is an urge for consensus and accuracy to allow the development of specific therapies for the different ASD subgroups. Finally, we can conclude that despite the research regarding ASD in constantly growing, the use of diagnostic tools is still heterogeneous. ADOS and ADI represent the two widest used instruments, but many other questionnaires not specifically designed for diagnostic purposes are frequently used for the diagnostic confirmation. This is particularly true for adult subject with suspected ASD. the scientific community should aim at reaching a consensus regarding the standardized instruments to confirm the diagnosis of people included in CCTs. It would be also ideal to develop a plausible battery of standardized instruments adequate for age, IQ and other patients' characteristics. Given the sensitization of the general population toward ASD in fact, the risk of a growing number of people inappropriately using screening tools for self-diagnoses could increase the risk of selection bias, especially in adults. This could potentially invalidate the results of CCTs, or scientific research in general.

3. Evaluation of the diagnostic reliability of ADOS-2 and ADI-R in adults with ASD without ID

3.1. Aims

As already discussed, ASD diagnosis in adulthood could represent a challenge for clinicians. However, even if guidelines strongly suggest supporting the clinical assessment of ASD with standardized instruments during a multistep procedure (91), this appear to be still far from clinical practice as we found with our extensive systematic review of clinical trials. Nevertheless, according with guidelines, our review confirmed that the most adopted tools for assessment of ASD are the Autism Diagnostic Observation Schedule-2 (ADOS-2) and the Autism Diagnostic Interview-Revised (ADI-R)., tools mainly developed for children that have been rarely evaluated, in terms of diagnostic accuracy in adult individuals (94).

The main aim of the present chapter is to test the accuracy of ADOS-2 and ADI-R for the diagnosis of ASD in adults in the normal range of intelligence (IQ ≥70). Secondly, we aimed at evaluating potential demographic and clinical predictors of the agreement between clinical consensus diagnosis and instrumental diagnosis (i.e. true positives vs false negatives) in ASD, in order to evaluate potential limitations of the standardized tools.

3.2. Methods

3.2.1. Setting

This research has been carried out at the Laboratorio Autismo, a research center belonging to the Brain and Behavioral Sciences Department of the University of Pavia. In more than a decade of activity, the Laboratorio Autismo has researched over several topics regarding ASD in adolescents and adults. Particularly, our group focused on the diagnosis of this condition and the research of potential biomarkers. The attention

toward the medical and psychiatric approach to the condition also allow to study other related aspect of ASD which represent current lines of research, such as medical and psychiatric comorbidities of the autistic condition. Furthermore, especially through the collaboration with Cascina Rossago, the first Italian farm community specifically designed for adults with ASD and ID, the Laboratorio Autismo is interested in the evaluation of educational interventions in an ecological context and the research of poorly understood clinical outcome, such as the quality of life, for people diagnosed with ASD.

Medical doctors, psychiatrists and trainees, composed the research staff led by Professor Pierluigi Politi. Referrals are mainly adults with a potential diagnosis of ASD and are brought to the attention of Laboratorio Autismo by other research groups, professionals, relatives, or by self-referral.

3.2.2. Clinical evaluation and diagnostic classification

After providing written informed consent, each person was extensively evaluated by a senior psychiatrist and at least one licensed medical doctor or psychiatrist with clinical expertise in diagnosing of adults with ASD during a multi-step clinical assessment. The clinical evaluation process included the collection of a complete psychopathological and clinical history also from caregivers (whenever possible), focusing on core symptoms of ASD both present or past. Clinicians focused on the following aspects: social communicative behaviors (both verbal and non-verbal), quantity and quality of social and personal relationships, presence of vocal or movement stereotypes or rituals, insistence on sameness, restrictive and pervasive interests, abnormal sensoriality. We performed a complete psychiatric assessment, also including, when needed,

standardized interviews (145, 146), to assess the presence of other psychiatric conditions. The diagnostic procedure also included the evaluation of the intelligence quotient (IQ) through the Wechsler Adult Intelligence Scale-Revised (147), the Raven's Standard Progressive Matrices (148), or the Leiter International Performance Scale-3 (149). Whenever possible, multiple IQ evaluation were performed, given the lack of consensus about the use of specific tools in this group of people. This first part of the assessment brought an interim diagnosis or exclusion of ASD according with DSM-5 criteria (81).

Furthermore, each participant was independently evaluated by two researchers, who were blind to the clinical diagnoses, with the ADOS-2 Module 4 and the ADI-R (if caregivers were available). As each interview or direct observation was performed by one assessor; no interrater reliability was computed.

Finally, a consensus meeting though clinicians involved in the assessments were performed, and the definitive clinical diagnosis according to the DSM-5 criteria was discussed and confirmed or rejected. If present, the diagnosis received also the evaluation of severity levels for criterion A and B. As already mentioned, the severity levels could vary, according with the level of support required by the individual, from 1 (require support) to 3 (require very substantial support) (81).

3.2.3. Participants

The recruitment started in June 2013 and the last assessment included in this report was conducted in August 2017. In total, 140 people referring to the Laboratorio Autismo were recruited based on the following inclusion criteria: (1) age of 18 years or above; (2) estimated IQ of 70 or above; (3) good comprehension of the Italian language. Our sample included 37 self-referrals, 56 individuals referred by relatives, and 47 referred by

specialists. The ADOS-2 was performed for all individuals, while ADI-R was administered to the caregivers of only 102 participants.

3.2.4. Statistical analysis

Mean and standard deviation were used to describe continuous variables, whereas categorical variables were presented as percentages and counts. We also verified the assumption of normal distribution and homogeneity of variance using visual inspection of the distribution plots and through Kolmogorov–Smirnov and Levine's tests.

The accuracy of the different diagnostic tools was evaluated with receiver operating characteristic (ROC) analyses. Results was interpreted according with Hosmer Jr, Lemeshow (150), considering AUC values (0.5: no discrimination; 0.7–0.79: acceptable; 0.8–0.89: excellent; ≥0.9 outstanding).

Diagnostic agreement between the assessment tools and clinical diagnosis were computed using Cohen's k. We used the Landis's cut-offs (151) to interpret Cohen's k value (0: no agreement; 0–0.2: slight; 0.21–0.40: fair; 0.41–0.60: moderate; 0.61–0.80: substantial; 0.81–1: almost perfect agreement).

To accomplish our second aim, we adopted a binary logistic regression analyses to determine independent demographic and clinical predictors (age, gender (female=0; male=1), IQ, and severity at criteria A and B) of the agreement between the clinical consensus diagnosis and the positivity to ADOS-2 and ADI-R. Hierarchical method were adopted.

Results were considered statistically significant at the $p \leq 0.05$ (two tailed tests). SPSS 24 software packages (SPSS, Chicago, IL) was used to perform all the statistical analysis.

3.3. Results

As already mentioned, the overall sample included 140 individuals. 95 subjects received a final diagnosis of ASD, whereas in 45 subject the diagnosis of ASD was excluded. The mean age at evaluation was 28.34 ± 10.80 years (range from 18 to 58) and the participants were mainly males (73%). As assessed with the instruments mentioned before, mean IQ was 111.14 ± 17.89 (range from 75 to 145). The characteristics of the sample have been reported in Table 3.1.

Table 3.1. Characteristics of the sample.

	ASD group	**Non-ASD group**	**Total sample**
	n = 95	**n = 45**	**n = 140**
Age	25 ± 8.46	35.40 ± 11.85	28.34 ± 10.80
Gender, male (%)	71 (74.7)	31 (68.9)	102 (72.9)
IQ	109.30 ± 17.89	115.02 ± 17.45	111.14 ± 17.89
ADOS-2 (original algorithm)	**n = 95**	**n = 45**	**n = 140**
Communication (C)	3.14 ± 1.50	1.78 ± 1.17	2.51 ± 1.67
Reciprocal Social Interaction (SI)	6.62 ± 2.46	3.22 ± 1.99	5.53 ± 2.81
C + SI	9.64 ± 3.80	4.40 ± 2.86	7.96 ± 4.29
Creativity	0.94 ± 0.74	0.67 ± 0.71	0.85 ± 0.74
RRB	1.50 ± 1.23	0.67 ± 1.00	1.24 ± 1.22
ADOS-2 (revised algorithm)	**n = 95**	**n = 45**	**n = 140**
Social affect	9.56 ± 3.78	4.53 ± 3.24	7.94 ± 4.30
RRB	1.98 ± 1.38	0.80 ± 0.92	1.60 ± 1.36
ADI-R	**n = 81**	**n = 21**	**n = 102**
Qualitative abnormalities in C	9.80 ± 3.71	5.90 ± 3.87	9.00 ± 4.04
Qualitative abnormalities in SI	12.73 ± 4.52	6.19 ± 3.29	11.38 ± 5.04
RRB	4.81 ± 2.31	3.09 ± 1.97	4.46 ± 2.34
Abnormalities ≤36 months	1.44 ± 1.38	0.09 ± 0.30	1.17 ± 1.35

Legend. *IQ*: Intelligence Quotient; *RRB*: Restricted and repetitive behaviors.

In the sub-sample receiving the diagnosis of ASD, severity levels were distributed as follows: criterion A, 48 individuals had level 1 (50.5%); 44 had level 2 (46.3%) and 3 level 3 (3.2%); The severity of the criterion B, was low for 56 individuals (58.9%, level 1), 37 had level 2 (38.9%) and only two subjects had level 3 (2.1%) (Figure 3.1). Subjects that did not received a diagnosis of ASD received a different psychiatric diagnosis in 78% of cases. Ten subjects did not receive any diagnosis. Other diagnoses are depicted in Figure 3.2.

Figure 3.1. Severity levels distributions in subjects receiving a diagnosis of ASD

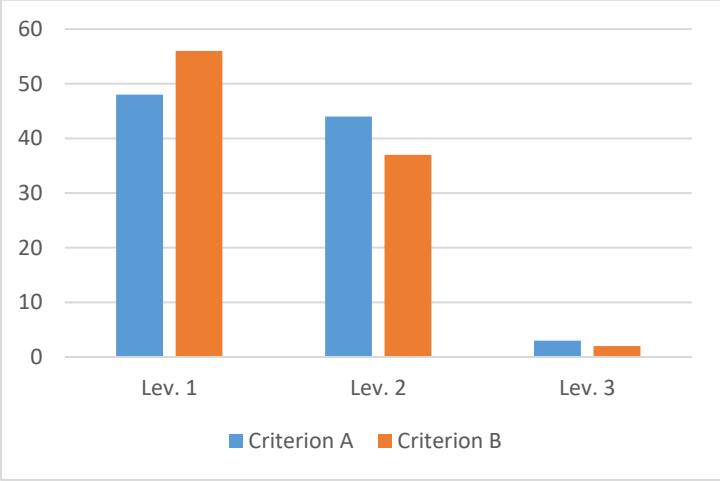
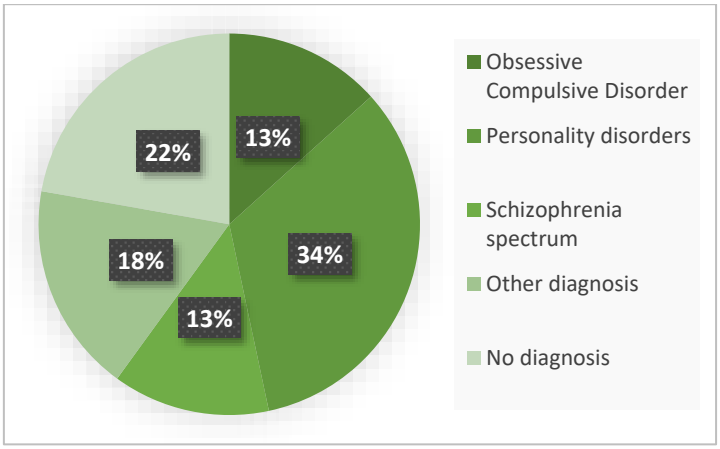


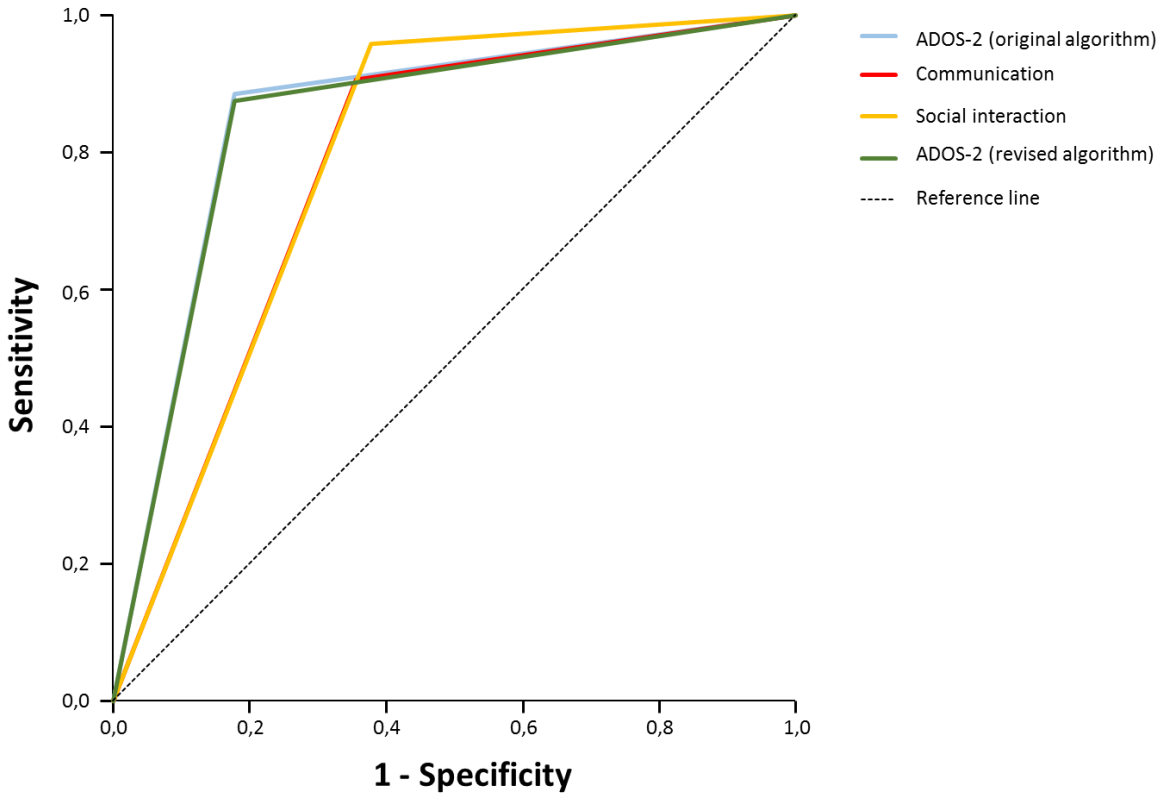
Figure 3.2. Alternative diagnosis distribution



3.3.1. Accuracy of diagnostic instruments and diagnostic agreement

ADOS-2 Module 4 was scored with both algorithms (as described in paragraph 1.7.3.), and both scoring have been tested. The discriminant validity for the combined Communication + Social Interaction domains (AUC=0.85, SE=0.04, p<0.001, 95% CI 0.78–0.93) of the original algorithm was excellent, as tested with the ROC curve. The accuracy was acceptable when the two domains were considered separately, with an AUC=0.79 (SE=0.05, p<0.001, 95% CI 0.70–0.88) for the Social Interaction domain and an AUC=0.77 (SE=0.05, p<0.001, 95% CI 0.68–0.87) for the Communication domain. The revised algorithm of ADOS-2, that has been developed after the publication of DSM-5 and included the evaluation of diagnostic criterion B, showed an excellent discriminant validity (AUC=0.85, SE=0.04, p<0.001, 95% CI 0.77–0.92) (see Figure 3.3).

Figure 3.3. ROC curves of ADOS-2



Clinical consensus diagnosis showed a substantial agreement both with traditional (k = 0.69, p<0.001) and revised algorithm (k = 0.68, p<0.001) of ADOS-2.

Table 3.2. reports sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of the ADOS-2 algorithms compared to the consensus clinical judgment.

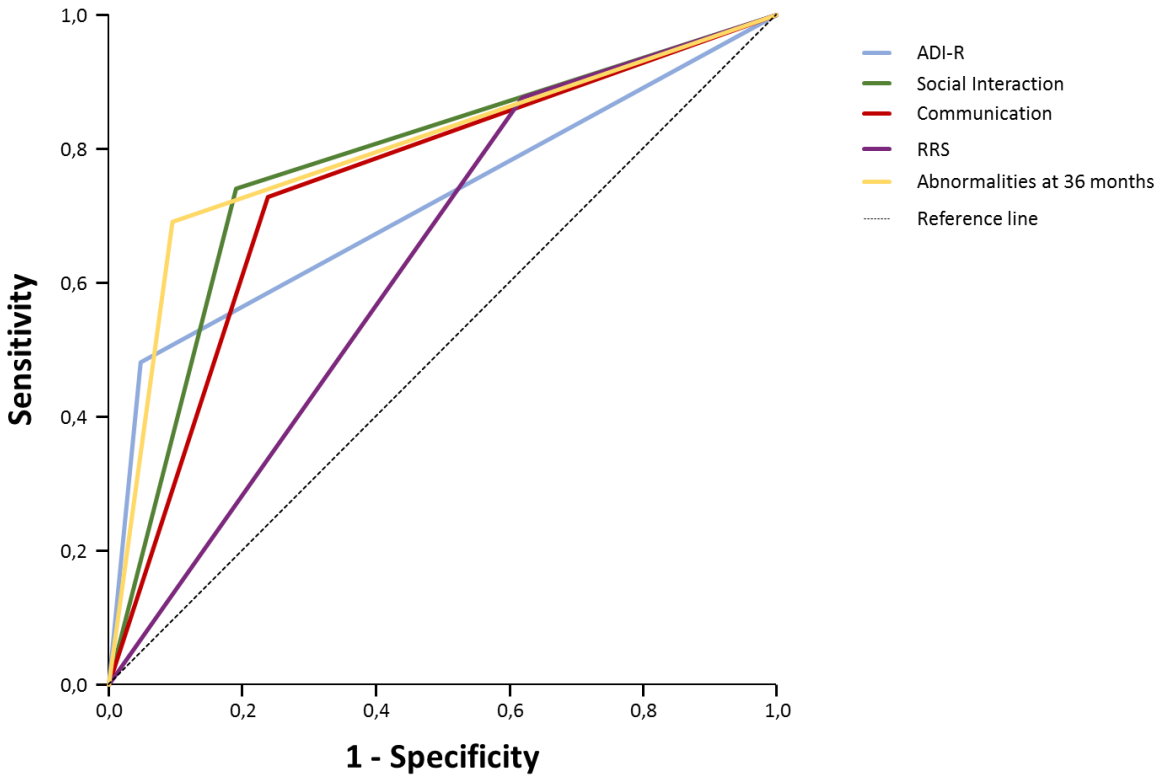
Table 3.2. ADOS-2 agreement with ASD diagnosis

	Sensitivity	**Specificity**	**Correct classification**	**PPV**	**NPV**	**Cohen's k**
ADOS-2 (original algorithm) (111)	88.4	82.2	86.43	91.3	77.1	0.694*
ADOS-2 (revised algorithm) (125)	87.4	82.2	85.71	91.2	75.5	0.68*

Legend: *NPV*: Negative Predictive Value; *PPV*: Positive Predictive Value

The diagnostic accuracy of the ADI-R was acceptable (AUC = 0.72, SE = 0.05, p < 0.001, 95% CI 0.61–0.82). The ADI-R sensitivity was 48.1 % and the specificity was 95.2%. Furthermore, we evaluated the accuracy of single ADI-R domains. This analysis was exploratory, giving that all domains must be over the cut-off for the scoring algorithm to be suggestive of ASD. Considering ADI-R single domains (Figure 3.3), an excellent discriminant validity was showed for the domain regarding the behavioral abnormalities evident at or before 36 months (AUC=0.80, SE=0.05, p<0.001, 95% CI 0.70–0.90). Acceptable accuracy was attributed for the domain of qualitative abnormalities of reciprocal social interaction (AUC=0.77, SE=0.06, p<0.001, 95% CI 0.66–0.89), and qualitative abnormalities in communication (AUC=0.75, SE=0.06, p<0.001, 95% CI 0.63–0.87). At the opposite, the repetitive and stereotyped patterns of behavior domain showed poor accuracy (AUC=0.63, SE=0.07, p=0.07, 95% CI 0.48–0.77).

Figure 3.3. ROC curves of the ADI-R and subscales.



The agreement between clinical consensus judgment and ADI-R was fair (k=0.25, p<0.001). A fair agreement was also found also with the subscales regarding communication (k=0.38, p<0.001) and repetitive behaviors (k=0.27, p<0.001). Both the domains of abnormalities in the early childhood and reciprocal social interaction moderately agreed with clinical judgment (k=0.42 p<0.001).

3.3.2. Predictors of diagnostic agreement

Among 95 individuals diagnosed with ASD, 84 exceeded the diagnostic treshold of the ADOS-2 (TP), while other 11 did not reach the cut-off scores (FN). The final regression model correctly classified the 88.1% of cases (χ^2=12.43, p=0.030; Cox and Snell pseudo R^2=0.123; n=95). Gender was a significant independent predictor so that males were

more likely to be correctly "diagnosed" with ADOS-2 than females (Table 3.3.).The revised algorithm of ADOS-2 correctly classified into the spectrum 83 people (87.4% of the sample) but the regression model was not significant (χ^2=8.02, p=0.15; Cox and Snell pseudo R^2=0.08; n=95).

Table 3.3. Predictors of diagnostic agreement of ADOS-2

	B	SE	OR (95% CI)	p-value
Gender	1.592	0.732	4.912 (1.170 to 20.629)	**0.030***
Age	-0.049	0.037	0.952 (0.885 to0 .024)	0.187
IQ	-0.005	0.027	0.995 (0.943 to 1.050)	0.851
Severity A	0.607	0.824	1.835 (0.365 to 9.230)	0.461
Severity B	0.099	0.851	1.104 (0.208 to 5.849)	0.907

Model χ^2 = 12.43, p = 0.03; Cox and Snell pseudo R^2 = 0.123; n = 95

Among the 95 subjects receiving a diagnosis of ASD, 81 ADI-R evaluations were available. Of these, 39 patients exceeded the ADI-R cut-off while 42 were not correctly identified as within the Autism Spectrum. The final logistic regression model fitted 68.3% of the sample (χ^2=15.23, p=0.009; Cox and Snell pseudo R^2=0.17; n=81). IQ and criterion B severity were significant predictors of diagnostic agreement according to the ADI-R (Table 3.4.). People diagnosed as ASD with higher IQ were less likely to be recognized as autistic using the ADI-R, while those showing higher severity in the RRB domain were more likely to be identified as having ASD at the ADI-R.

Table 3.4. Predictors of diagnostic agreement of ADI-R

	B	SE	OR (95% CI)	p-value
Gender	0.028	0.649	0.973 (0.273 to 3.467)	0.966
Age	-0.015	0.040	0.986 (0.911 to 1.066)	0.715
IQ	-0.033	0.016	0.968 (0.939 to 0.998)	**0.037***
Severity A	-323	0.516	0.724 (0.263 to 1.991)	0.531
Severity B	1.202	0.539	3.326 (1.156 to 9.565)	**0.026***

Model $\chi^2 = 15.23$, $p = 0.009$; Cox and Snell pseudo $R^2 = 0.17$; $n = 81$

3.4. Discussion

According to guidelines, ASD diagnosis in adulthood should include standardized tools. However, few studies investigated the reliability of these instrument in adults suffering from ASD. Here I reported the data of the evaluation of adult subjects with average or above average intelligence referring to the Laboratorio Autismo for a formal diagnosis of ASD (4). The first aim of this study was to assess the accuracy of the clinical observation ADOS-2 and the diagnostic interview ADI-R for the diagnosis of ASD in adults, and their agreement with clinical judgment. Secondly, we performed a secondary analysis aiming at evaluating the potential predictors of the agreement. Our results indicated substantial agreement between the clinical diagnosis and the ADOS-2 scores (both according with original and the revised diagnostic algorithm). Furthermore, the ADOS-2 showed good sensitivity and specificity. Using ROC curves, AUC values for the Communication + Social Interaction domain (original algorithm (111)) and for the

SA + RRB domain (revised algorithm (125)) indicated excellent accuracy. These results are consistent with previous studies which evaluating the discriminant validity of ADOS-2 Module 4 in adults without ID (94, 125, 127, 128, 131, 152), cautiously suggesting that ADOS-2 could be a reliable instrument also for first evaluations in adults (4).

The predictors analysis underlined that, in the ASD sample, the diagnostic agreement between clinical criteria and ADOS-2 was more likely in males as compared to females. These findings are in line with literature data, which reported a minor accuracy of standardized observational instruments in detecting ASD in females. In fact, the construct of diagnostic and screening tests to date mainly rely on the typical male phenotype of ASD, thus excluding some of the specific features of girls with autism (118, 119). Citing DSM-5, "girls without accompanying intellectual disability or language delays may go unrecognized, perhaps because of subtler manifestation of social and communication difficulties" (81). It has been reported that females with ASD tend to suffer more from less evident "internalizing" (e.g. anxiety, depression) rather than "externalizing" (e.g. hyperactivity, conduct problems) difficulties (122). Higher camouflaging behavior has been observed in women with ASD (153). In their study, the Authors showed that given comparable scores at the ADI-R, females subjects suffering from ASD tended to show lower scores on the ADOS. It has also been suggested that females with autism used gestures more vividly than males with autism during the ADOS-2 (119). However, our regression analysis was not significant when considering the revised diagnostic algorithm. This could be associated with the inclusion of DSM-5's criterion B as part of the ADOS-2 scoring, attenuating the relevance of criterion A in which female subjects appear to show greater differences (119).

As expected, the agreement between ADI-R and clinical diagnosis was poor, correctly classifying only 58% of our sample. However, we need to acknowledge that our sample was composed by adults (mean age = 28.34) and, unfortunately, only for 73% of the subjects the ADI-R could be completed. Furthermore, most of the items of the ADI-R focuses on the childhood period between 4 and 5 years of age (113). The time laps between the interview and the children behavior was thus very large (in mean, more than two decades) and the quality of informant's memory might not be detailed or reliable for this reason, as already acknowledged by other Authors (13). However, this assumption is merely theoretical as, surprisingly, the agreement between ADI-R and clinical judgment in our sample was not predicted by the age of the subjects. Interestingly, IQ appeared to be negatively correlated with the agreement of ADI-R and ASD diagnosis in our sample. It is plausible that lower cognitive abilities and coping skills could be associated with more severe symptoms in infancy as individuals with higher IQ could have developed camouflaging or compensating strategies since their childhood. These results are consistent with the study from Hus and Lord (154). The Authors showed that greater cognitive impairment was associated with more severe impairment on most behavioral measures. However, this study included also children with ID and specific assessment are needed to understand if more subtle IQ differences could impact the presentation of ASD symptoms in youngster. Unfortunately, we did not perform interaction analyses, due to low statistical power. The possible entanglement between IQ, coming abilities and age at the assessment will deserve further investigation.

Another research supporting the hypothesis that ID could be associated with more pronounced symptoms in infancy and higher parents' recall later in life could be found

in the study of Sappok, Diefenbacher (136). In this study conducted on adults with ASD and a concomitant diagnosis of ID and a long history of developmental delay, the accuracy of ADI-R in adults was higher. Sappok, Diefenbacher (136), in fact, found less specificity (80%), but extremely higher sensitivity (88%) compared to our sample. In this case, parents or caregivers were probably able to easily recall information about the troublesome developmental history of the patients, also because that may have undergone through several previous evaluations (4). This hypothesis is consistent with the study of Talari, Balaji (155). In line with our results, the Authors found a low specificity (37.5%) of ADI-R in a clinical sample of adults with heterogeneous cognitive profiles. Our findings partially reflect the conclusions of recent studies assessing the discriminant validity of ADI-R in children, which have found high specificity, but moderate to low sensitivity (156, 157).

Criterion B severity was a positive and independent predictor of the diagnostic agreement of ADI-R and clinical diagnosis. Apparently, people with more stereotypes and numerous or pervasive restricted interests were more likely to exceed all the cut-off scores for the ADI-R domains. Again the findings of Talari, Balaji (155) agreed with our observation. The Authors have recently demonstrated the strong predictability of RRB domain for clinical diagnosis of autism. This is particularly true for early presence of these symptoms: previous studies showed an improvement in the RRB domain in adults with ASD (158) but their presence in childhood appeared a relevant anamnestic indicator of ASD. This observation is also confirmed by evidences reporting that scores on RRB domain of the ADI-R are more indicative of ASD, particularly in males (159, 160).

3.4.1. Strengths and limitations

One of the main strength of this study is that the included sample is well described by the concept of the “lost generation” of autistic adults (13). In fact, individuals included in our sample represent the higher end of the spectrum, individuals with higher cognitive abilities and milder symptoms who became aware of the possible explanation of their experiences and behaviors after the broadening of ASD diagnostic criteria and the increased mediatic awareness towards this condition. As already suggested by other Authors, an accurate diagnosis could be challenging in this population (13) thus research in this field appear to be of crucial relevance. Another point of strength is that ADOS-2 and ADI-R were administered, in our study, by personnel blind to the clinical diagnoses. In addition, including self-referrals and people looking for the first time a possible diagnosis of ASD, we have avoided the risk of generating low specificity values. The opposite has been suggested by previous studies that evaluated the ADOS in clinical settings (94, 129, 133, 136).

It is also important to acknowledge some limitations. We are aware that the sample is relatively small. Especially considering the variety of clinical presentations along the spectrum, multicenter studies and data sharing appeared to be crucial to include larger populations; however, our sample is rapidly growing and the expected prevalence of 1 individual with ASD over 58 people is far to be reached. Secondly, the assessment process was not conducted by a multidisciplinary team (91), as assessors were psychiatrists and medical doctors with expertise in the evaluation of ASD in adulthood but no developmental psychologist or child psychiatrist and developmental therapist could have been involved in the evaluation. Thirdly, our main strength is also a significant limitation as limiting the analysis to people belonging to the higher-

functioning part of the spectrum, reduced the generalizability of our findings, as the spectrum also include subjects with ID. This decision was based on the need to maintain, as much as possible, a limited heterogeneity to avoid loss of statistical power. Finally, we decided to restrict our regression analysis to subjects receiving a diagnosis of ASD, thus limiting the disagreement with standardized tools to “false negatives” (people who did not met the score threshold to be included in the autism spectrum according to diagnostic instrument but met DSM-5 criteria for ASD). This methodological choice reflected the observation that that very few individuals positively scored using the diagnostic instruments despite not receiving an ASD clinical diagnosis (0.48% for the ADI-R and 17.78% for the ADOS).

4. Increased CNTF levels in adults with autism spectrum disorders with ID: a new potential biomarker.

4.1. Aims

If diagnostic standardized instruments have been proved to effectively support the diagnosis of ASD in subjects without ID, some diagnostic difficulties remain when considering the population of ASD subjects with ID, especially in adulthood. In fact, while the ADOS-2 require good language abilities in adults, frequently lacking in ASD associated with ID, ADI-R has been tested in this population with conflicting results (136, 155). This is consistent with the results of our systematic research on CCTs in ASD and ID revealing that only 20% of the retrieved studies adopted ADOS or ADI-R for the diagnostic assessment. As already discussed, the recent estimate of ID prevalence in ASD was 31%, while 23% presented with borderline range of intelligence (7). The paucity of diagnostic tools specifically designed for this population and the scarce confidence of their use in CCT highlighted the need for reliable and practical biomarkers to be included in the clinical routine. As discussed in chapter 1, several general pathways have been linked to the pathogenesis of ASD, such as impaired neurogenesis, neuronal differentiation, and synaptic plasticity (29). In general, all these processes are known to be regulated by NTF and possible alteration of these molecules have been reported in ASD. However, conflicting evidences emerged. For instance, the most studied peptide in this family, the BDNF, is not yet recognized as reliable biomarker (31, 32), supporting the need for further research. The ciliary neurotrophic factor (CNTF) is a 22kDa NTF which belongs to the interleukin-6 family. As many neurokines, it is mainly expressed in the brain, principally on astrocytes and other glial cells. However, other studies found the CNTF also in the liver, the muscle and the bone tissue (161). Opposite to others NTF, CNTF is not secreted by the Golgi apparatus, but it is probably released after cellular

stress or injury (162). CNTF is supposed to exerts pleiotropic effects. It appears to promote the survival of autonomic, motor, sensory and hippocampal neurons. For this reason, CNTF and analogous molecules have been tested in CCT in motor neuron disorders and macular degeneration with conflicting results (163, 164). However, CNTF could also cause proinflammatory central and peripheral immune responses (161). Its potential role as biomarker of diagnosis and disease progression in neurology has been evaluated in amyotrophic lateral sclerosis (165), and epilepsy (166). To present, no psychiatric condition has been associated to altered CNTF levels, as no studied has been published on this topic. This chapter is based on a study conducted at the Laboratorio Autismo during my doctoral course, which has recently been published on a peer reviewed journal (5).The aim of the present study is to evaluate the potential usefulness of CNTF serum levels in the diagnosis and characterization of ASD associated with ID.

4.2. Methods

4.2.1. Setting and partecipants

This research has been carried out at the Laboratorio Autismo (see paragraph 3.2.1.), in collaboration with Cascina Rossago, the first Italian farm community specifically designed for adults with ASD and ID (70), Interactive S.C.S, an Italian social cooperative society collaborating with the national health system providing residential facility services for people suffering from ID or psychiatric disorders, and with Fondazione Istituto Ospedaliero Sopiro – ONLUS, a service dedicated to people with mental and intellectual disability and ASD. Our plan was to recruit three group of participants and to compare serum levels of CNTF between them:

- People with ASD and ID (ASD+ID group);

- People with ID without ASD (ID group);
- People without ASD or ID (typically developing group, T group);

All recruited subjects were Caucasian adults Italians, unrelated, nonsmokers, and free of both chronic and acute physical illnesses. Exclusion criteria were common among all study groups: 1) presence of a known genetic syndrome (i.e. Fragile X, Rett syndrome), 2) presence of comorbid medical conditions known to alter CNTF levels (i.e. rheumatoid arthritis, infections, pregnancy, neurodegenerative disorders, gastrointestinal problems, chronic use of anti-inflammatory medications).

Inclusion criteria varied according to the study group. To enter the ADS+ID group the participant received a multistep evaluation as already described in paragraph 3.2.2. Briefly, the diagnosis was made according to DSM 5 criteria for autism spectrum disorders and the severity of the condition was rated according to DSM 5 standards. All participants in the ASD+ID group met level 3 of severity. Additionally, before entering the study, a psychiatrist administered the Autism Diagnostic Interview-Revised (ADI-R) (113) to parents or caregivers of each subject to improve diagnostic accuracy. The presence of ID (IQ<70) was evaluated with the Leiter International Performance Scale – Revised (Leiter-R) (167). This scale was preferred to other neuropsychological tools given the non-verbal setting. Additional characterization of the subjects was carried out by Childhood Autism Rating Scale (CARS) (112) which is a 15-item clinician-rated scale evaluating the presence and severity of autistic symptoms. Higher scores indicated higher impairment.

To enter the ID group, the presence of ASD was ruled out administering the ADI-R to each subject caregiver. Furthermore, only subjects with CARS score < 30 were included in the study and the presence of ID was again confirmed with the Leiter-R.

The T group was recruited among healthy volunteers matched for age and gender. The presence of any DSM-5 (6) psychiatric diagnosis and use of any type of medications on a regular basis represented an exclusion criterion for this group. A senior psychiatrist screened all subjects to exclude the presence of psychiatric disorders. IQ of all participants was evaluated through the Leiter-R to exclude ID and the absence of autistic-like traits was evaluated by means of the Autism Spectrum Quotient (AQ) (101). All controls scored <20 at the AQ. Each participant, caregiver or parent was provided with information about the study and provided written inform consent for participants before enrollment. The study was conducted according to the Declaration of Helsinki.

4.2.2. CNTF evaluation

To ensure consistence and minimize circadian variation, blood samples were drawn from the antecubital vein between 8:00 and 10:00 am after an overnight fast. Blood samples were collected in serum plastic tubes and centrifuged at 3000 rpm per ten minutes. Sera were aliquoted and stored at -80°C until the assay. All samples were assessed in duplicate. CNTF was measured using an ELISA (Quantikine, Human CNTF; R & D Systems Europe), according to manufacturer's instruction. The intra- and inter-assay coefficients of variation were 2.5% and 7.8%, respectively. The sensitivity of the assay is typically less than 8 pg/mL. When CNTF levels were below the lower reference threshold, we estimated the CNTF concentration calculating the standard curve according to Keizer, Jansen (168).

4.2.3. Statistical analysis

Descriptive statistics were reported for all variables. Assumption of normal distribution and homogeneity of variance were tested using Kolmogorov-Smirnov and Levene's tests before statistical procedures were applied. As the distribution of all variables was not normal, we used the nonparametric Kruskal-Wallis H test for the difference in CNTF serum levels in the three groups. To allow for multiple comparison, Bonferroni's correction was applied. Given the lack of robustness toward outliers of this test, we looked for outliers to minimize risk of bias. Two outliers were identified, and we decided to perform a sensitivity analysis by dropping them and checking if results or assumptions (i.e. normality) changed. As no significant change occurred, we decided to maintain the outliers in the analysis. A power analysis was conducted before the recruitment using the open source G*Power 3.1 software. Sixteen participants per group had an 80% power and an alpha error of 0.1 to detect a difference in CNTF levels between the three groups with an effect size of 0.4. All tests were two tailed and we adopted a statistical threshold of $p \leq 0.05$ for significance. Statistical analysis was performed using SPSS 24.0 software packages (SPSS, Chicago, IL).

4.3. Results

Sixty-nine subjects were enrolled in this study, including 54 males and 15 females. Demographic variables and clinical measures of the three groups have been reported in table 4.1. The ASD+ID group was composed by twenty-three adults with ASD and intellectual disability (19 males and 4 females; Mage= 30.69 years, SD = 7.07; range: 20–44 years). The ID group included twenty age- and gender-matched ID subjects (15 males and 5 females; Mage= 30.55 years, SD = 6.56; range: 20–44 years). All patients included

in these two groups were stable on neuroleptic and psychoactive therapy. The T group included twenty-six age- and gender-matched typical adults (20 males and 6 females; Mage= 28.88 years, SD = 5.59; range: 19–42 years). To check for age (p=0.369) and gender matching non-parametric Kruskal-Wallis test was performed for continuous variables and exact Fisher's test was applied for categorical variables: no significant differences were found. Furthermore, body weight did not differ among the three groups (p=0.473). IQ scores were significantly higher in typical adults compared to the ASD+ID and the ID groups, as expected. No significant differences were observed in IQ scores between ASD+ID and ID subjects.

Table 4.1. General characteristics of the study groups

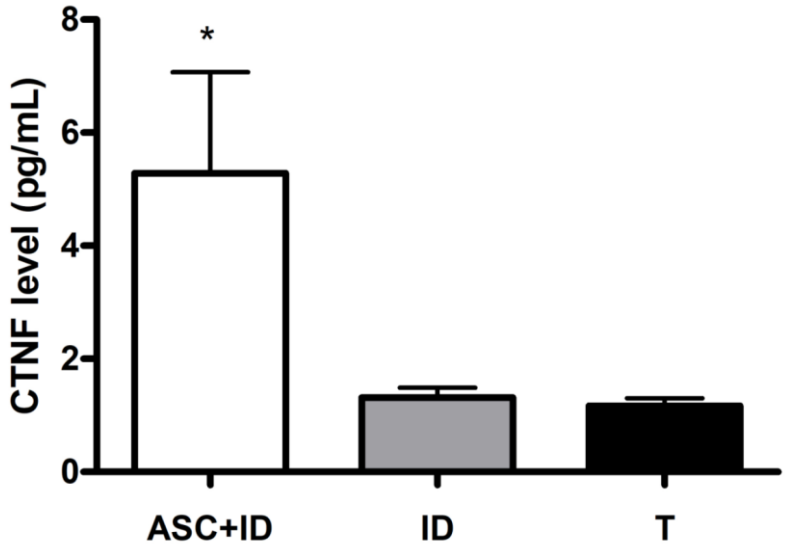
	ASD+ID (n=23)	**ID (n=20)**	**T group (n= 26)**	**p-value**
Age (year)	30.69 ± 7.07	30.55 ± 6.56	28.88 ± 5.59	0.369
Gender (M, %)	19 (79.2%)	15 (83.3%)	20 (76.9%)	0.808
IQ	40.00 ± 5.50	44.12 ± 10.16	100.78 ± 5.94	**P<0.001***
Body weight (kg)	72.67 ± 12.88	73.48 ± 14.26	68.52 ± 13.97	0.473
AQ			15.15 ± 6.64	
CARS	41.37 ± 9.53		20.13 ± 4.71	

***T group significantly different from both ASC+ID and ID group; no difference between the ASC+ID and the ID group (p=0.250)

Legend. *ABC*: Aberrant Behavioral Checklist; *AQ*: Autism Spectrum Quotient; *CARS*: Childhood Autism Rating Scale; *IQ*: Intelligence Quotient; *VABS*: Vineland Adaptive Behavioral Scales.

Serum CNTF levels were significantly different between the three groups (H(2)=27.67, p<.001, effect size ε^2=0.42). Specifically, we found elevated serum CNTF levels in the ASD+ID group (mean rank 52.96, median=2.81 pg/ml, M=5.22 pg/ml, SD=8.58 pg/ml) compared to the ID group (mean rank 25.98, median=1.08 pg/ml, M=1.22 pg/ml, SD=0.69 pg/ml, Bonferroni's correction, p < .001) or the T group (mean rank 26.06, median=1.19 pg/ml, M = 1.20 pg/ml, SD = 0.85 pg/ml, Bonferroni's correction, p < .001) (figure 1). No significant differences in CNTF levels were observed between the ID and the T groups (p = 1.00) (Figure 4.1.).

Figure 4.1. Serum CNTF levels in ASD+ID, ID and T group.



4.4. Discussion

Our study is the first to report a significant difference in serum CNTF levels between subjects with severe ASD and ID as compared with subjects diagnosed with ID without ASD or typically developing individuals. The increase of CNTF seemed specific of severe

ASD and comorbid intellectual disability. CNTF is a neurotrophic factor that exerts numerous neuroprotective effects, especially against glutamate excitotoxicity (169). Excitotoxicity is a Calcium and NO• dependent pathological event associated with neuronal excitation through overstimulation of neurons by excitatory amino acids. This mechanism has been suggested as a potential pathogenic mechanism in ASD (170). In this regard, elevated CNTF levels could represent a counteracting mechanism for excessive glutamate spillover by increasing astrocytes uptake (169). On the other hand, CNTF acts as a weak proinflammatory neurokine interacting with the IL-6 receptor, and therefore it may be partly held responsible for the increase oxidative stress observed in ASD brain (36). The supposed increased in pro-inflammatory cytokines in ASD has been recently supported by a meta-analysis from Masi, Quintana (171). The Authors reported an increase in blood concentration of several inflammatory cytokines, including IL-1β, IL-6, IL-8, interferon-gamma, eotaxin and monocyte chemotactic protein-1. This report is in line with our result, reporting increased CNTF levels which were not tested in any study included in the meta-analysis. However, our findings are in contrast with a small study, investigating CNTF levels in the sera of three children with ASD (172). This incongruence could be due to several factors: 1) the study adopted Western Blot analysis which appears a less sensitive technique to detect small amounts of CNTF, as expected in in normal samples; 2) potential explanation of this difference may rely on the very small sample size (three subject per group) (172) and be associated with random effect; 3) the neurodevelopmental stage of the two samples is different as we focused on adults whose brain has already reached its maturity, while Kazim, Cardenas-Aguayo (172) included only children. Neurotrophins levels seem to vary along neurodevelopment. It has been reported, for instance, that BNDF showed a progressive

increase in serum levels during childhood and adolescent with a peak in the thirties and subsequently decreasing in older adults (173).

In our study we acknowledged some limitations. Firstly, the sample size is still small and our results should be replicated in larger sample to allow parametric testing and improve generalizability. However, we performed power analysis to and reached the expected sample size. Additionally, we focused only on severe ASD with comorbid severe and profound ID. Even if this decision was agreed to increase the specificity of the results, it has limited the result generalizability. For this reason, we are planning in replicating our results in adults with ASD but without ID. This however was not the main aim of this study, as we investigated a potential biomarker in a group were the paucity of specifically designed standardized test limit the multistep assessment approach. Additionally, even if CNTF was higher in ASD than in both control groups, the concentrations for ASD laid still in the normal range according to the other two studies investigating CNTF with ELISA (165, 174). Of note, our data are less dispersed than the aforementioned studies which could have suffered the larger presence of outliers. Furthermore, there is no widely accepted normal range for CNTF levels, which, accordingly to current available literature, are consistently elevated only in neurodegenerative disorders (165, 174). In conclusion, our data are in line with previous evidence suggesting a neurotrophic imbalance in ASD, and CNTF may potentially represent a candidate biomarker for detecting severe ASD in people with ID.

5. Conclusions

In this thesis I have detailed several aspects of the complex and time-consuming process of diagnosis ASD. As frequently stated, the complexity reaches its maximum in adulthood, and both subjects with and without intellectual disability present critical and specific challenges for a correct diagnostic assessment. This is true for the absence of reliable biomarkers and for the presence of similar symptomatology in different psychiatric conditions along with the limitations in collecting reliable information about the early development of the patients (13).

Focusing on the diagnostic assessment of ASD in adults in the mildest form of the spectrum, it appears desirable to systematically assess the presence and the developmental evolution of core symptoms of ASD. The results of our systematic research revealed the trend towards a more frequent use of these tools, especially ADOS and ADI in clinical controlled trials. With our original research, we confirmed the diagnostic reliability of ADOS-2 Module 4 in adults without ID. However, it should be cautiously asserted that the ADI-R algorithm lacks of accuracy in the diagnosis of adults without intellectual disability seeking first formal diagnosis of ASD (4). Furthermore, female gender and higher IQ were associated with worst accuracy of the standardized tools. On the contrary, higher severity of repetitive behaviors and restricted interest was associated with better accuracy using the standardized tools investigated. Finally, our research group suggested the introduction of a potential serum biomarker to support the diagnosis of ASD in adults with comorbid intellectual disability. CNTF were tested for the first time, to our knowledge, in this population ad appeared to be significantly

correlated to the diagnosis of ASD in subject with ID as compared with ID subjects without ASD and typically developing individuals.

In conclusion, accurate diagnosis of ASD in adulthood is fundamental to guarantee adequate interventions. The accuracy of the diagnosis needs to be supported both with standardized assessment tools and hopefully with reliable biomarkers. Additionally, considering the expanding number of CCT in ASD, as reported in the systematic review included in the present dissertation, there is the necessity to include in clinical trials only people whose diagnosis has been confirmed by professionals with specific expertise and adequate assessment procedure. This issue is important to limit selection bias that would compromise the results and limit the progress in the critical field of treatment for ASD.

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Appendix A

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Adams, 2004	Nut	R-P	20	Unspec.	United St.
Adams, 2011	Nut	R-P	104	Unspec.	United St.
Adkins, 2012	Edu	R-P	36	DSM-IV-TR, ADOS	United St.
Akhondzadeh, 2004	Ph	R-P	40	DSM-IV	Iran
Akhondzadeh, 2008	Ph	R-P	40	DSM-IV	Iran
Akhondzadeh, 2010	Ph	R-P	40	DSM-IV-TR	Iran
Al-Ayadhi, 2013	Nut	R-H2H	60	DSM-IV-TR	Saudi Arabia
Al-Ayadhi, 2015	Nut	R-H2H	65	DSM-IV-TR	Saudi Arabia
Aldred, 2004	Edu	R-P	28	ADI, ADOS	UK
Allam, 2008	Misc	R-P	20	DSM-IV-TR, CARS	Egypt
Almirall, 2016	Edu	R-SMART	61	ADOS	United St.
Aman, 2010	Ph	R-P	308	DSM-IV-TR, ADI	United St.
Amatachaya, 2014	Misc	R-C	20	DSM-IV-TR	Thailand
Amatachaya, 2015	Misc	R-C	20	DSM-IV-TR	Thailand
Amminger, 2007	Nut	R-P	12	DSM-IV, ADI, ADOS	Austria
Anagnostou, 2012	Ph	R-P	19	DSM-IV, ADI, ADOS	United St.
Anderson, 1984	Ph	R-C	40	DSM-III	United St.
Anderson, 1989	Ph	R-C	45	DSM-III	United St.
Andrews, 2013	Psy	R-P	58	ASDI	Australia
Anninos, 2016	Misc	R-C	10	Unspec.	Greece
Arnold, 2006	Ph	R-C	16	DSM-IV, ADI	United St.
Arnold, 2012	Ph	R-P	20	DSM-IV, ADI	United St.
Asadabadi, 2013	Ph	R-P	40	DSM-IV-TR, ADI	Iran
August, 1987	Ph	R-C	10	DSM-III	United St.
Baghdadli, 2013	Edu	R-P	14	ICD-10, ADI, ADOS	France
Barthelmey, 1981	Nut	R-C	21	DSM-III	France
Barthelmey, 1981	Nut	R-C	35	DSM-III	France
Barthelmey, 1981	Nut	R-C	37	DSM-III	France
Bass, 2009	Misc	R-P	34	DSM-IV-TR	United St.
Beaumont, 2008	Edu	R-P	49	DSM-IV-TR, CAST	Australia
Beaumont, 2015	Edu	Non-R	69	Unspec.	Australia
Beeghly, 1987	Ph	R-C	7	DSM-III	United St.
Begeer, 2011	Edu	R-P	36	DSM-IV-TR	Netherlands
Begeer, 2015	Edu	R-P	97	DSM-IV-TR	Netherlands
Beisler, 1986	Ph	R-C	6	DSM-III	United St.
Belsito, 2001	Ph	R-P	28	ADI	United St.
Bent, 2011	Nut	R-P	25	DSM-IV-TR, ADI, SCQ	United St.
Bent, 2014	Nut	R-P	57	SCQ	United St.

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Bernard-Opitz, 2004	Edu	Non-R	8	ADI	Singapore
Bertoglio, 2010	Nut	R-C	30	DSM-IV-TR, ADOS, ADI	United St.
Bettison, 1996	Misc	R-P	80	Unspec.	Australia
Birnbrauer, 1993	Edu	Non-R	14	DSM-III-R	Australia
Bolman, 1999	Nut	Non-R	8	DSM-III-R	United St.
Borgi, 2016	Misc	R-P	26	ICD-10, DSM-IV-TR	Italy
Bouvard, 1995	Ph	R-C	10	DSM-III-R, ADI	France
Boyd, 2014	Edu	Non-R	198	ADOS, SCQ	United St.
Buchsbaum, 2001	Ph	R-C	6	DSM-IV, ADI	United St.
Buday, 1995	Misc	R-C	10	Unspec.	United St.
Buitelaar, 1990	Ph	R-C	14	DSM-III	Netherlands
Buitelaar, 1992	Ph	R-C	14	DSM-III-R	Netherlands
Buitelaar, 1996	Ph	R-P	47	DSM-III-R	Netherlands
Campbell, 1982	Ph	R-C	33	DSM-III	United St.
Campbell, 1988	Ph	R-P	28	DSM-III	United St.
Campbell, 1990	Ph	R-P	18	DSM-III-R	United St.
Campbell, 1993	Ph	R-P	41	DSM-III-R	United St.
Carey, 2002	Ph	R-C	8	DSM-IV	United St.
Carminati, 2016	Ph	R-P	13	CARS, ADI, ICD-10	Switzerland
Casenhiser, 2013	Edu	R-P	51	ADOS, ADI	Canada
Chalfant, 2007	Psy	R-P	47	Unspec.	Australia
Chan, 2009	Misc	R-P	32	Unspec.	Hong kong
Chan, 2012	Nut	R-P	24	DSM-IV-TR, ADI	Hong kong
Chan, 2013	Misc	R-H2H	40	DSM-IV-TR, ADI	Hong kong
Chez, 2000	Ph	R-C	25	DSM-IV	United St.
Chez, 2002	Nut	R-P	31	DSM-IV-TR	United St.
Chez, 2003	Ph	R-P	43	DSM-IV	United St.
Chugani, 2016	Ph	R-P	142	DSM-IV-TR, ADI, ADOS	United St.
Coben, 2007	Misc	Non-R	49	Unspec.	United St.
Coggins, 1988	Ph	R-C	5	Unspec.	United St.
Cohen, 1980	Ph	R-C	10	DSM-III	United St.
Cohen, 2006	Edu	Non-R	37	ADI	United St.
Coniglio, 2001	Ph	R-P	57	DSM-IV	United St.
Corbett, 2001	Ph	R-C	12	DSM-IV, ADI, ADOS	United St.
Corbett, 2008	Misc	R-C	11	DSM-IV, ADOS	United St.
Corbett, 2016	Misc	R-P	30	DSM-5, ADOS	United St.
Cortesi, 2012	Ph	R-P	134	DSM-IV-TR, ADI, ADOS	Italy
Dadds, 2014	Ph	R-P	38	DSM-IV-TR, CARS, DISCAP-ASD, OARS, OAGIS	Australia

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Danfors, 2005	Nut	R-C	12	DSM-IV	Sweden
Dawson, 2010	Edu	R-P	45	DSM-IV, ADI, ADOS	United St.
Dawson, 2012	Edu	R-P	29	DSM-IV, ADI, ADOS	United St.
de Vries, 2015	Misc	R-P	90	DSM-IV-TR, SRS, ADI	Netherlands
D'Elia, 2014	Edu	Non-R	30	DSM-IV-TR, ADI, ADOS	Italy
DeRosier, 2011	Edu	R-H2H	52	ASSQ, CAST, SCQ	United St.
Dollfus, 1992	Ph	R-H2H	18	DSM-III	France
Dolske, 1993	Nut	R-C	18	DSM-III-R	United St.
Domes, 2014	Ph	R-C	14	DSM-IV, ADI, ADOS	Germany
Drahota, 2011	Psy	R-P	40	Unspec.	United St.
Duker, 1991	Ph	Non-R	22	DSM-III-R	Netherlands
Dunn-Geier, 2000	Ph	R-P	95	DSM-IV, CARS	Canada
Edelson, 1999	Misc	R-P	19	RDEC	United St.
Edelson, 1999	Misc	R-P	12	Unspec.	United St.
Eikeseth, 2002	Edu	Non-R	25	ICD-10, ADI	Norway
Eikeseth, 2007	Edu	Non-R	25	ICD-10, ADI	Norway
Ekman, 1989	Ph	R-C	20	DSM-III-R	Sweden
Elder, 2006	Nut	R-C	15	DSM-IV, ADI	United St.
Enticott, 2014	Misc	R-P	30	DSM-IV	Australia
Escalona, 2001	Misc	R-P	20	DSM-III-R	United St.
Fahmy, 2013	Nut	R-P	30	Unspec.	Egypt
Fankhauser, 1992	Ph	R-C	9	DSM-III-R	United St.
Fazlioğlu, 2008	Edu	R-P	30	DSM-IV	Turkey
Feldman, 1999	Ph	R-C	24	DSM-III-R, CARS	United St.
Fernell, 2011	Edu	Non-R	198	DSM-IV	Sweden
Findling, 1997	Nut	R-C	10	DSM-III-R	United St.
Fletcher-Watson, 2016	Edu	R-P	54	ADOS	UK
Flores, 2014	Edu	R-H2H	13	Unspec.	United St.
Frankel, 2010	Edu	R-P	68	ADOS, ADI, ASSQ	United St.
Freitag, 2016	Psy	R-P	209	ICD-10, ADI, ADOS	Germany
Frye, 2016	Nut	R-P	48	ADOS, ADI, DSM-5	United St.
Fujii, 2013	Psy	R-P	12	ADOS, ADI	United St.
Gabriels, 2015	Misc	R-H2H	116	ADOS	United St.
Gantman, 2012	Edu	R-P	17	AQ	United St.
Garstang, 2006	Ph	R-C	7	Unspec.	UK
Gattino, 2011	Misc	R-P	24	DSM-IV-TR, ADI, CARS	Brazil
Geier, 2011	Nut	R-P	27	Unspec.	United St.
Geretsegger, 2016	Misc	R-P	15	ICD-10, ADOS, ADI	Austria
Gev, 2016	Misc	R-P	67	DSM-IV-TR, ADOS	Israel

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Ghaleiha, 2013	Ph	R-P	40	DSM-IV-TR, ADI	Iran
Ghaleiha, 2014	Ph	R-P	40	DSM-IV-TR, ADI	Iran
Ghaleiha, 2015	Ph	R-P	40	DSM-IV-TR, ADI	Iran
Ghaleiha, 2016	Ph	R-P	46	DSM-IV-TR, ADI	Iran
Ghalichi, 2016	Nut	R-P	76	ADI	Iran
Ghanizadeh, 2013	Nut	R-P	31	DSM-IV-TR, ADI	Iran
Ghanizadeh, 2014	Ph	R-H2H	59	DSM-IV-TR, ADI	Iran
Ghanizadeh, 2015	Ph	R-P	34	DSM-IV-TR, ADI	Iran
Ghasemtabar, 2015	Misc	Non-R	27	CARS	Iran
Gillberg, 1986	Nut	R-C	4	DSM-III	Sweden
Golan, 2006	Misc	Non-R	26	DSM-IV	UK
Golan, 2006	Misc	R-P	41	DSM-IV	UK
Golan, 2010	Misc	R-P	38	ADI, CAST	UK
Goods, 2013	Edu	R-H2H	11	ADOS	United St.
Gordon, 1992	Ph	R-H2H	14	DSM-III-R, ADI	United St.
Gordon, 1993	Ph	R-C	12	DSM-III-R, ADI	United St.
Gordon, 1993	Ph	R-C	12	DSM-III-R, ADI	United St.
Gordon, 2011	Edu	R-P	83	ADOS	UK
Gordon, 2015	Edu	R-P	48	3Di	UK
Granpeesheh, 2010	Misc	R-P	29	DSM-IV, ADOS	United St.
Green, 2010	Edu	R-P	152	ADOS, ADI	UK
Gringras, 2014	Misc	R-C	67	ADI, ADOS	UK
Groden, 1987	Ph	Non-R	8	DSM-III, NSAC	United St.
Guastella, 2010	Ph	R-C	15	DSM-IV-TR	Australia
Guastella, 2015	Ph	R-P	50	DSM-IV-TR, ADOS	Australia
Gulsrud, 2010	Edu	R-P	38	DSM-IV, ADI	United St.
Handen, 2000	Ph	R-C	12	CARS	United St.
Handen, 2005	Ph	R-C	8	ADOS, ADI	United St.
Handen, 2009	Ph	R-P	111	DSM-IV-TR, ADI	United St.
Handen, 2011	Ph	R-P	34	ADI, ADOS	United St.
Handen, 2015	Ph	R-P	64	DSM-IV-TR, ADI	United St.
Hardan, 2012	Nut	R-P	29	DSM-IV-TR, ADOS, ADI	United St.
Hardan, 2015	Edu	R-H2H	47	DSM-IV-TR, ADI, ADOS	United St.
Harfterkamp, 2012	Ph	R-P	97	ADI	Netherlands
Hasanzadeh, 2012	Nut	R-P	47	DSM-IV-TR, ADI	Iran
Hayward, 2009	Edu	Non-R	44	ICD-10, ADI	UK
Hellings, 2005	Ph	R-P	30	DSM-IV, ADI, ADOS	United St.
Hendren, 2016	Nut	R-P	50	ADI, ADOS	United St.
Hesselmark, 2014	Psy	R-H2H	68	ADOS	Sweden
Hildebrandt, 2016	Misc	R-P	43	ICD-10	Germany

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Hochhauser, 2016	Edu	R-P	61	SCQ	Israel
Hollander, 2003	Ph	R-C	15	DSM-IV, ADI	United St.
Hollander, 2005	Ph	R-C	39	DSM-IV-TR, ADI, ADOS	United St.
Hollander, 2006	Ph	R-P	13	DSM-IV, ADI, ADOS	United St.
Hollander, 2006	Ph	R-P	11	DSM-IV, ADOS, ADI	United St.
Hollander, 2007	Ph	R-C	15	DSM-IV, ADI	United St.
Hollander, 2010	Ph	R-P	27	DSM-IV-TR, ADI, ADOS	United St.
Hollander, 2012	Ph	R-P	34	DSM-IV, ADOS, ADI	United St.
Honomichl, 2002	Ph	R-C	14	DSM-IV, ADOS, ADI	United St.
Hopkins, 2011	Edu	R-P	49	DSM-IV, CARS	United St.
Howard, 2005	Psy	Non-R	61	DSM-IV	United St.
Howlin, 2007	Misc	R-P	84	ADOS	UK
Hyman, 2016	Nut	R-C	14	DSM-IV-TR, ADI, ADOS	United St.
Ichikawa, 2013	Edu	R-P	11	ICD-10	Japan
Ichikawa, 2016	Ph	R-P	92	DSM-IV-TR	Japan
Ingersoll, 2010	Edu	R-P	21	DSM-IV-TR, ADOS	United St.
Ingersoll, 2012	Edu	R-P	27	DSM-IV-TR, ADOS	United St.
Ingersoll, 2016	Edu	R-P	19	DSM-IV-TR, ADOS	United St.
Isong, 2014	Edu	R-P	69	Unspec.	United St.
Iwanaga, 2014	Edu	Non-R	20	DSM-IV	Japan
Jarusiewicz, 2002	Misc	R-P	24	Unspec.	United St.
Jaselskis, 1992	Ph	R-C	8	DSM-III-R	United St.
Jocelyn, 1998	Edu	R-P	35	DSM-III-R	Canada
Johnson, 2010	Nut	R-P	23	DSM-IV-TR, ADOS	United St.
Kaale, 2012	Edu	R-P	61	ICD-10, ADI, ADOS	Norway
Kalyva, 2005	Edu	Non-R	5	Unspec.	UK
Kamps, 2015	Edu	R-P	94	Unspec.	United St.
Kaplan, 1998	Misc	R-C	18	Unspec.	United St.
Kasari, 2006	Edu	R-P	58	ADOS, ADI	United St.
Kasari, 2010	Edu	R-P	38	DSM-IV, ADI	United St.
Kasari, 2012	Edu	R-P	60	ADOS, ADI	United St.
Kasari, 2014	Edu	R-SMART	61	ADOS	United St.
Kasari, 2014	Edu	R-H2H	107	ADOS	United St.
Kasari, 2015	Edu	R-H2H	83	ADOS, ADI	United St.
Kasari, 2016	Edu	R-H2H	133	ADOS, SCQ	United St.
Keehn, 2013	Psy	R-P	22	DSM-IV-TR, ADI, ADOS	United St.
Kent, 2013	Ph	R-P	92	DSM-IV-TR, ADI	United St.
Kenworthy, 2014	Edu	R-H2H	60	DSM-IV-TR, ADOS	United St.
Kern, 2001	Nut	R-P	37	DSM-IV	United St.

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Kern, 2002	Ph	R-C	19	DSM-IV	United St.
Kern, 2013	Edu	R-C	10	CARS, M-CHAT	United St.
Khorshid, 2006	Misc	R-H2H	14	Unspec.	United St.
Kim, 2008	Misc	R-C	10	DSM-IV, CARS, ADOS	South korea
King, 2001	Ph	R-P	39	DSM-IV, ICD-10, ADI, ADOS	United St.
King, 2009	Ph	R-P	149	DSM-IV-TR, ADOS, ADI	United St.
Klaiman, 2013	Nut	R-P	46	DSM-IV-TR, ADI, ADOS	United St.
Knivsberg, 2002	Nut	R-P	20	DIPAB	Norway
Koch, 2015	Misc	Non-R	31	ICD-10	Germany
Koehne, 2016	Misc	R-P	51	DSM-IV, ICD-10, ADOS, ADI	Germany
Koenig, 2010	Edu	R-P	41	ADOS, SCQ, PDDBI	United St.
Kok, 2002	Edu	Non-R	8	AUBC	Singapore
Kolmen, 1995	Ph	R-C	13	DSM-III-R, CARS	United St.
Kolmen, 1997	Ph	R-C	11	DSM-III-R, CARS	United St.
Koning, 2013	Psy	R-P	15	DSM-IV-TR, ADOS	Canada
Kosaka, 2016	Ph	R-P	60	DSM-IV-TR, DISCO	Japan
Kouijzer, 2013	Misc	R-P	38	DSM-IV-TR, ADI, SCQ	Netherlands
Kretzmann, 2015	Edu	R-P	24	DSM-IV-TR, ADOS	United St.
Kroeger, 2007	Edu	Non-R	25	Unspec.	United St.
Kuriyama, 2002	Nut	R-P	8	DSM-IV	Japan
Lamberti, 2016	Ph	R-H2H	44	DSM-5, ADOS, ADI	Italy
Landa, 2011	Edu	R-H2H	48	ADOS	United St.
Langdon, 2016	Psy	R-C	45	ADO	UK
Laugeson, 2009	Edu	R-P	33	Unspec.	United St.
Laugeson, 2014	Edu	Non-R	73	DSM-IV-TR	United St.
Laugeson, 2015	Edu	R-P	17	AQ	United St.
Lawton, 2012	Edu	R-P	16	ADO	United St.
Layton, 1988	Edu	R-H2H	60	CARS	United St.
Leboyer, 1992	Ph	R-C	4	DSM-III-R	France
LeGoff, 2004	Edu	Non-R	47	Unspec.	United St.
Lelord, 1981	Nut	R-C	21	Unspec.	France
Lemonnier, 2012 #218	Ph	R-P	54	ICD-10, ADOS, ADI, CARS	France
Lerna, 2012	Edu	Non-R	18	DSM-IV-TR, ADOS	Italy
Lerna, 2014	Edu	Non-R	14	DSM-IV-TR, ADOS	Italy
Lerner, 2012	Edu	R-H2H	13	SRS, SCQ	United St.
Levine, 1997	Nut	R-C	9	DSM-III-R	Israel
Levy, 2003	Ph	R-P	61	ADI	United St.

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Loebel, 2016	Ph	R-P	148	DSM-IV-TR, ADI	United St.
Lopata, 2008	Edu	R-H2H	54	DSM-IV-TR	United St.
Lopata, 2010	Edu	R-P	35	Unspec.	United St.
Lopata, 2015	Edu	R-H2H	47	ADI	United St.
Lopata, 2016	Edu	R-P	36	ADI	United St.
Lovaas, 1987	Edu	Non-R	38	DSM-III	United St.
Luby, 2006	Ph	R-P	23	DSM-IV	United St.
Maddox, 2016	Psy	R-P	25	ADOS, ADI	United St.
Magiati, 2007	Edu	Non-R	44	ADI	UK
Malone, 2001	Ph	R-H2H	12	DSM-I	United St.
Mandell, 2013	Edu	R-P	119	ADOS	United St.
Mankad, 2015	Nut	R-P	38	DSM-IV-TR, ADI, ADOS	Canada
Marcus, 2009	Ph	R-P	178	DSM-IV-TR, ADI	United St.
Marshall, 2016	Edu	R-P	37	ICD-10, DSM-IV-TR	UK
Martineau, 1985	Nut	R-C	60	DSM-III	France
McConachie, 2014	Psy	R-P	32	ADOS	UK
McCracken, 2002	Ph	R-P	101	DSM-IV, ADI	United St.
McDougle, 1996	Nut	R-C	17	DSM-III-R, ICD-10, ADOS, ADI	United St.
McDougle, 1996	Ph	R-P	30	DSM-III-R, ICD-10, ADI, ADOS	United St.
McDougle, 1998	Ph	Non-R	31	DSM-IV, ADI, ADOS	United St.
McGillivray, 2014	Psy	Non-R	42	Unspec.	Australia
McKeel, 2015	Edu	R-P	27	Unspec.	United St.
McNally Keehn, 2013	Psy	R-P	22	ADOS, ADI, DSM-IV-TR	United St.
McVey, 2016	Edu	R-P	47	ADOS	United St.
Minshawi, 2016	Ph	R-P	66	ADOS, ADI, DSM-IV-TR	United St.
Miral, 2008	Ph	R-H2H	28	DSM-IV	Turkey
Miyajima, 2016	Misc	R-P	14	DSM-5, PARS	Japan
Mohammadi, 2013	Ph	R-P	40	DSM-IV-TR, ADI	Iran
Mohammadzaheri, 2014	Edu	R-H2H	30	DSM-IV-TR	Iran
Molloy, 2002	Ph	R-C	42	DSM-IV	United St.
Morgan, 2014	Edu	R-P	28	DSM-IV-TR, ADOS	United St.
Mudford, 2000	Misc	R-C	16	DSM-IV, ICD-10	UK
Munasinghe, 2010	Nut	R-C	43	DSM-IV-TR	Australia
Munesue, 2016	Ph	R-C	29	DSM-IV-TR, DISCO	Japan
Nagaraj, 2006	Ph	R-P	39	DSM-IV	India
Navarro, 2015	Nut	R-P	12	DSM-IV, ADI, ADOS	United St.
Nazni, 2008	Nut	Non-R	20	Unspec.	India
Niederhofer, 2003	Ph	R-C	12	ICD-10	Austria

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Niederhofer, 2004	Ph	R-C	14	ICD-10	Italy
Nikoo, 2015	Nut	R-P	40	DSM-IV-TR, ADI, ABC	Iran
Owen, 2009	Ph	R-P	98	DSM-IV-TR, ADI	United St.
Owens, 2008	Edu	R-P	47	ADI, SCQ	UK
Owley, 1999	Ph	R-C	20	ADI, ADOS, DSM-IV	United St.
Owley, 2001	Ph	R-C	56	DSM-IV, ADOS, ADI	United St.
Ozonoff, 1998	Edu	Non-R	22	Unspec.	United St.
Pahnke, 2014	Psy	R-P	28	DSM-IV	Sweden
Pajareya, 2011	Edu	R-P	31	DSM-IV	Thailand
Panerai, 2009	Edu	Non-R	34	DSM-IV-TR, CARS, ADI	Italy
Pearson, 2013	Ph	R-C	24	DSM-IV-TR, ADI, ADOS	United St.
Peters-Scheffer, 2010	Edu	Non-R	34	DSM-IV	Netherlands
Peters-Scheffer, 2013	Edu	Non-R	40	ICD-10, DSM-IV-TR, CARS, ADOS	Netherlands
Pfeiffer, 2011	Misc	R-H2H	37	DSM-IV-TR	United St.
Pineda, 2008	Misc	R-P	19	ADI, ADOS	United St.
Piravej, 2009	Misc	R-P	60	DSM-IV	Thailand
Porges, 2014	Misc	R-P	114	ICD-10, DSM-IV-TR, ADI	United St.
Posey, 2004	Ph	Non-R	20	DSM-IV-TR, ADI	United St.
Poslawsky, 2015	Edu	R-P	77	DSM-IV-TR, ADOS	Netherlands
Pusponegoro, 2015	Nut	R-P	50	DSM-IV	Indonesia
Quintana, 1995	Ph	R-C	10	DSM-III-R, CARS	United St.
Quirmbach, 2009	Edu	R-H2H	45	ADOS	United St.
Ratcliffe, 2014	Edu	Non-R	217	DSM-IV-TR	Australia
Ratliff-Schaub, 2005	Ph	R-C	15	DSM-IV	United St.
Realmuto, 1986	Ph	R-C	12	DSM-III	United St.
Reaven, 2009	Psy	Non-R	31	ADOS, SCQ	United St.
Reaven, 2012	Psy	R-P	50	ADOS, SCQ, DSM-IV-TR	United St.
Reed, 2007	Edu	Non-R	27	Unspec.	UK
Reitzel, 2013	Edu	R-P	15	DSM-IV-TR, ADI, ADOS	Canada
Remington, 2001	Ph	R-C	36	DSM-IV	Canada
Remington, 2007	Edu	Non-R	44	ADI	UK
Research Units on Pediatric PsychoPhacology Autism, 2005	Ph	R-C	66	DSM-IV, ADI	United St.
Rezaei, 2010	Ph	R-P	40	DSM-IV-TR, ADI	Iran
Rice, 2015	Edu	R-P	31	Unspec.	United St.

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Rickards, 2007	Edu	R-P	59	DSM-IV, ADI, ADOS	Australia
Rimland, 1995	Misc	R-P	16	RDEC	United St.
Roberts, 2001	Ph	R-P	64	ADI, ADOS, DSM-IV	Canada
Roberts, 2011	Edu	R-P	56	DSM-IV, ADOS	Australia
Rodgers, 2015	Edu	R-P	60	ADI	United St.
Roeyers, 1996	Edu	R-P	85	DSM-III-R	Belgium
Rogers, 2006	Edu	R-H2H	10	ADOS, SCQ, DSM-IV	United St.
Rossignol, 2009	Misc	R-P	56	DSM-IV, ADI, ADOS	Australia
Russell, 2013	Psy	R-H2H	40	ADI, ADOS	UK
Saad, 2015	Nut	R-P	92	DSM-IV-TR	Egypt
Sallows, 2005	Psy	R-H2H	23	DSM-IV, ADI	United St.
Sampanthavivat, 2012	Misc	R-P	58	DSM-IV-TR	Thailand
Sandler, 1999	Ph	R-P	52	DSM-IV, CARS, AUBC	United St.
Santomauro, 2016	Psy	R-P	20	ASDI, ASASC	Australia
Scahill, 2015	Ph	R-P	62	DSM-IV, SCQ, ADOS	United St.
Scarpa, 2011	Psy	R-P	11	ADOS	United St.
Schaaf, 2014	Edu	R-P	31	ADI, ADOS	United St.
Schohl, 2014	Edu	R-P	58	ADOS	United St.
Schreibman, 2014	Edu	R-H2H	39	DSM-IV, ADI, ADOS	United St.
Schwartzberg, 2013	Edu	R-H2H	30	Unspec.	United St.
Schwartzberg, 2016	Edu	R-H2H	29	Unspec.	United St.
Scifo, 1991	Ph	R-C	11	DSM-III-R, CARS, BSE	Italy
Shea, 2004	Ph	R-P	77	DSM-IV, CARS	Canada
Sheinkopf, 1998	Edu	Non-R	22	Unspec.	United St.
Sherman, 198	Ph	R-C	15	DSM-III, NSAC	Canada
Silva, 2007	Misc	R-P	15	DSM-IV	United St.
Silva, 2009	Misc	R-P	46	Unspec.	United St.
Silver, 2001	Edu	R-P	22	Unspec.	UK
Singh, 2014	Nut	R-P	36	ADOS, DSM-IV	United St.
Smith, 1985	Misc	R-C	14	Unspec.	United St.
Smith, 1997	Edu	Non-R	21	DSM-III	Norway
Smith, 2014	Edu	R-P	26	SRS	United St.
Smith, 2016	Ph	R-P	22	DSM-IV-TR, ADI	United St.
Sofronoff, 2005	Psy	R-P	71	DSM-IV, CAST	Australia
Sofronoff, 2007	Psy	R-P	45	DSM-IV, CAST	Australia
Solomon, 2004	Edu	R-P	18	DSM-IV, ADI, ADOS	United St.
Solomon, 2008	Edu	R-P	19	DSM-IV-TR, ADI, ADOS	United St.
Solomon, 2014	Edu	R-P	121	DSM-IV, ADOS, SCQ	United St.

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Soorya, 2015	Edu	R-H2H	67	DSM-IV-TR, ADOS, ADI	United St.
Spek, 2013	Edu	R-P	41	DSM-IV-TR, ADI	Netherlands
Spjut Jansson, 2016	Edu	Non-R	71	ADOS, DISCO	Sweden
Sponheim, 2002	Ph	R-C	6	ADI, ICD-10	Norway
Srinivasan, 2015	Misc	R-P	33	ADOS	United St.
Srinivasan, 2016	Misc	R-P	33	ADOS	United St.
Stern, 1990	Ph	R-C	19	DSM-III	Australia
Storch, 2013	Psy	R-P	45	ADI, ADOS	United St.
Storch, 2015	Psy	R-P	31	ADI, ADOS, CARS	United St.
Strain, 2011	Edu	R-H2H	294	Unspec.	United St.
Strickland, 2013	Edu	R-P	22	Unspec.	United St.
Sugie, 2005	Ph	R-C	18	DSM-IV	Japan
Sun, 2016	Nut	Non-R	66	DSM-IV	China
Sung, 2011	Psy	R-H2H	70	DSM-IV, ADOS	Singapore
Tanaka, 2010	Edu	R-P	79	DSM-IV, ADI, ADOS	Canada
Thomeer, 2012	Edu	R-P	34	ADI	United St.
Thomeer, 2015	Edu	R-P	43	ADI	United St.
Thomeer, 2016	Edu	R-P	57	SCQ	United St.
Thompson, 2014	Misc	R-P	21	DSM-IV-TR	Australia
Tolbert, 1993	Nut	R-C	15	DSM-III-R	United St.
Troost, 2005	Ph	R-P	24	DSM-IV-TR, ADI	Netherlands
Tsang, 2007	Edu	Non-R	34	DSM-IV	Hong kong
Unis, 2002	Ph	R-P	85	DSM-IV, ADOS	United St.
Urbano, 2014	Ph	R-H2H	20	DSM-IV-TR	United St.
Urbano, 2015	Ph	R-H2H	20	DSM-IV-TR	United St.
Van Bourgondien, 2003	Edu	Non-R	32	Unspec.	United St.
Van Hecke, 2015	Edu	R-P	57	ADOS,	United St.
van Steensel, 2014	Psy	Non-R	49	DSM-IV-TR, ADI	Netherlands
van Steensel, 2015	Psy	Non-R	79	DSM-IV-TR, ADI	Netherlands
Veenstra-VanderWeele, 2016	Ph	R-P	150	DSM-IV-TR, ADOS	United St.
Voigt, 2014	Nut	R-P	48	DSM-IV-TR, CARS	United St.
Wasserman, 2006	Ph	R-P	20	DSM-IV, ADOS, ADI	United St.
Watanabe, 2015	Ph	R-C	9	DSM-IV-TR, ADI, ADOS	Japan
Wehman, 2014	Edu	R-P	40	Unspec.	United St.
Welterlin, 2012	Edu	R-P	20	Unspec.	United St.
Wetherby, 2014	Edu	R-H2H	82	ADOS	United St.
White, 2013	Edu	R-P	30	ADOS, ADI	United St.
White, 2016	Edu	R-H2H	8	ADOS	United St.
Whiteley, 2010	Edu	R-P	59	ICD-10, ADOS, ADI	Denmark

Reference	Intervention	Design	Total ASD sample	Diagnostic tool	Study location
Willemsen-Swinkels, 1995	Ph	R-C	24	DSM-III	Netherlands
Willemsen-Swinkels, 1995	Ph	R-C	17	DSM-III-R	Netherlands
Willemsen-Swinkels, 1996	Ph	R-C	20	DSM-III-R	Netherlands
Williams, 2012	Edu	R-P	55	ADOS, DSM-IV-TR	Australia
Wink, 2014	Ph	Non-R	142	DSM-IV-TR	United St.
Wink, 2016	Nut	R-P	25	DSM-IV, ADI	United St.
Wong, 2007	Edu	R-H2H	41	ADOS, ADI	United St.
Wong, 2010	Misc	R-P	55	DSM-IV, ADI, ADOS	Hong kong
Wong, 2010	Edu	R-P	17	ADI, ADOS, DSM-IV-TR	Hong kong
Wong, 2010	Misc	R-P	50	DSM-IV, ADI, CARS	Hong kong
Wong, 2013	Edu	R-P	33	CARS	United St.
Woo, 2013	Misc	R-P	28	ADOS	United St.
Woo, 2015	Misc	R-P	50	DSM-IV-TR, ADOS	United St.
Wood, 2009	Psy	R-P	40	ADI, ADOS	United St.
Wood, 2015	Psy	R-P	33	ADI, ADOS	United St.
Wright, 2011	Ph	R-C	17	ICD-10, ADI, ADOS	UK
Wu, 2016	Edu	R-P	20	DSM-IV	Taiwan
Yarbrough, 1987	Ph	R-C	20	DSM-III	United St.
Yatawara, 2016	Ph	R-C	31	DSM-IV-TR, ADOS, SRS, DBC	Australia
Yoder, 2006	Edu	R-H2H	36	ADOS	United St.
Yoo, 2014	Edu	R-P	47	DSM-IV, ADI, ADOS	South korea
Young, 2016	Edu	R-P	255	CARS	United St.
Yui, 2012	Nut	R-P	13	DSM-IV, ADI	Japan
Zachor, 2007	Edu	Non-R	39	DSM-IV, ADI	Israel
Zachor, 2010	Edu	Non-R	78	DSM-IV, ADI	Israel
Zhang, 2012	Misc	Non-R	76	DSM-IV, CARS	China

Legend: *Edu:* Educational; *Misc:* Miscellaneous; *Non-R*: Non-randomized trial; *Nut:* Nutraceutical: *Ph:* Pharmacological; *Psy:* Psychotherapy; *R-C*: Randomized controlled trial - Crossover; *R-H2H*: Randomized controlled trial – Head-to-head; *R-P*: Randomized controlled trial – Parallel group; *R-SMART:* Randomized controlled trial – SMART design.