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MOTOR IMPAIRMENTS AS A BIO-BEHAVIORAL MARKER OF AUTISM SPECTRUM DISORDER

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In order to arrive at what you do not know
You must go by a way which is the way of ignorance.
In order to possess what you do not possess
You must go by the way of dispossession.
In order to arrive at what you are not
You must go through the way in which you are not.
And what you do not know is the only thing you know
And what you own is what you do not own
And where you are is where you are not.

from EAST COKER
(No. 2 of 'Four Quartets')
T. S. Eliot

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ABSTRACT

Autism Spectrum Disorder (ASD) is a highly heterogeneous neurodevelopmental disorder with multiple causes, courses, and a wide range in symptom severity. Although the etiology of the disorder is generally considered multifactorial, high heritability estimates suggest a critical role for genetic factors. However, the notable clinical heterogeneity within the broad behavioral phenotype has been a major obstacle to gene identification. Furthermore, the hallmark heterogeneity of ASD makes the quest for personalized treatment and potential precision medicine inherently difficult. Starting from these considerations, in the last years there has been an increasing need for developing a reliable marker for ASD, currently diagnosed on the basis of the clinical judgment of symptoms.

The purpose of the present project was to provide further evidence supporting the use of motor impairments as a bio-behavioral marker of ASD. Indeed, abnormalities in motor behavior are one of the features most frequently associated to the disorder and can have a significant impact on quality of life and social development. Specifically, in this thesis we investigated the gait pattern and the motor adaptation to discrete gait perturbations in school-aged children with ASD using an innovative multi-sensor platform based on immersive virtual reality (Chapter 1). Further, we developed a supervised machine-learning method to identify and correctly discriminate preschool children with ASD from typically developing children by means of kinematic analysis of a simple reach, grasp and drop task (Chapter 2). Finally, using diffusion tensor imaging (DTI), we explored the hypothesis of reduced long-range connectivity between frontal lobes and posterior brain regions in ASD given the key role of these pathways for language, praxis, imitation, and basic motor coordination (Chapter 3).

Results highlighted an altered gait pattern in children with ASD and slower rates of adaptation to the perturbation. Diminished learning adaptation was also significantly related with more severe autistic traits. With respect to classification based on kinematics analysis, our machine-learning method reached a good mean individual classification in the comparisons between children with ASD and healthy controls (overall mean accuracy = 84.9%). Thus, we demonstrated that machine-learning classification approach might be helpful for supporting the clinical practice of diagnosing ASD, even

fostering a computer-aided diagnosis perspective. Last, the DTI study provided evidence for alterations in white matter diffusivity of the left superior longitudinal fasciculus in a well-characterized group of high-functioning children with ASD.

All in all, our findings offer insight on a possible, multi-domain (i.e., behavioral, computational, and imaging) motor signature of ASD that is potentially useful to identify a well-defined subset of patients, thus reducing the clinical heterogeneity within the broad behavioral phenotype. This may guide further exploration of neuropathology of the disorder adding power to genetic analysis.

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by persistent social impairment, communication abnormalities, and restricted and repetitive behaviors (Diagnostic and Statistical Manual, Fifth Edition, DSM-5, American Psychiatric Association, 2013). ASD is a highly heterogeneous condition with multiple causes, courses, and a wide range in symptom severity (Amaral et al., 2008), with an estimated prevalence of 1% worldwide (Elsabbagh et al., 2012), one in 68 U.S.A. children (Centers for Disease Control and Prevention, CDC, 2016). Although the etiology of the disorder is generally considered multifactorial, high heritability estimates suggest a critical role for genetic factors (Jeste and Geschwind, 2014). However, the notable clinical heterogeneity within the broad behavioral phenotype has been a major obstacle to genes identification. Furthermore, the hallmark heterogeneity of ASD makes the quest for personalized treatments and potential precision medicine inherently difficult. Starting from these considerations, in the last years there has been an increasing need for developing a reliable marker for neurodevelopmental disorders such as ASD, currently diagnosed on the basis of the clinical judgment of symptoms (Yerys and Pennington, 2011).

One feature frequently associated to ASD that has been proposed as one of the most consistent markers for the disorder alongside the socio-communicative difficulties is abnormalities in motor behavior (Dowd et al. 2012; Esposito and Pasca, 2013; Mosconi and Sweeney, 2015; Anzulewicz et al., 2016). Indeed, although the core features of ASD are persistent deficits in social communication and interaction and the presence of restricted, repetitive patterns of behavior, interests, or activities, it is of great importance not to ignore the motor impairments associated with ASD as they are highly prevalent, at 79%, and can have a significant impact on quality of life and social development (Lai et al. 2014). Already in the earliest descriptions of the disorder (Kanner, 1943; Asperger, 1944), widespread anomalies of movement have been described including atypical postural control, gait,

reach-to-grasp movements, gross and fine motor control. Although Kanner and Asperger focused on different traits, they also both recognized atypicalities in the motor domain. In "Autistic Disturbances of affective contact" (1943), Kanner reported that "several of the children were somewhat clumsy in gait and gross motor performances, but all were very skillful in terms of finer muscle coordination", and he noticed a "failure to assume at any time an anticipatory posture preparatory to being picked up". In "The Autistic Psychopaths in Childhood" (1944; Asperger's original work was translated by Uta Frith, 1991), Asperger described four children all presenting motor delay and clumsiness: "motor milestones were rather delayed […] and for a long time was extremely clumsy and unable to do things for himself", "since he was very clumsy, could not control his movements and had no idea where to aim, he often allegedly caused injury to others", and again "as is to be expected from his whole appearance, he was clumsy to an extraordinary degree". Since these two seminal and fascinating clinical pictures, a number of empirical works have reported different motor deficits in ASD, including anomalies in walking patterns (e.g., Vilensky et al., 1981; Rinehart et al., 2006; Nobile et al., 2011), hand movements such as reaching (e.g., Mari et al., 2003; Glazebrook et al., 2006; Forti et al., 2011), eye-hand coordination (e.g., Glazebrook et al., 2009; Crippa et al., 2013), and oculomotor function (Mosconi and Sweeney, 2015). A relatively recent meta-analysis (Fournier et al., 2010) concluded that ASD is significantly associated with extensive alterations in motor performance, with a noteworthy effect size of 1.20. As the literature regarding motor difficulties in ASD has been thoroughly reviewed elsewhere (e.g., Gowen and Hamilton, 2013; Sacrey et al., 2014; Kindegran et al., 2015; Cook, 2016), here we briefly make reference to a part of these works, in order to illustrate why the study of motor functions offers several advantages for research on the pathophysiology of ASD (Minshew et al., 2004; Dowd et al., 2010; Esposito and Pasca, 2013; Mosconi and Sweeney, 2015).

A first reason is that motor abnormalities in ASD may occur very early in development (Teitelbaum et al. 1998, Brian et al. 2008), with a number of studies suggesting that motor difficulties could be one of the earliest identifiable clinical manifestations of ASD (Bryson et al., 2007; Esposito

et al., 2008; Phagava et al., 2008; Esposito et al., 2011; Sacrey et al., 2015). Early motor disturbances could even provide crucial information to delineate different trajectories for typical development, "early-onset", and regressive forms of ASD, starting from 6 months of age (Landa et al., 2012; Landa et al., 2013). Nevertheless, motor impairments may be apparent over time (Fournier et al. 2010; Van Waelvelde et al. 2010) being a pervasive feature of the disorder. Recent studies have also provided evidence for the specificity of motor impairments identified in high functioning children with ASD compared to children with attention deficit/hyperactivity disorder (ADHD) (Izawa et al. 2012; Ament et al. 2014; although with some commonalities, see Biscaldi et al., 2015) and to typically developing children matched by nonverbal IQ and receptive language (Whyatt and Craig 2013). Finally, the severity of motor deficits correlates with the degree of social withdrawal and the severity of symptoms (Freitag et al. 2007). Motor control has even been speculated to be crucial for communication and social interaction (Leary and Hill 1996).

Another advantage of studying the motor function is that motor system can be more easily evaluated than other more complex system (e.g., cognitive functions) given the limited language and cognitive demands of motor paradigms. This allows to study also very challenging cohort of participants, such as very young children or low-functioning patients. It is worth to remember that the prevalence of intellectual disability in children and adolescents with ASD can be considerable (e.g., 47.6%. in an Italian sample, Postorino et al., 2016). Moreover, motor abilities can be quantified precisely and represent more objective and reliable measures in comparison with symptoms as judged by clinicians and with semi-structured behavioral observations.

Finally, a last reason why it could be fruitful to use the motor impairments as a marker of ASD is that neurophysiological correlates of motor system are well-characterized on the basis of animal and human lesion studies. Previous findings regarding motor abnormalities in ASD suggest alterations to cortico-cerebellar, fronto-striatal, and long-distance fronto-parietal pathways (Fournier et al., 2010).

Within this frame of reference, the research presented in the current dissertation aimed to provide further evidence supporting the use of motor impairments as a bio-behavioral marker of ASD. To investigate the motor system and its neurophysiological correlates in children with ASD we made use of innovative tools (Chapter 1 and Chapter 3) and of a new methodology (Chapter 2), respectively.

In the experiment presented in Chapter 1, we described the gait pattern and the motor adaptation to discrete gait perturbations of drug naïve, school-aged children with ASD compared to typically developing peers. We decided to focus on the study of gait since locomotor activity represents a milestone for the progressive development of the children's communication. Further, we additionally focused on motor adaptation during a split-belt walking task because we know that this split-belt adaptation relies on cerebellum, one of the anatomical structure primarily implicated in ASD. We examined locomotion using the Gait Real-time Analysis Interactive Lab (GRAIL), an innovative multi-sensor platform based on immersive virtual reality.

In Chapter 2, we presented a proof-of-concept study that we have undertaken to investigate whether a simple upper-limb movement could be useful to accurately classify low-functioning children with ASD who are between the ages of two to four. In order to answer this question, we developed a supervised machine-learning method to identify and correctly discriminate preschool children with ASD from typically developing children by means of kinematic analysis of a simple reach, grasp and drop task.

Finally, in the study described in Chapter 3, we aimed to investigate whether a well-characterized group of high-functioning children with ASD would demonstrate reduced white matter integrity, as indirectly measured by diffusion tensor imaging, in three long-distance frontal-posterior tracts ―namely, inferior fronto-occipital fasciculus, inferior and superior longitudinal fasciculus. To achieve this goal, we used Automated Fiber Quantification (AFQ), a relatively novel tool that measures diffusivity measures along the trajectories of major white matter tracts of the brain network. We further aimed to explore the relationship between potential differences in diffusion properties and core features of ASD, as well as measures of motor function and praxis. Exploring the hypothesis of

an anterior-posterior underconnectivity in ASD might provide useful insight into the mechanisms of social communication, given the key role of these pathways for language, praxis, imitation, and basic motor coordination.

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CHAPTER 1:

GAIT PATTERN AND MOTOR PERFORMANCE DURING DISCRETE GAIT PERTURBATION IN CHILDREN WITH AUTISM SPECTRUM DISORDERS[1]

Aims of the chapter

- Locomotor activity represents a milestone for the progressive development of the children's communication.
- Quantitative assessment of locomotor activity, such as kinematic analysis of gait, has been considered a useful tool to identify subtle signs of motor system dysfunction in ASD.
- In the present work, we aimed to investigate the gait pattern of school-aged children with ASD using the Gait Real-time Analysis Interactive Lab (GRAIL), a multi-sensor platform based on immersive virtual reality.
- Further, we additionally assessed the motor adaptation during a split-belt walking task because we know that this split-belt adaptation might rely on cerebellum, one of the anatomical structure primarily implicated in ASD.

[1] The study presented in this chapter was carried out at Scientific Institute, IRCCS Eugenio Medea (Bosisio Parini, Italy), in collaboration with Dr. Emilia Biffi, Cristina Costantini, Silvia Busti Ceccarelli, Dr Gian Marco Marzocchi, Dr. Maria Nobile, and Massimo Molteni. A previous version of the present study has been presented as a poster at the 2017 International Meeting for Autism Research (IMFAR), San Francisco, USA.

Introduction

It has been hypothesized that studies on motor function could have significant potential in providing critical insights into the neurobiological basis of Autism Spectrum Disorder (ASD) and in improving its diagnostic characterization (Minshew et al., 2004). Already in the earliest descriptions of the disorder, a range of movement anomalies have been described including atypical postural control, gait, reach-to-grasp movements, gross and fine motor control. Among these domains, the study of gait has drawn growing interest in the last decades in ASD. Indeed, locomotion is a complex and crucial motor activity that enables children to explore the surrounding space and to integrate their own behavior in the context acting on the environment. Thus, locomotor activity represents a milestone for the progressive development of the children's communication. Given these considerations, quantitative assessment of locomotor activity employing measures sensitive to subtle gait anomalies, such as kinematic analysis of gait, has been considered a useful tool to identify subtle signs of motor system dysfunction in ASD (Nayate et al., 2005) and to investigate possible neural correlates of motor atypicalities. Moreover, retrospective analyses of home videos of infants and toddlers later diagnosed with ASD have suggested that abnormal gait patterns may precede social and communicative difficulties (Teitelbaum et al., 1998; Esposito and Venuti, 2008; Esposito et al., 2011). Since the first quantitative study of gait in children with ASD (Vilensky et al., 1981), a number of empirical research on locomotor activity have been reported, with mixed findings in the types of movement anomalies described in ASD (see Kindregan et al., 2015, for a recent review). The causes of these puzzling results might be various, as the studies differed in many important methodological aspects, such as the technologies used, sample sizes, and participants' ages. Furthermore, at this stage of the scientific literature, these is a lack of empirical studies which used simultaneously kinetic and kinematic gait parameters to investigate locomotion in children with ASD.

The present study aimed to describe the gait pattern of drug naïve, school-aged children with ASD compared to typically developing peers using the Gait Real-time Analysis Interactive Lab

(GRAIL), a multi-sensor platform based on immersive virtual reality, with an instrumented dual-belt treadmill, that acquires and process in real time kinetic and kinematic data. To the best of our knowledge, no studies have employed yet this innovative, integrated system to examine the locomotion in children with neurodevelopmental disorders. On the basis of the findings of a previous study of overground gait from our group (Nobile et al., 2011), we expected children with ASD to have: (1) with respect to spatio-temporal parameters, reduced stride length and increased time in the stance phase; (2) with respect to kinematic parameters, reduced range of motion of joints. Further, we aimed to explore the relationship between potential differences in gait parameters and core features of ASD.

Finally, a second main goal of the present study was to examine the motor performance and adaptation during discrete gait perturbation in the same cohort of participants. The discrete perturbation was administered using the GRAIL split-belt treadmill, with the independent belt under the dominant leg suddenly accelerating at a single toe-off phase of twenty gait trials. We aimed to investigate walking adaptation because we know from previous studies that this split-belt adaptation might rely on cerebellum (Morton and Bastian, 2006). On the basis of decades of anatomical and imaging data (Bauman & Kemper, 1985; Courchesne et al., 1988; Becker & Stoodley, 2013), it has been suggested that the cerebellum could be primarily implicated in ASD, with cognitive and behavioral effects beyond the difficulties in the motor domain. With particular reference to gait, evidence from previous studies in ASD are consistent with a cerebellar involvement (Vilensky et al., 1981; Hallett et al., 1993; Ambrosini et al., 1998; Rinehart et al., 2006; Nobile et al., 2011). To date, no studies have investigated this type of adaptation to discrete gait perturbations in children with ASD. On the basis of the literature regarding upper limb motor learning and adaptation in ASD (Haswell et al., 2009; Izawa et al., 2012; Marko et al., 2015), we expected children with ASD to have slower rate or atypical pattern of adaptation.

Methods

Participants

Thirteen children with ASD (mean age: 9.9 ± 1.5 years; 12 males, 1 female) and thirteen typically developing children (TD) (mean age: 10.2 ± 1.0 years; 12 males, 1 female) matched by gender and age were enrolled. All participants were Caucasian, had normal or corrected-to-normal vision, and were not taking any medication. Children in the ASD group were recruited at Child Psychopathology Unit of Scientific Institute, IRCCS Eugenio Medea (Lecco, Italy). All participants in the clinical group had been previously diagnosed according to the DSM-IV TR (American Psychiatry Association, 2000) at admission by a medical doctor specialized in child neuropsychiatry with expertise in autism. The diagnoses were then confirmed using the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2002). Children in the healthy control group were recruited by local pediatricians and from kindergartens in the vicinity of our institute to be gender- and age-matched to the clinical sample from the typically developing population. The TD children had no previous history of social/communicative disorders, developmental abnormalities, or medical disorders with central nervous system implications. At least five subtests of the WISC-IV (Vocabulary, Similarities, Block Design, and Matrix reasoning, Picture Concepts; Wechsler, 2012) were administered to all children to determine the general IQ level (Crawford et al, 2010). All participants were required to have estimated full-scale IQ (FSIQ) of 80 or above. One children with ASD with FSIQ of 76 was included on the basis of discrepant subscores, having a Perceptual Reasoning Index (PRI) of 80. In the present study, perceptual reasoning index (PRI) was used as the measure of intellectual reasoning ability to match the two groups of participants. Being a measure of nonverbal abilities, PRI may better estimate cognitive abilities in subject with ASD than the FSIQ (Mottron, 2004). Parents completed the Social Responsiveness Scales (SRS) (Constantino & Gruber, 2005) to measure general autistic symptoms/traits and behavior difficulties across participants, and the Social Communication Questionnaire–Lifetime (SCQ) (Rutter et al., 2003) to check for ASD symptoms in the TD children. Last, data on parental employment were used as a measure of

socioeconomic status (SES) and coded according to the Hollingshead 9-point scale for parental occupation (Hollingshead, 1975). The study was explained to both children and their parent(s) or caregivers, and all of the participants' legal guardians signed the informed written consent before the children's participation. The research received approval from the ethic committee of our institute and was therefore performed in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments.

Motor assessment

Motor skills of the participants were assessed using the Movement Assessment Battery for Children 2 (MABC 2: Henderson, Sugden, & Barnett, 2007). The MABC 2 consists of eight subtests that evaluate three components of motor proficiency: manual dexterity, ball skills, and static and dynamic balance. For MABC 2, higher scores are indicative of better motor performance. In addition, parents completed the Developmental Coordination Disorder Questionnaire (DCDQ) (Wilson et al., 2007). The DCDQ is a 15-item questionnaire that investigates gross and fine motor skill impairments. The DCDQ yields a raw total score (score range 15–75); higher scores indicate better motor functioning as rated by parents.

Instrumentation

The GRAIL system is a dedicated solution for gait analysis and gait training in motivating environments (Sloot et al., 2014a). It is an integrated platform made up of an instrumented dual-belt treadmill, a two degrees of freedom motion frame and integrated force plates (16 channels, sample frequency 1000 Hz) to capture useful gait data during the trial. The system is equipped with 10 optoelectronic cameras (sample frequency 100 Hz) for kinematic data acquisition, a motion-capture system and 3 video cameras (Figure 1.1).

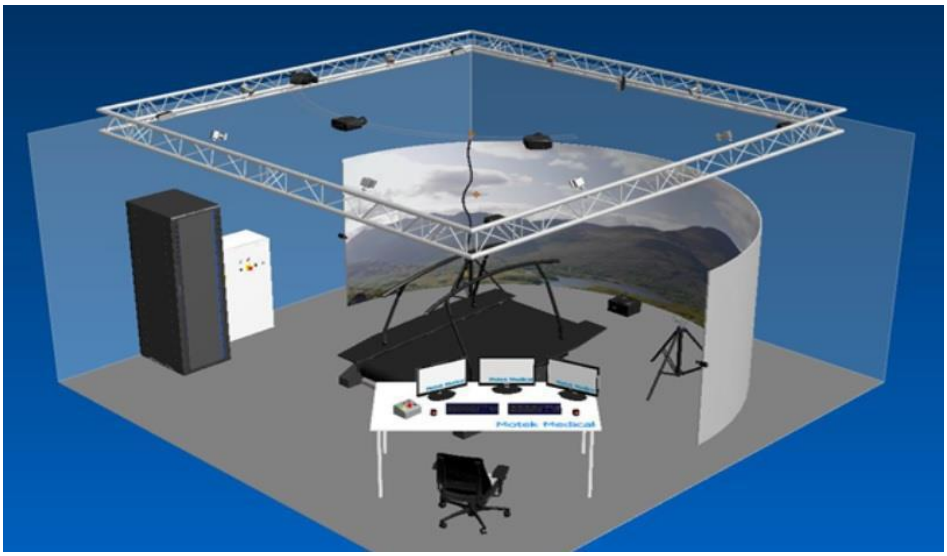

Fig 1.1. The GRAIL system: general environment.

The integration with synchronized VR environments which are projected on a 180° cylindrical projection screen allows the subject to walk and move in natural and attractive settings. The motion frame can translate in longitudinal and lateral direction to assess compensatory strategies and investigate dynamic stability. Moreover, the self-paced functionality of the treadmill is designed to simulate a more realistic walking environment in which the subject determines his own walking speed. The system automatically adjusts the speed of the treadmill and of the VR scenery to the walking ability of the subject allowing more realistic gait training in a safe environment. GRAIL acquires kinematic and kinetic data and then processes them in real time by means of the 25-marker Human Body Model (van den Bogert et al., 2013) This feature gives the possibility of using gait and movement parameters directly within virtual games and for the creation of multisensory stimulations. Furthermore, it makes available useful information during the session for a personalized training of static and dynamic postural control. Real-time filtering is performed with a 2nd order Butterworth filter, with a cut-off frequency equal to 6 Hz and then gait traces are saved in a csv file. It is possible to compute the mean and standard deviation of every gait parameters by acquiring many steps during the trial, which also extremely reduces data acquisition and processing time.

The whole system is controlled by the D-flow, a software that oversees the relationship between the subject, the scenario and the interactive feedbacks and stimulations. The D-Flow runs on Microsoft Windows and it is designed for the development of interactive and immersive virtual reality applications, for the purpose of clinical research and rehabilitation. The subject is a central part of a real-time feedback loop, in which multi-sensory input devices (e.g., motion capture systems, force plates and electromyography) measure the behavior of the subject, while output devices (e.g. motion platforms, treadmills, audio devices and displays) return motor sensory, visual and auditory feedback to the subject. The system operator defines feedback strategies through a flexible and extensible application development framework, based on visual programming. The D-Flow programming, indeed, is based on the concept of modules, which are components with a specific functionality, which can be combined to create complex, interactive virtual reality applications. Some modules directly control specific hardware devices, such as a treadmill or a motion base. Other modules provide access to real-time data streams from live input devices. Others manipulate virtual objects or detect collisions between them, thus allowing the interaction between the subject and the virtual environment. Finally, D-Flow also contains a general-purpose scripting module and a module for expression parsing. In addition to databased communication, the D-Flow kernel framework allows for event-based communication between modules. The operator can define a set of global events, which can be broadcast by modules at specific occurrences, while each module exposes a set of module actions that affect the behavior of the module in a specific way, enabling maximum flexibility in event-based communication (D-flow, 2011).

Procedure

Gait analysis was carried out in the above-described immersive virtual environment using a 3D motion analysis system with a dual-belt, instrumented treadmill (GRAIL) (Figure 1.2).

Fig 1.2. Picture of the experimental setup. The participants were instructed to walk barefoot at their own natural speed following the street in front of them presented on a speed-matched virtual environment with 180° projection. Children wore a safety harness over legs and shoulders loosely hanging from the ceiling, to prevent injury in case of an accidental fall. The safety harness did not support body weight during walking.

The participants were instructed to walk at their own natural speed for 6 min in the mediolateral middle of the treadmill, placing one foot on each separate belt. During this habituation time, the speed of the belt was real-time adjusted to meet the children's time-varying walking pace, by means of a self-paced (SP) speed algorithm (Sloot et al., 2014b). After this 6-min period of adaptation to the treadmill, the virtual environment, and the SP speed algorithm, a 20-steps cycle was recorded as baseline (T0). Subsequently, each participant was exposed to 20 trials with a discrete gait perturbation: after a random number of steps, a single perturbation was applied to the dominant side at toe-off, using a split-belt acceleration. Immediately afterwards, a 20-steps cycle was acquired. Finally, at the end of the perturbed trials, we recorded a 20-steps cycle as post perturbation trial (T1). In Figure 1.3, the experiment protocol diagram is shown.

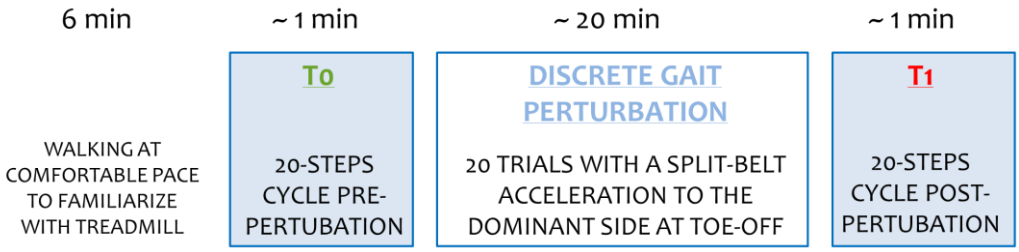

Fig 1.3. Time course for the experimental paradigm.

Data processing and statistical analysis

Between-group differences on the demographic variables, questionnaires, cognitive and motor measures were analyzed using independent-samples t test, according to the distributional nature of the data.

Gait parameters describing spatio-temporal information, kinematic and kinetic data were extracted as previously done at the Bioengineering Lab of our Institute (Biffi et al., 2015; Biffi et al., 2017). In brief, the Gait Off-line Analysis Tool (GOAT) automatically recognized strides with foot placement on both belts and excluded those from further analyses. For T0 and T1, all the valid strides, as assessed by GOAT, were considered for the statistical analysis. With respect to the 20 discrete gait perturbations, only the perturbed stride was selected for the statistical analysis. For each step cycle, stride length and time (i.e., duration between two contacts of the same foot), walking speed, step width (i.e., the mediolateral distance between the left and right heel), stance duration (which begins with initial contact and ends at toe-off of the same limb) and stance percentage per stride, swing duration (i.e., the time during which the foot is not in contact with the ground) and swing percentage per stride were calculated by GOAT from the foot marker data. Kinetic and kinematic information were calculated by GOAT from the ankle, the knee, the hip, and the pelvis marker data. Kinematic and kinetic parameters were extracted with custom-made software (MatLab, The Mathworks®). Medians and interquantile ranges (IQRs) were computed across the collected steps. A paired-samples t test was performed to compare the left and the right side of the gait data. When no statistical difference was found between the right and the left limb, the data from both sides were pooled for

between-group comparisons. In accordance with the normal distribution of data, an independent-samples t test was used to compare the two groups of children on all experimental measures at T0. The possible modifications of gait pattern between T0 and T1 were evaluated using the absolute mean variation for each variable. According to the non-normal distributional nature of those data, between-group differences were explored using the Mann-Whitney test.

In order to compare the adaptation rate across groups when the participants were exposed to a perturbation, we examined whether a linear ($[y = b1 + b2 * x]$, where $b1$ is the slope intercept, $b2$, being the angular coefficient of the slope, represents the adaptation rate, x is the trial number; Rand et al., 2004) or an exponential function ($[y = b1 + b2 * e^{(b3*x)}]$, where $b1$ is the exponential asymptote, $b2$, being the exponential decay, represents the adaptation rate, x is the trial number; Orban de Xivry & Lefèvre, 2015; Lago-Rodriguez & Miall, 2016) fit the data derived for each variable on all the perturbed steps best. The Mann-Whitney test was used to match the R^2 values for the models' fitting across participants. Finally, for gait parameters that showed significant differences between groups, bivariate Spearman's rho correlations were conducted to determine associations between gait data and ASD symptom measures (ADOS and SRS).The significance level was two tailed, $p < .05$, for all the analyses. Because this study was exploratory, no correction was applied for family-wise error rate.

Results

As shown in Table 1.1, the ASD and TD groups were balanced on sex, age, PRI, footedness, and SES. There was a slight trend toward significance for the between-group difference in IQ, with TD children having higher scores, although all participants had the IQ in the average range. As expected, children with ASD have higher scores on Social Responsiveness Scale compared to healthy controls. With respect of motor skills, in comparison to the TD group, the ASD group had lower scores on MABC2 manual aiming and catching, balance, and total score, and lower DCDQ scores. Differences between the left and right foot were found for less than 10% of considered experimental

gait variables in both groups. Therefore, the data from both sides was pooled. Table 1.2 shows gait data at T0 of two groups of children included in the study (ASD vs. TD) and the results of t-test calculated on all parameters.

Table 1.1. Demographic and clinical characteristics of the participants

	ASD (*n* = 13)	**TD (*n* = 13)**	**T-test**	***p*-value**
Sex	12M/1F	12M/1F		
Footedness	12R/1L	11R/2L		
Age	9.88 ± 1.53	10.19 ± 1.05	2.760	.557
IQ	105.85 ± 17.88	117.08 ± 14.99	0.719	.095
PRI	108.00 ± 17.99	116.23 ± 21.94	0.207	.306
SES	67.31 ± 16.15	62.31 ± 17.39	0.356	.455
SRS*	73.85 ± 13.86	49.00 ± 10.57	1.617	**.000**
DCDQ	46.0 ± 13.94	69.31 ± 17.41	0.074	**.001**
MABC 2 – Manual Dexterity	26.38 ± 23.81	34.85 ± 24.02	0.023	.376
MABC 2 – Aiming and Catching	28.50 ± 24.58	67.08 ± 22.22	0.018	**.000**
MABC 2 – Balance	21.08 ± 24.60	48.00 ± 21.61	0.026	**.007**
MABC 2 – Total Score	17.00 ± 13.47	49.08 ± 24.42	3.547	**.000**
ADOS**	6.92 ± 1.75	-		
SCQ***	-	3.15 ± 3.00		

* clinical cut off = 60; ** comparison score; *** clinical cut off = 15; IQ = Intellectual Quotient; PRI = Perceptual Reasoning Index; SES = Socioeconomic Status; SRS = Social Responsiveness Scale; DCDQ = Developmental Coordination Disorder Questionnaire*;* MABC 2= Movement Assessment Battery for Children 2; ADOS = Autism Diagnostic Observation Schedule; SCQ = Social Communication Questionnaire.

Table 1.2. Differences between the two samples for each parameter at T0 based on T-test, with mean and standard deviation (SD).

[b]ROM = Range of Motion

		ASD (13)			TD (13)				
		mean	**±**	**SD**	**mean**	**±**	**SD**	**T-test**	***p*-value**
Spatio-temporal									
Stance-swing	%	68.20	±	1.18	66.62	±	2.04	1.305	**0.024**
Stance time	s	0.76	±	0.07	0.72	±	0.07	0.094	0.179
Swing time	s	0.35	±	0.03	0.36	±	0.02	0.815	0.429
Step width	m	0.17	±	0.03	0.15	±	0.04	0.073	0.402
Step length	m	0.40	±	0.08	0.45	±	0.07	0.069	0.087
Stride time	s	1.12	±	0.09	1.08	±	0.08	0.002	0.391
Walking speed	m/s	0.80	±	0.16	0.93	±	0.20	0.593	0.073
Kinetics									
Ankle flexion moment max	Nm/kg	1.13	±	0.17	1.27	±	0.13	0.955	**0.027**
Hip flexion moment max	Nm/kg	0.56	±	0.16	0.68	±	0.18	0.171	0.073
Hip flexion moment min	Nm/kg	-0.21	±	0.09	-0.27	±	0.12	1.809	0.113
Knee flexion moment max	Nm/kg	0.16	±	0.07	0.23	±	0.17	11.248	0.187
Knee flexion moment min	Nm/kg	-0.43	±	0.11	-0.47	±	0.10	1.422	0.304
Ankle flexion power max	W/kg	1.07	±	0.47	1.66	±	0.83	9.489	**0.033**
Ankle flexion power max time	s	0.68	±	0.06	0.64	±	0.08	0.356	0.208
Ankle flexion power min	W/kg	-0.80	±	0.22	-0.97	±	0.23	0.050	0.075
Ankle flexion power min time	s	0.48	±	0.13	0.45	±	0.12	0.097	0.541
Hip flexion power max	W/kg	0.78	±	0.30	0.89	±	0.31	0.120	0.366
Knee flexion power max	W/kg	0.75	±	0.25	0.95	±	0.22	0.624	**0.042**
Kinematics									
Ankle flexion initial contact	°	1.91	±	2.75	2.06	±	2.58	0.232	0.884
Ankle flexion max stance	°	16.13	±	3.57	16.57	±	2.77	0.782	0.729
Ankle flexion min stance	°	-3.36	±	3.64	-5.49	±	3.31	0.006	0.132
Ankle flexion ROM[b] stance	°	19.49	±	3.31	22.06	±	3.86	0.241	0.081
Ankle flexion max swing	°	7.30	±	3.32	6.05	±	2.77	0.216	0.305
Ankle flexion min swing	°	-1.66	±	4.31	-6.09	±	5.55	2.188	**0.033**
Ankle flexion ROM swing	°	8.97	±	2.98	12.13	±	4.34	3.584	**0.040**
Ankle flexion max	°	16.14	±	3.58	16.57	±	2.77	0.825	0.736
Ankle flexion max time	s	0.61	±	0.11	0.57	±	0.10	0.048	0.380
Ankle flexion min	°	-4.09	±	3.88	-7.67	±	4.62	0.993	0.043
Ankle flexion min time	s	0.38	±	0.21	0.51	±	0.24	0.832	0.142
Foot progression	°	-3.06	±	6.18	-0.83	±	6.89	0.106	0.393
Knee flexion initial contact	°	1.67	±	3.07	0.34	±	4.03	0.625	0.351
Knee flexion max	°	62.45	±	4.64	63.84	±	3.57	1.028	0.398
Knee flexion max time	s	0.85	±	0.08	0.81	±	0.07	0.001	0.250
Knee flexion min	°	-0.73	±	2.75	-1.30	±	3.96	1.325	0.675
Knee flexion min time	s	0.61	±	0.09	0.51	±	0.14	1.008	**0.043**
Knee flexion ROM	°	63.18	±	4.05	65.14	±	5.38	2.531	0.303
Hip flexion initial contact	°	30.99	±	6.30	27.16	±	5.23	0.231	0.104
Hip flexion max	°	35. 74	±	6.34	33.43	±	5.07	0.396	0.314
Hip flexion max time	s	0.88	±	0.11	0.87	±	0.19	1.083	0.891
Hip flexion min	°	0.55	±	7.24	-2.34	±	6.31	0.431	0.288
Hip flexion min time	s	0.63	±	0.07	0.59	±	0.06	0.039	0.123
Hip flexion ROM	°	35.20	±	5.69	35.77	±	4.06	1.242	0.770
Hip Abduction-Adduction stance	°	2.78	±	2.73	1.67	±	2.10	3.055	0.254
Hip Abduction-Adduction swing	°	-2.20	±	2.16	-3.34	±	2.14	0.095	0.190
Hip Abduction-Adduction ROM	°	11.40	±	2.64	11.41	±	1.91	2.252	0.991
Hip rotation initial contact	°	1.36	±	6.35	-4.09	±	4.81	0.518	**0.021**
Hip rotation stance	°	3.63	±	4.42	2.88	±	4.58	0.000	0.672
Hip rotation swing	°	-2.15	±	2.13	-2.16	±	2.58	0.824	0.997
Pelvic tilt initial contact	°	13.23	±	4.09	9.20	±	5.31	0.690	**0.041**
Pelvic tilt	°	12.24	±	4.31	8.88	±	5.14	0.168	0.084
Pelvic tilt ROM	°	4.36	±	1.18	4.05	±	0.57	6.120	0.398
Pelvic Obliquity	°	-0.37	±	1.70	-0.15	±	2.21	0.456	0.777
Pelvic Obliquity ROM	°	6.03	±	1.68	6.29	±	1.63	0.005	0.689
Pelvic rotation	°	0.32	±	4.39	0.52	±	4.21	0.033	0.908
Pelvic rotation ROM	°	9.71	±	3.06	9.19	±	2.82	0.012	0.659

With respect to the spatio-temporal parameters of gait, stance/swing ratio at T0 was higher in children with ASD. In reference to kinetic variables, TD children showed greater ankle flexion

moment and greater ankle and knee power at push off. With respect to the kinematic parameters, the ankle minimum flexion was 4.42° and 3.58° lower in children with ASD during swing and throughout the gait cycle, respectively. Those differences resulted in a 3.16° decrease of range of ankle flexion during swing in ASD group compared to TD peers. In addition, time to knee minimum flexion was 12% delayed in children with ASD. ASD group also presented at initial contact a more internally rotated hip, in comparison to the typical externally rotated hip displayed by healthy controls. Last, the mean pelvic tilt at initial contact was approximately 3.5° more anterior in ASD group compared to TD group. The results of the analysis of absolute mean variation for each variable between T0 and T1 are depicted in Table 1.3. After the discrete gait perturbations, TD group presented a significant 1.50° increase in range of hip flexion in comparison to the slight decrease of ASD group (-0.45°). Children with ASD showed a greater increase in ankle power at push off and in hip adduction during stance phase, both in the direction of TD gait pattern.

Table 1.3. Differences between the two samples for absolute mean variation of each parameter based on Mann-Whitney U Test, with median and interquartile range (IQR).

		ASD (13)	TD (13)		
		median (IQR)	median (IQR)	Mann-Whitney U	*p*-value
Spatio-temporal					
Stance-swing	%	0.19 (0.56)	0.30 (1.42)	57.500	0.166
Stance time	s	0.00 (0.06)	0.03 (0.06)	72.500	0.536
Swing time	s	0.00 (0.01)	0.00 (0.02)	74.000	0.581
Step width	m	0.01 (0.02)	0.00 (0.02)	80.000	0.814
Step length	m	0.00 (0.03)	0.01 (0.02)	71.500	0.500
Stride time	s	0.00 (0.08)	0.04 (0.07)	73.000	0.554
Walking speed	m/s	-0.01 (0.02)	0.00 (0.03)	60.000	0.200
Kinetics					
Ankle flexion moment max	Nm/kg	0.01 (0.04)	0.00 (0.03)	59.500	0.197
Hip flexion moment max	Nm/kg	0.01 (0.14)	0.06 (0.05)	57.000	0.157
Hip flexion moment min	Nm/kg	0.00 (0.05)	0.02 (0.04)	60.500	0.214
Knee flexion moment max	Nm/kg	0.00 (0.04)	0.00 (0.05)	72.000	0.519
Knee flexion moment min	Nm/kg	0.00 (0.02)	0.00 (0.02)	73.000	0.548
Ankle flexion power max	W/kg	0.13 (0.17)	0.02 (0.09)	32.000	**0.007**
Ankle flexion power max time	s	0.00 (0.01)	0.02 (0.04)	61.000	0.224
Ankle flexion power min	W/kg	-0.03 (0.09)	-0.03 (0.26)	72.500	0.537
Ankle flexion power min time	s	0.02 (0.06)	0.01 (0.07)	78.500	0.757
Hip flexion power max	W/kg	0.03 (0.15)	0.07 (0.08)	57.000	0.158
Knee flexion power max	W/kg	0.01 (0.15)	0.04 (0.18)	78.500	0.758
Kinematics					
Ankle flexion initial contact	°	-0.25 (1.49)	-0.24 (2.48)	82.000	0.898
Ankle flexion max stance	°	0.40 (1.64)	0.43 (0.29)	80.000	0.817
Ankle flexion min stance	°	-0.48 (2.84)	-1.23 (2.88)	70.000	0.457
Ankle flexion ROM[b] stance	°	0.88 (1.45)	1.76 (3.49)	65.000	0.317
Ankle flexion max swing	°	-0.52 (1.30)	0.21 (1.90)	61.000	0.228
Ankle flexion min swing	°	-1.18 (2.36)	-0.63 (2.89)	81.500	0.878
Ankle flexion ROM swing	°	0.42 (2.22)	1.07 (2.64)	72.000	0.522
Ankle flexion max	°	0.40 (1.47)	0.43 (0.29)	81.000	0.858
Ankle flexion max time	s	0.00 (0.06)	0.02 (0.03)	80.500	0.836
Ankle flexion min	°	-0.81 (3.15)	-0.59 (3.75)	82.000	0.898
Ankle flexion min time	s	-0.02 (0.10)	0.02 (0.03)	83.000	0.939
Foot progression	°	-1.73 (3.69)	-0.60 (4.48)	79.000	0.778
Knee flexion initial contact	°	-0.66 (2.04)	0.05 (0.61)	55.500	0.137
Knee flexion max	°	-0.12 (3.07)	0.68 (1.79)	66.000	0.343
Knee flexion max time	s	0.00 (0.08)	0.03 (0.04)	70.000	0.453
Knee flexion min	°	-0.83 (1.64)	0.11 (1.59)	64.000	0.293
Knee flexion min time	s	0.03 (0.11)	0.09 (0.19)	60.500	0.217
Knee flexion ROM	°	0.17 (3.51)	1.19 (2.32)	73.000	0.555
Hip flexion initial contact	°	-0.95 (1.57)	-0.13 (1.51)	55.000	0.130
Hip flexion max	°	-1.04 (2.73)	-0.47 (1.29)	62.000	0.249
Hip flexion max time	s	-0.10 (0.22)	-0.01 (0.08)	61.000	0.227
Hip flexion min	°	-1.65 (3.93)	-1.80 (1.03)	71.000	0.489
Hip flexion min time	s	0.00 (0.07)	0.03 (0.04)	71.000	0.486
Hip flexion ROM	°	-0.06 (2.73)	1.36 (1.09)	36.000	**0.013**
Hip Abduction-Adduction stance	°	-0.72 (0.79)	-0.07 (0.67)	46.000	**0.048**
Hip Abduction-Adduction swing	°	-1.09 (1.26)	-0.77 (0.89)	77.000	0.700
Hip Abduction-Adduction ROM	°	0.77 (0.89)	1.43 (1.25)	51.500	0.091
Hip rotation initial contact	°	-1.57 (2.94)	0.67 (1.61)	56.000	0.144
Hip rotation stance	°	-1.26 (1.90)	-0.13 (2.13)	61.000	0.228
Hip rotation swing	°	-0.14 (2.51)	0.18 (1.31)	71.000	0.489
Pelvic tilt initial contact	°	-1.11 (0.76)	-1.21 (1.08)	78.000	0.739
Pelvic tilt	°	-1.04 (1.39)	-1.16 (1.68)	73.000	0.555
Pelvic tilt ROM	°	0.22 (0.51)	0.37 (1.10)	72.000	0.522
Pelvic Obliquity	°	0.25 (1.01)	-0.13 (0.81)	61.000	0.228
Pelvic Obliquity ROM	°	0.51 (1.08)	0.75 (0.65)	60.500	0.218
Pelvic rotation	°	0.56 (2.60)	-0.23 (2.49)	84.000	0.980
Pelvic rotation ROM	°	0.65 (4.68)	0.78 (2.68)	73.000	0.555

[b]ROM = Range of Motion

Next, the adaptation rate of participants to the discrete perturbations was explored using a linear or an exponential function to fit the data derived for each variable on all the perturbed steps. At this stage of the present project, data regarding adaptation are available for 12 children with ASD and 11 TD participants. Mann-Whitney test showed a significant between-group difference ($p = 0.017$) when linear function was used for fitting, with TD group showing a greater adaptation compared to ASD group (median R^2 TD = 0.12; median R^2 ASD = 0.09). This results was confirmed also using an exponential function (Mann-Whitney test, $p = 0.007$), with TD children having a better adaptation in comparison to participants with ASD (median R^2 TD = 0.16; median R^2 ASD = 0.12).

Finally, the possible relationship between gait parameters, demographics of participants and autism symptomatology was investigated. A significant positive correlation was found between age and ankle maximum flexion moment ($\rho = +.664$, $p < 0.001$), and time to knee minimum flexion ($\rho = +.413$, $p < 0.05$). Furthermore, the absolute mean variation between T0 and T1 of range of hip motion negatively correlated with the T scores on Social Responsiveness Scale across participants ($\rho = -.411$, $p = 0.037$; Figure 1.4), and with ADOS comparison scores ($\rho = -.672$, $p = 0.012$; Figure 1.5).

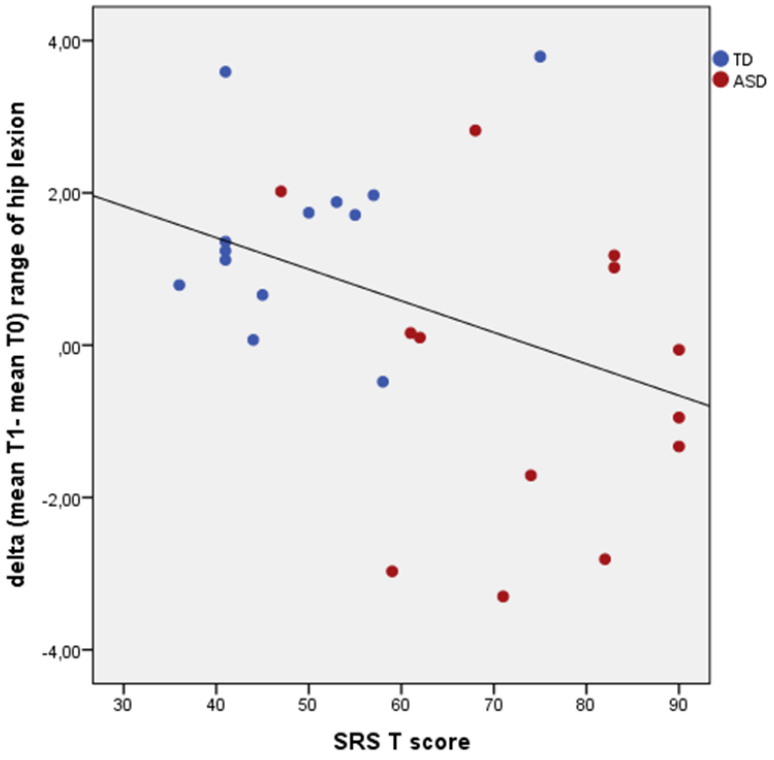

Fig 1.4. Plot showing the correlation between variation of range of hip motion between T0 and T1 and the T scores on Social Responsiveness Scale (ρ = -.411, p = 0.037).

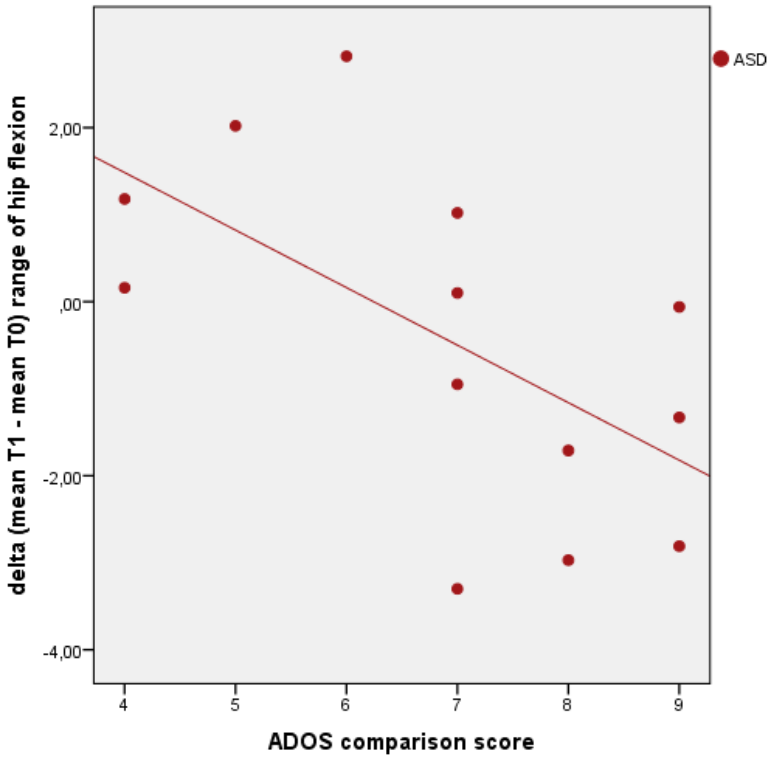

Fig 1.5. Plot showing the correlation between variation of range of hip motion between T0 and T1 and ADOS comparison scores in children with ASD (ρ = -.672, p = 0.012).

Discussion

The purpose of the present study was twofold. The first aim was to evaluate the gait pattern of drug naïve, school-aged children with ASD compared to a group of peers with typical development using the Gait Real-time Analysis Interactive Lab (GRAIL). To date, this was the first study that makes use in a sample with ASD of this innovative and motivating environment dedicated to gait analysis. Second, we intended to investigate across participants the motor adaptation to discrete gait perturbations administered with a single, unexpected acceleration of the belt under the dominant leg at toe-off. We hypothesized that children with ASD would present: (1) with respect to spatio-temporal parameters, reduced stride length and increased time in the stance phase; (2) with respect to kinematic parameters, reduced range of motion of joints. Further, we hypothesized that children with ASD would show a reduced or altered adaptation rate.

With regard to the first goal of the study, the present findings extended an earlier investigation of our group (Nobile et al., 2011), depicting an altered gait pattern in children with ASD with a wide range of atypical gait parameters, including spatio-temporal, kinetic, and kinematic features. In line with previous studies (Vilensky et al., 1981; Weiss et al., 2013), children with ASD spent more time in the stance phase of gait cycle compared to typical peers (68.1% vs. 66.9%). Further, in accordance with the study of Ambrosini and colleagues (1998), children with ASD showed reduced ground reaction forces (both moments and power) during terminal stance at ankle and knee. Finally, kinematic data suggested a global reduction of range of motion for the ankle, a delayed knee flexion, an atypical internal rotation of the hip coupled with an anterior displacement of pelvis at foot initial contact. As a whole, these data indicated a pathological gait pattern, where children with ASD tend to augment their own locomotion stability. We did not find in the present study a between-group difference in the step length, even though we observed a trend to significance with patients showing slightly shorter steps.

With regard to the second aim of this study, our results indicated a weak but significant adaptation to the perturbation in both groups. These findings were also confirmed by the slight

modifications of unperturbed gait pattern recorded after the discrete gait perturbations. TD children presented an augmented range of hip flex-extension as if they tended to respond to the perturbation with more flexible joint. On the other hand, after the perturbation children with ASD increased reaction force at ankle and hip adduction during stance phase of gait cycle. However, the direct between-group comparison showed that TD children had a better rate of adaptation compared to children with ASD. To the best of our knowledge, no studies have examined yet the walking adaptation in ASD by means of the type of discrete, single-leg perturbation used in the present study. Nevertheless, our findings relative to gait motor adaptation were in line with the observations deriving from upper limb motor learning and adaptation in ASD (Haswell et al., 2009; Izawa et al., 2012; Marko et al., 2015). These studies have consistently reported at least some degree of adaptation to the perturbation in children with ASD, although with slower rates or atypical pattern of adaptation. Moreover, the abnormal pattern of motor learning, characterized in children with ASD by an increased sensitivity to proprioceptive error and a decreased sensitivity to visual error, has been directly linked to anomalies in the cerebellum (Marko et al., 2015).

Finally, the present study also found a positive relationship between the increase of range of hip flex-extension after the perturbation trials and better social abilities participants, as measured by Social Responsiveness Scale, or lower ADOS scores in children with ASD. This relationship suggested that children who displayed a diminished learning adaptation also presented more severe autistic traits.

The current study underlined the usefulness of employing standardized measures (MABC2) to identify the presence of motor dysfunction. In line with several other studies (e.g. Grace et al., 2017), children with ASD showed motor impairments at MABC2, in particular with respect to aiming and catching abilities and balance. On the other hand, the present work also demonstrated that standardized measures can be limited in identifying motor impairments significantly related to the clinical symptomatology of the disorders. Indeed, the innovative multi-sensor platform utilized here for automatic analysis locomotion was able to recognize a parameter relative to walking adaptation

linked to the severity of autistic traits. The present results should also be regarded with some limitations in mind. First, this study was limited by its small sample size, although we recruited well-characterized, drug naïve children with ASD of school age and controls matched by gender, age, and intellectual functioning. A second limitation was that study was restricted to a group of children with high-functioning ASD, and the findings may not be directly generalizable to children with ASD and intellectual disability or to adult patients. Future investigations should also extend the present innovative instrumentation of gait analysis also to younger populations, so that an accurate longitudinal pattern of the locomotor trajectories in ASD can be identified. Last, although we do not consider appropriate to apply corrections to multiple comparisons because this study was exploratory, the present results require replication on a larger scale to verify the generalizability of the novel findings we documented.

In conclusion, using the Gait Real-time Analysis Interactive Lab (GRAIL), an innovative multi-sensor platform based on immersive virtual reality, we described a stiffer gait in children with ASD, with reduced ground forces and atypical joints posture. In addition, a limited walking adaptation to discrete perturbations was found in children with ASD. Reduced mobility of joints was directly related to poorer socio-communicative abilities and to the gravity of autistic symptomatology. The pattern of the present findings could be compatible with a possible involvement of cerebellum. Future research based on both neuroimaging and detailed kinematic characterization of gait could help to better understand the neural correlates of the motor system in ASD.

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CHAPTER 2:
USE OF MACHINE LEARNING TO IDENTIFY CHILDREN WITH AUTISM AND THEIR MOTOR ABNORMALITIES[2]

Aims of the chapter

- Autism spectrum disorder is currently diagnosed on the basis of symptoms as qualitatively judged by clinicians and by means of semistructured observations and standardized interviews or questionnaires.
- The use of pattern recognition methods to predict group membership has recently attracted strong attention, not only from a computer-aided diagnosis perspective, but also as suitable tool to define objective, quantitative measures of the disorder.
- The purpose of the present study was to explore the ability of the kinematic analysis of a simple upper-limb movement to correctly discriminate young low functioning children with ASD from typically developing children.
- To achieve this goal, we applied our validated supervised machine-learning procedure to the kinematic analysis of a simple reach, grasp, and drop task performed by preschool children with ASD in comparison to their mental-age-matched, typically developing peers.

[2] A slightly modified version of this chapter has been published as Crippa A, Salvatore C, Perego P, Forti S, Nobile M, Molteni M & Castiglioni I. (2015). Use of machine learning to identify children with autism and their motor abnormalities. *Journal of Autism and Developmental Disorders*; 45(7):2146-2156.

Introduction

Autism spectrum disorder (ASD) is a highly heterogeneous neurodevelopmental disorder with multiple causes, courses, and a wide range of symptoms severity (Amaral et al. 2008). The diagnostic criteria for ASD include behavioral descriptions of symptoms, which often overlap with the manifestations of many other psychopathologies. Currently, despite the fact that brain structural abnormalities have been consistently described in subjects with ASD (see, for example, Di Martino et al., 2014), the diagnostic procedure include the clinical judgment of behaviors by a qualified multi-disciplinary team personnel based on direct observation, parent-report information, and rating scales (Falkmer et al., 2013). Indeed, the "gold standard" for the assessment has been formalized with the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2002) ―a semi-structured, play-based behavioral observation―, and with the Autism Diagnostic Interview-Revised (ADI-R; Rutter et al., 1995) ―a standardized interview for caregivers―. These procedures are long term and heavily rely on experiences and practical knowledge of clinicians who administer the measurements. Furthermore, the diagnosis of ASD can be difficult because of the notable heterogeneity of ASD and due to the age-related changes of symptoms (Huerta & Lord, 2012).

Given these reasons, the diagnostic process of ASD is still challenging, and clinicians strongly demand more objective and reliable measures for diagnosing individuals with ASD. Recent studies have started to investigate the predictive value of neurobiological, as well as behavioral, measures in ASD in order to identify a well-defined phenotype of subjects and—possibly—to enable a computer-aided diagnosis perspective. These works typically make use of pattern classification methods that are based on machine-learning algorithms to predict or classify individuals of different groups by maximizing the distance between groups of datasets. Machine learning commonly refers to all procedures that train a computer algorithm to identify a complex pattern of data (i.e., "features") that can then be used to predict group membership of new subjects (e.g., patients vs. controls). Machine-learning techniques based, for

example, on support vector machines (SVMs; Vapnik, 1995) require a well-characterized dataset in the training phase in order to extract the classification algorithm that best separates the groups (i.e., the "hyperplane" or "decision function"). In the testing phase, the classification algorithm can be used to predict the class membership of a participant not involved in the training procedure (e.g., whether a new child has ASD). Pattern classification methods can also identify complex patterns of anomalies not efficiently recognized by other univariate statistical methods. Thus, the use of pattern recognition methods to predict group membership should not be considered simply in a potentially "diagnostic" perspective but, also, as an useful tool used to develop objective measures for each individual from a set of sample data. Most of the studies have applied pattern classification methods to neuroanatomical data measured by structural magnetic resonance (Ecker et al., 2010a; Ecker et al., 2010b) or by diffusion tensor imaging (Lange et al., 2010; Ingalhalikar et al., 2011; Deshpande et al., 2013), although Oller and colleagues' (2010) analysis of data regarding automated vocal analysis produced promising results.

In the present work, we have undertaken a proof-of-concept study to investigate whether a simple upper-limb movement could be useful to accurately classify low-functioning children with ASD who are between the ages of two to four. In order to answer this question, we developed a supervised machine-learning method to identify and correctly discriminate preschool children with ASD from typically developing children by means of kinematic analysis of a simple reach, grasp and drop task (Forti et al., 2011). We decide to analyze this simple motor task since the motor system can be more easily probed in low-functioning autistic children than systems that underlie complex cognitive functions (Minshew et al., 2004). Our validated supervised machine-learning method (Salvatore et al., 2013) involved two different steps: 1) feature selection, which was performed by using a Fisher discriminant ratio (FDR)-based technique (Padilla et al., 2012); and 2) classification, performed using support vector machines (SVMs) (Scholkopf & Smola, 2001; López et al., 2011). Hence, in addition to the potential predictive value of the SVM classification in investigating the clinical relevance of simple upper-limb movement

measures in ASD, the feature selection could identify a limited set of kinematic characteristics that even suggests the hypothesis of a motor signature of autism.

Methods

Participants

Fifteen preschool-aged children with autism (ASD) were compared to fifteen typically developing children (TD) who were matched by mental age. IQ and mental age were assessed by using the Griffiths Mental Development Scales (Griffiths, 1970) as a part of the routine clinical practice with low-functioning children. A poor score on the Griffiths scales at 1 and/or 2 years has been demonstrated to be a good predictor of impairment at school age (Barnett et al., 2004). All participants were Caucasian, had normal or corrected-to-normal vision, and were not taking any medication.

The participants in the ASD group were recruited at Child Psychopathology Unit of Scientific Institute, IRCCS Eugenio Medea (Lecco, Italy) over an 18-month period. All participants in the clinical group had been previously diagnosed according to the DSM-IV TR (American Psychiatry Association, 2000) at admission by a medical doctor specialized in child neuropsychiatry with expertise in autism. The diagnoses were then confirmed independently by a child psychologist through direct observation and discussion with each child's parents. The children's hospitalization lasted 15 days on average, and during this time a multidisciplinary team including child neuropsychiatrists, child psychologists, speech therapists, occupational therapists and special educators used a diagnostic and assessment protocol in accordance with guidelines suggested by the Child Neurology Society and by the American Academy of Neurology (2000). Seven children had been administered the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2002). The participants in the control group were recruited by local pediatricians and from kindergartens in the vicinity of our institute to be mentally age-matched to the clinical sample from the normally developing population. We decided to include, as a comparison group, typically

developing children matched by mental age, following the assumption that mental age usually predict ability to understand task instructions, use appropriate strategies and inhibit inappropriate responses (Jarrold & Brock, 2004). The TD children had no previous history of social/communicative disorders, developmental abnormalities, or medical disorders with central nervous system implications. The study was explained to both children and their parent(s) or caregivers, and all of the participants' legal guardians signed the informed written consent before the children's participation. The research received approval from the ethic committee of our institute and was therefore performed in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments.

Procedure

The participants sat in front of a table of variable height, which was adjusted to the base of the children's trunk. The experimenter sat at the opposite side of the table and one parent was present in the room. All trials started with the children's hands resting at a set position 20 cm away from the ball support. The experimental task consisted of grasping a rubber ball (6-cm diameter) that was placed over a support (see Figure 2.1, 1a); that is, a reach-to-grasp movement before they dropped it in a hole (7-cm diameter). The hole (see Figure 2.1, 1b) was located inside a see-through square box (21 cm high, 20 cm wide, see Figure 2.1) and was large enough not to require fine movements. Ten trials per participant were conducted: five consecutive trials on the left side (and left hand) and five consecutive trials on the right side (and right hand). The trial blocks order was counterbalanced between subjects. The experimenter performed the task first in order to overtly illustrate the task demand (i.e., reach for the ball, grasp it and drop it in the hole) without any verbal cue. Practice trials, the number of which varied individually, were given to participants before recording in order to verify the children's understanding of the task. The participants were allowed to interrupt the experiment at will in order to rest. The experimental task was simple and interesting enough to ensure the full motivation and compliance of all participants across groups.

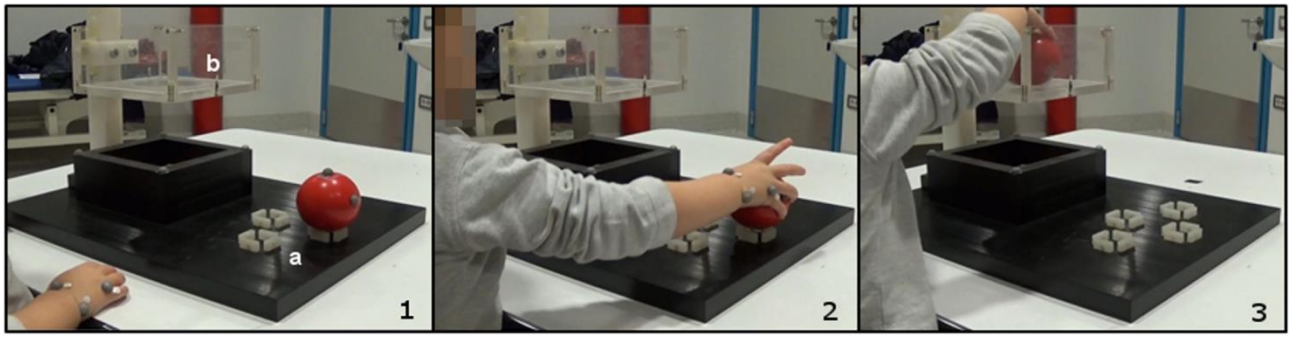

Fig 2.1. Experimental setting. The ball is initially positioned on the support (A). The child has to reach the ball, grasp it and do a second reach to throw the ball in the goal area (B) and through the hole (C). The goal area is transparent to allow seeing through. 4 markers (black points) are placed on the basket under the goal area, 2 on the ball and 3 on each hand (attached to the ulnar and radial surfaces of the participant's wrist and to the hand dorsum on the 4th and 5th metacarpals).

Apparatus

An optoelectronic system (The SMART D from BTS Bioengineering® Garbagnate Milanese, Italy) was used to acquire the kinematics data. 3D kinematic data were collected by eight infrared-motion analysis cameras at 60 Hz (spatial accuracy less than 0.2 mm), located four per side at 2.5 m from the participants. Passive markers (1 cm) were attached to the ulnar and radial surfaces of the participant's wrists and to the hand dorsum on the fourth and fifth metacarpals (see Figure 1). Moreover, two markers were placed on the ball and four on the box edges under the goal area. All raw data were firstly pre-processed with Matlab (Mathworks® Natick, MA, USA): a fifth order Butterworth, 8-Hz low-pass filter was applied and movement segmentation and parameters estimation were computed with self-written software.

The overall movement was divided into two sub-movements: *Sub-movement 1*—the movement necessary to reach the ball and place it on its support; *Sub-movement 2*— the movement to transport the

ball from its support to the target box hole where the ball was to be dropped. For each of these sub-movements, statistics that pertain to a set of dependent measures was collected: (a) total movement duration (TD); (b) number of movement units[3] (MU); (c) peak velocity (PV); (d) time of PV from sub-movement onset (tPV); (e) peak acceleration (PA); (f) time of PA (tPA); (g) peak deceleration (PD); and (h) time of peak deceleration (tPD). Moreover, final movement accuracy was evaluated by the wrist inclination at the time of the ball drop (delta_WA), calculated as the angle between the palm and the vertical axis of the coordinate system (more precisely, the difference between the wrist angle at the end of the transport phase and the wrist angle at the time of peak deceleration). These 17 kinematic measures were used as input features for the pattern classification procedure.

Data Analysis

After checking that the assumptions were not violated, an analysis of covariance (ANCOVA) was carried out to compare the two groups of children on all kinematic measures with Group (ASD vs. TD) as a between-participant factor, and with IQ and chronological age as between-participant covariates. The alpha level was set to .05 for all data analyses. Effect sizes for ANCOVA are reported using partial eta squared (η_p^2).

The Machine-Learning Method

A pattern classification method based on a machine-learning algorithm was used to classify ASD versus TD by maximizing the distance between the two groups of datasets. A validated supervised machine-learning method (Salvatore et al., 2013) was used. The method involves two different steps: 1) feature selection, the process of selecting a subset of relevant features to be used for classification, and 2) classification, the process of using the selected features to separate the two considered groups of subjects (ASD versus TD).

[3] Movement units are defined as an acceleration phase followed by a deceleration phase higher than 10mm/s, starting from the moment at which the increase or decrease in cumulative velocity is over 20mm/s (Von Hofsten, 1991; Thelen, Corbetta, & Spencer, 1996).

Feature Selection

In order to understand which of the collected kinematic features were more discriminative for the ASD versus TD comparison, feature selection was implemented by using a Fisher discriminant ratio (FDR)-based technique (Padilla et al., 2012).

By this technique, for each subject, the collected features[4] and the "label" associated to that subject on a clinical diagnosis basis (*i.e.*, ASD or TD) were considered to calculate a score (FDR score) for each feature.

Specifically, for the feature *i*, the FDR score was calculated using the following formula:

$$FDR_i = \frac{(\mu_{i-ASD} - \mu_{i-TD})^2}{\sigma^2_{i-ASD} + \sigma^2_{i-TD}}$$

Where μ_{i-ASD} and μ_{i-TD} are the mean value of the feature *i* calculated across the whole ASD and TD datasets, respectively. σ^2_{i-ASD} and σ^2_{i-TD} are the variance of the feature *i* calculated across the whole ASD and TD datasets, respectively.

Ranked features were then sorted in a decreasing order, from the most to the least discriminative, according to their FDR score.

Classification algorithm

Classification of ASD and TD subjects was performed using a Support Vector Machine (SVM) approach (Scholkopf et al., 2002; Vapkin, 1995; Vapkin, 1998; Vapkin, 1999, López et al., 2011), already optimized and validated in a clinical setting (Salvatore et al., 2013). SVM are the most widely used classification technique. They are computationally efficient and provide reliable predictions even for high-dimensional data (Debruyne, 2009). A comparison between classification techniques showed that SVM frequently resulted in the best performing group of classifiers when applied to different tasks or

[4] In the present work, the features used for the pattern classification procedure correspond to the 17 kinematic measures collected as dependent measures.

datasets (Van Gestel et al., 2004). Finally, a last advantage SVM has over other data mining techniques is that it provides the best results in classification in case of a linear distribution of data and for binary classification (e.g., patients vs. controls), such as in the present work. In the last years, another traditional machine learning technique, Deep Learning, has attracted considerable attention in biomedical image analysis. Deep learning is emerging as a leading tool in particular in computer vision where it performs better than other "classical" methods (Razavian et al., 2014). However, Deep learning requires a large amount of unlabeled training data (in the region of thousands for each class) to make concise conclusions, whereas SVM can use small datasets provided by the users. This last attribute limited, as yet, the application of deep learning to the field of medicine.

The aim of the considered SVM is to generate a model able to (1) learn from the selected features of labeled subjects how to discriminate subjects of different groups (binary labeled training datasets, ASD and TD), and (2) correctly classify, by means of the same selected features, new unlabeled subjects, left out from the training phase, as belonging to one of the two groups (ASD or TD).

The learning process of the classifier consists of a training phase in which the selected features of the ASD and TD subjects are two training datasets (consisting of the 17 kinematic variables) associated to the ASD and TD labels, respectively.

Mathematically, if we have training data consisting of a vector $x_i \in R^N, i = 1, \dots N$ and the associated binary label $y_i \in \{\pm 1\}$ (e.g. +1 for ASD, -1 for TD), then SVM uses the principle of structural risk minimization to design an optimal hyperplane (OH) that maximizes the distance between the two training groups and that separates them. The lower the distance of a training subject from the OH, the more important that training subject to define the OH. Thus, the distance identifies the "weight" of that training subject in the definition of OH.

The OH can then be used as model to classify new subjects, *i.e.*, subjects for which the label is unknown.

Mathematically, the model used for the identification of the binary label y' of a new subject x, as a result of the classification of that new subject, is given by the following function:

$$y'(x) = \sum_{i=1}^{N} a_i \cdot y_i \cdot k(x, x_i) + b$$

a_i being the weight of the training subject x_i, y_i being the binary label of the training subject i, $k(x, x_i)$ being a linear kernel function, b being a threshold parameter called bias, and *N* being the number of training subjects. We chose to employ a linear kernel because it represents the more general form of a decision function and because it ensures better computational efficiency.

In this study, the whole machine-learning method was implemented on the Matlab platform (Matlab version R2013b, The MathWorks, Natick, MA). In particular, we used functions of the biolearning toolbox of Matlab to implement the classification algorithm.

Performance of the classification algorithm

Performance of the classification algorithm was assessed by using a cross-validation strategy. In general, cross validation involves splitting the original dataset into two complementary subsets: a training set and a testing set. The training set is a set of data associated to a label and used to perform the training of the classifier (as already described in the previous section); the testing set is a set of data not associated to a label and used to perform the validation of the classifier. By considering different partitions of the data, multiple rounds of cross-validation can then be performed.

In a particular case of cross-validation, called leave-one-out (LOO) cross-validation, the testing set is solely composed of one sample of the original dataset and the training set is made up of the remaining samples of the original dataset (*N–1*). Therefore, if we want to test all *N* samples in the original dataset, then it is sufficient that the number of rounds to be performed equals the number N of samples in the original dataset. LOO is a widely used validation approach in literature because it has been proven

able to return an almost unbiased estimate of the probability of error (e.g., Vapkin, 1998; Chapelle et al., 1999).

In this study, validation of the classifier for the ASD versus TD comparison was performed by using an LOO cross-validation strategy for a number *i* of selected features running from one to the whole number of features (*i.e.*, 17). A schematic description of the whole procedure is shown in Figure 2.2.

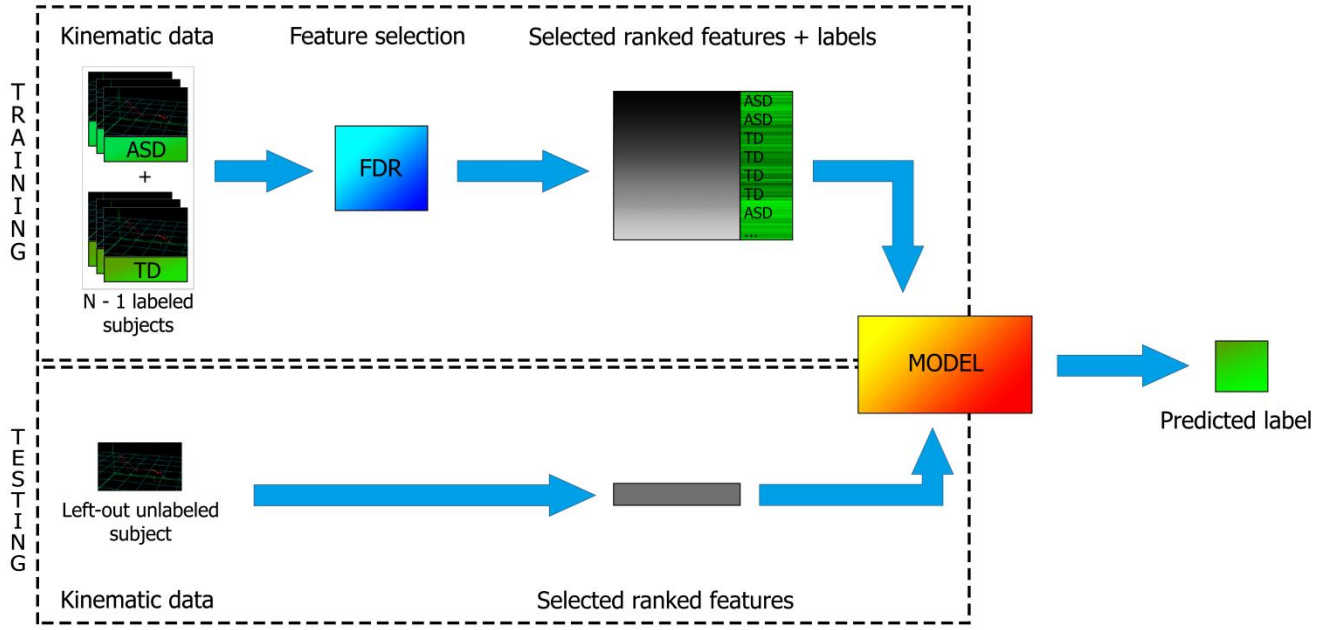

Fig. 2.2. Flowchart of preprocessing, support vector regression and leave-onesubject-out procedures.

In order to quantify the performance of the proposed classification algorithm, the accuracy, specificity, and sensitivity rates were computed. Accuracy of classification measures the rate of correctly classified samples in both positive (ASD) and negative (TD) classes. Specificity and sensitivity measure the rate of correctly classified samples in the positive (ASD) and in the negative (TD) class, respectively.

Mathematically, the accuracy, specificity and sensitivity of the classifier when the first *i* selected features are used, were computed as follows:

$$Accuracy_i = \frac{N^{CC}}{N}$$

$$Specificity_i = \frac{N_{TD}^{CC}}{N_{TD}^{CC} + N_{TD}^{IC}}$$

$$Sensitivity_i = \frac{N_{ASD}^{CC}}{N_{ASD}^{CC} + N_{ASD}^{IC}}$$

where N is the total number of classified subjects; N^{CC} is the total number of correctly classified (CC) subjects, N_{TD}^{CC} is the number of TD samples that were CC as belonging to the TD gr (true negatives), N_{TD}^{IC} is the number of TD samples that were incorrectly classified (IC) as belonging to the ASD class (false positives); N_{ASD}^{CC} is the number of ASD samples that were CC as belonging to the ASD class (true positives), N_{ASD}^{IC} is the number of ASD samples that were IC as belonging to the TD class (false negatives).

We then studied the dependency of accuracy, specificity, and sensitivity on the number i of selected features.

The maximum values reached for accuracy, specificity, and sensitivity, referred to as maximum accuracy, specificity, and sensitivity, allowed the definition of the most discriminative features.

Overall mean accuracy, specificity, and sensitivity rates were calculated as mean values of accuracy, specificity, and sensitivity as follows:

$$Overall\ Mean\ Accuracy = \frac{1}{F} \cdot \sum_{i=1}^{F} Accuracy_i$$

$$Overall\ Mean\ Specificity = \frac{1}{F} \cdot \sum_{i=1}^{F} Specificity_i$$

$$Overall\ Mean\ Sensitivity = \frac{1}{F} \cdot \sum_{i=1}^{F} Sensitivity_i$$

where F is the whole number of features (17).

Results

Data on the demographical, cognitive, and clinical characteristics of the participants are summarized in Table 2.1.

Table 2.1. Demographics of the participants

	ASD	**TD**	**t (1, 28)**	***p***
N	15	15		
Females : Males	3:12	2:13		
Chronological Age[a]	3;5 ± 7,7	2;6 ± 5,2	-4.55	< .001
	(2;8 - 4;6)	(1;7 - 2;9)		
Mental Age[a]	2;6 ± 5,7	2,7 ± 5,9	.513	n.s.
	(1;7 - 3;4)	(1;6 - 3;2)		
IQ[b]	75 ± 13,4	105 ± 12,7	6.52	< .001
	(51 - 96)	(81 - 119)		
ADOS[c]				
Social	11 ± 2,2	–		
Communication	7 ± 1,5	–		
SBRI[d]	2 ± 1,6	–		

ASD = autism group; TD = typically developing group; IQ and mental age were assessed using the Griffiths Mental Development Scales (Griffiths, 1970). [a] Mean years; months ± standard deviation (range); [b] Mean ± standard deviation (range); [c] ADOS autism diagnostic observation schedule, Lord et al. (2000); [d] Stereotyped Behavior and Restricted Interests scale.

The validity of mental age matching was confirmed ($p > 0.05$). Gender was also balanced between groups, as there were 3 girls in ASD group and 2 girls in healthy control group ($\chi 2(1) = .240$; $p > 0.05$). As expected, IQ and chronological age were not balanced across groups (both $p < 0.001$). Table 2.2 shows kinematics features values of two groups of children included in the study (ASD vs. TD) and the

results of ANCOVA calculated on all kinematic measures. We found several significant group differences based on the kinematic variables even after having controlled for between-participant differences in IQ and chronological age.

Table 2.2. Kinematic data were initially analyzed through an ANCOVA with Group (ASD vs. TD) as a between-participant factor, and with IQ and chronological age as covariates. The alpha level was set to .05 for all data analyses. Table depicts group means and standard deviations for kinematic variables, values of F test, p values and effect sizes reported using partial eta squared (η_p^2).

Submovement 1		**ASD**	**TD**	**F (1,26)**	**Sig.**	**η_p^2**
Movement Units	M (SD)	1,91 (0,62)	1,70 (0,37)	<1,0	n.s.	,012
Total Movement Duration	M (SD)	0,69 (0,14)	0,66 (0,12)	<1,0	n.s.	,010
Peak Velocity	M (SD)	0,46 (0,12)	0,59 (0,17)	5,626	**<0,05**	,178
Time of Peak Velocity	M (SD)	0,34 (0,07)	0,31 (0,04)	<1,0	n.s.	,036
Peak Acceleration	M (SD)	3,18 (0,93)	4,26 (1,52)	7,884	**<0,01**	,233
Time of Peak Acceleration	M (SD)	0,21 (0,07)	0,16 (0,05)	<1,0	n.s.	,031
Peak Deceleration	M (SD)	-3,59 (1,28)	-3,93 (1,44)	<1,0	n.s.	,067
Time of Peak Deceleration	M (SD)	0,47 (0,08)	0,44 (0,06)	<1,0	n.s.	,017
Submovement 2						
Movement Units	M (SD)	3,45 (1,78)	1,76 (0,39)	4,408	**<0.05**	,145
Total Movement Duration	M (SD)	1,35 (0,44)	0,79 (0,15)	13,832	**=0,001**	,347
Peak Velocity	M (SD)	0,61 (0,15)	0,76 (0,16)	13,475	**=0,001**	,341
Time of Peak Velocity	M (SD)	0,41 (0,14)	0,31 (0,05)	18,501	**<0,001**	,416
Peak Acceleration	M (SD)	3,85 (1,13)	5,58 (1,94)	12,416	**<0,01**	,323
Time of Peak Acceleration	M (SD)	0,23 (0,20)	0,13 (0,04)	6,303	**<0.05**	,195
Peak Deceleration	M (SD)	-3,29 (1,15)	-4,27 (1,88)	2,632	n.s.	,092
Time of Peak Deceleration	M (SD)	0,75 (0,24)	0,51 (0,11)	26,652	**<0,001**	,506
Wrist Angle	M (SD)	-4,25 (16,34)	-25 (12,40)	6,604	**<0.05**	,203

The Machine-learning Method

Classification algorithm. In Figure 2.3, the optimal hyper-plane separating ASD from TD participants is shown as representative example of the training phase of the machine-learning method.

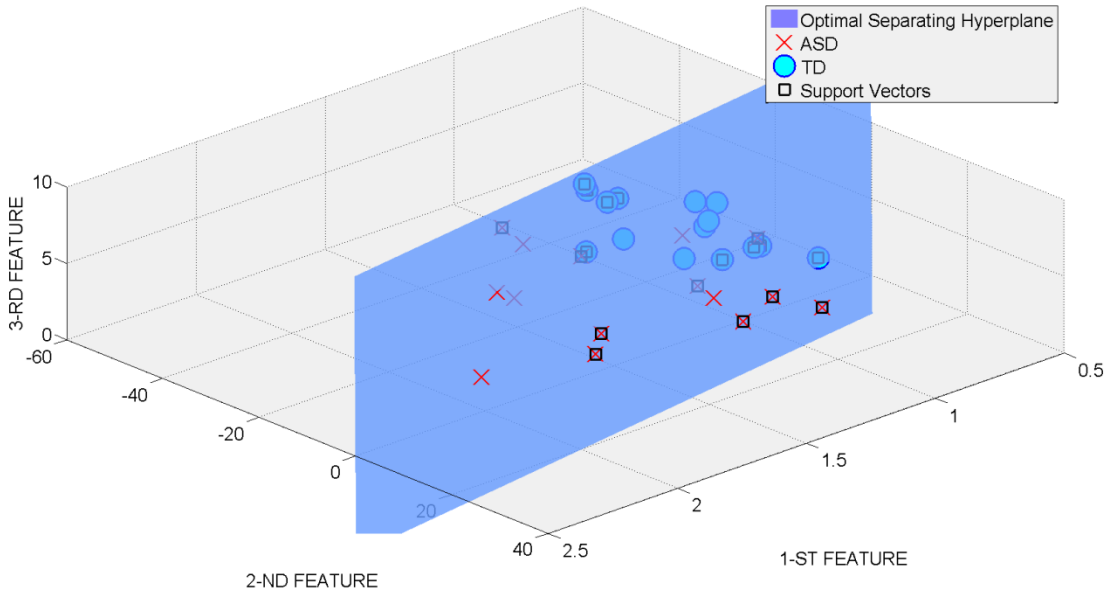

Fig 2.3. Optimal separating hyper-plane for the Autism group (ASD) versus typically developing groups (TD) (1st, 2nd and 3rd components) is shown as a representative example of the training phase of the machine-learning method.

Performance of the Classification Algorithm

In Table 2.3, the accuracy, specificity, and sensitivity of the machine-learning method for the ASD versus TD comparison are reported.

Table 2.3. Accuracy, Specificity and Sensitivity rates of SVM using LOO validation.

	Maximum Accuracy (%) **(# selected features)**	**Maximum Specificity (%)** **(# selected features)**	**Maximum Sensitivity (%)** **(# selected features)**
	Overall Mean Accuracy (%)	**Overall Mean Specificity (%)**	**Overall Mean Sensitivity (%)**
ASD vs. TD	96.7 (7)	93.8 (7)	100.0 (7)
	84.9	89.1	82.2

ASD = autism group; TD = typically developing group. The maximum values reached by Accuracy, Specificity and Sensitivity were referred to as Maximum Accuracy, Specificity and Sensitivity rates. Accuracy, Specificity and Sensitivity reached their maximum values using 7 features, all related to the second part of the movement -Sub movement 2-: (1) Total Duration; (2) delta Wrist Angle; (3) number of Movement Units; (4) time of Peak Deceleration; (5) Peak Acceleration; (6) time of Peak Velocity; (7) Peak Velocity.

The machine-learning method was able to successfully classify participants by diagnosis. The classification accuracy reached a maximum accuracy of 96.7% (specificity 93.8% and sensitivity 100%) by using seven features selected by Fisher discriminant ratio-based technique. Overall mean accuracy, specificity, and sensitivity rates were also calculated over a number of selected features ranging from one to 17 (the whole number of features). The overall mean classification accuracy (specificity/sensitivity) was 84.9% (mean specificity 89.1% and mean sensitivity 82.2%).

In Figure 2.4, the dependence of the metrics on the number of considered features is shown. The resulting data are shown for a number of features ranging from one to 17. As expected, accuracy, specificity, and sensitivity rates increase with the number of selected features, reaching their maximum values when considering seven selected features.

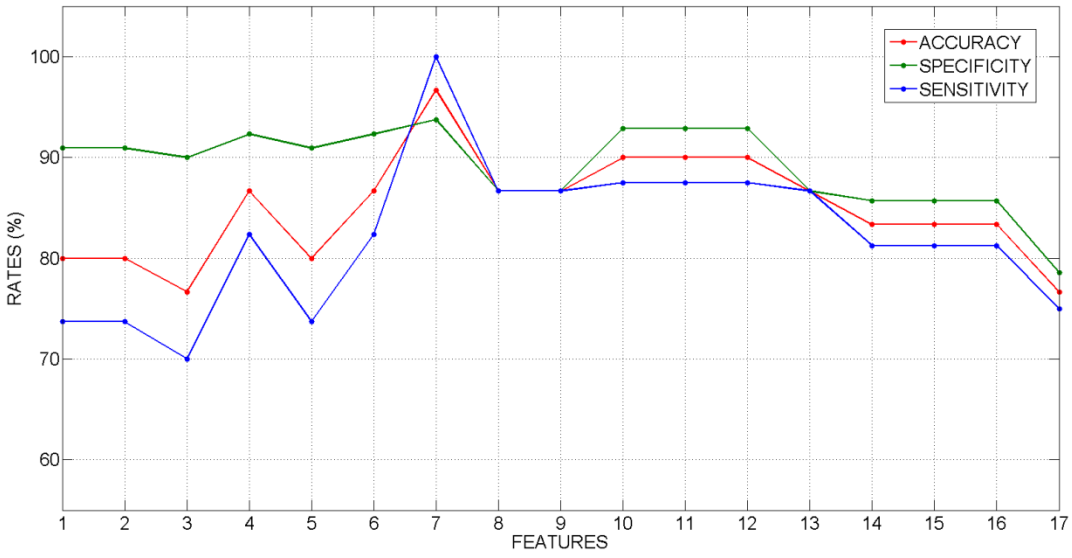

Fig 2.4. Graph showing classification Accuracy, Specificity and Sensitivity rates (%) of SVM (Y-axis) in relation of the number of considered features (X-axis). As expected, Accuracy, Specificity and Sensitivity rates increased with the number of selected features. The classification accuracy reached a Maximum Accuracy of 96.7% (Specificity 93.8%, and Sensitivity 100%) utilizing seven features. All of these seven kinematic features are related to the second part of the movement—sub-movement 2—the movement to transport the ball from a support to the target hole in which the ball was to be dropped. Such suggests that goal-oriented movements may be critical in separating children with ASD from typically developing children.

Besides calculating the accuracy of the SVM method, we were particularly interested in identifying which kinematic features contributed toward the classification. Our analysis showed that seven of 17 features were sufficient to classify autism with a 96.7% accuracy rate. All of these seven kinematic features are related to the second part of the movement, *sub-movement 2* (i.e., the movement to transport the ball from a support to the target hole in which the ball was to be dropped): (1) total duration; (2) delta wrist angle; (3) number of movement units; (4) time of peak deceleration; (5) peak acceleration; (6) time of peak velocity; and (7) peak velocity. Finally, the most discriminative features between the two groups when considering all of the N rounds (30) of the LOO cross-validation strategy

are reported here in descending order: Total Duration sub movement 2, Delta Wrist Angle, Movement Units sub movement 2, time of Peak Deceleration sub movement 2, Peak Acceleration sub movement 2, time of Peak Velocity sub movement 2, Peak Velocity sub movement 2, Peak Velocity sub movement 1, time of Peak Acceleration sub movement 1, Peak Acceleration sub movement 1, time of Peak Acceleration sub movement 2, Peak Deceleration sub movement 2, time of Peak Velocity sub movement 1, Movement Units sub movement 1, time of Peak Deceleration sub movement 1, Peak Deceleration sub movement 1, Total Duration sub movement 1.

Discussion

Autism spectrum disorder is currently diagnosed by a qualified multi-disciplinary clinical team and by means of semi-structured observations (ADOS) and standardized interviews for caregivers (ADI-R). Given that these gold standards for the diagnosis of ASD are qualitative measures of symptoms, the use of machine learning methods to make predictions about the group membership of new individuals (e.g., patients vs. controls) has recently attracted strong attention, not only as a potential "diagnostic" tool, but also as efficient instrument to describe objective, quantitative measures of the disorder. Since previous studies have explored the predictive value of neurobiological and behavioral measures in patients with ASD, the aim of the present study was to investigate the efficiency of the kinematic analysis of a simple upper-limb movement to correctly differentiate young low-functioning children with ASD from peers with typical development. For this purpose, we applied our validated supervised machine-learning procedure (Salvatore et al., 2013) to the kinematic analysis of a reach, grasp, and drop task performed by preschool children with ASD in comparison to their mental-age-matched, typically developing peers.

The SVM algorithm attained a good mean individual accuracy in discriminating children with ASD from healthy controls (overall mean accuracy = 84.9%, with overall mean specificity = 89.1% and

overall mean sensitivity = 82.2%), with a maximum accuracy of 96.7% (with maximum specificity of 93.8% and maximum sensitivity of 100%). The classification accuracy achieved in this work is consistent with previous SVM applications to structural magnetic resonance imaging data (Ecker et al., 2010a; Ecker et al., 2010b) and to diffusion tensor imaging data (Ingalhalikar et al., 2011; Deshpande et al., 2013) or with quadratic discriminant function application on diffusion tensor asymmetries (Lange et al., 2010). Furthermore, the accuracy of the present algorithm is also in line with the results of Oller and colleagues (2010), who derived algorithms that were based on linear discriminant analysis by using an automated analysis of the acoustic characteristics of babble and early language to discriminate typical from language disordered development, such as autism or language delay. Thus, the feasibility and the applicability of our SVM method in correctly classifying preschool children with ASD on the basis of a motor task is evidently shown by the present findings. Taking into account that the diagnosis of ASD, even using the gold standard diagnostic procedure, is innately difficult in young, low-functioning children with autism, our motor measure might have potential clinical application in such cases, thus providing useful information for clinicians to support a diagnostic decision. Indeed, because of the easiness and self-explanatory nature of the task, all participants were able to fully understand the experimental demand and to complete the movement successfully. Therefore, a point of relevance of the present work, is that we decided to study the predictive value of a simple reach, grasp, and drop task, because the motor system can be more easily assessed (i.e., even in young low-functioning children with ASD) than other more complex systems (e.g., cognitive functions). Furthermore, kinematics acquisition needs a constraint-free, non-intrusive environment for a challenging clinical population such as ASD, in comparison with a magnetic resonance examination that is mostly used in previous pattern-recognition applications. Lastly, kinematic analysis is also a more convenient and less expensive technology than MRI, requiring a clinical setting equipped with an optoelectronic system to acquire kinematic data. The

task can be easily administered by any professional who works with children: testing sessions last 15 min, and data analysis can be performed by a trained operator in approximately 30 min for each subject.

By means of feature selection, we found the best classification accuracy of 96.7% identifying seven features which had the highest discriminative proficiency between the groups. These seven kinematic features are all related to the second part of the movement —sub-movement 2— in which the participant transported the ball from a support to the target hole where the ball was to be dropped. This finding suggests that goal-oriented movements may be crucial in distinguishing children with ASD from typically developing children. In particular, the top three features within the seven kinematic characteristics of sub-movement 2—time duration, movement units, and wrist angle—depict in children with ASD slower and more fragmented movements with improper hand inclination for ball-drops during the last phase of sub-movement 2. Thus, these findings support and extend previous investigations in ASD that highlight the difficulty of converting intention into a motor chain leading to the action goal (Cattaneo et al., 2007; Fabbri-Destro et al., 2009; Forti et al., 2011), and they demonstrate that a limited set of kinematic characteristics could reliably identify children with ASD describing a well-defined phenotype of subjects within a complex and highly heterogeneous disorder. Therefore, these results suggest the existence of a possible motor signature of autism related to disrupted planning movement sequences.

Although our results seem promising, some methodological caveats should be considered. The main limitation concerns the small sample sizes of participant groups; therefore, it is necessary to replicate the present findings in a larger sample in order to validate the present SVM method by using a data set upon which it has not trained. Another limitation of this study is that our SVM classification is highly specific to the sample implicated in training the classifier (i.e., preschool children with ASD). To generalize our findings to the heterogeneous spectrum of the disorder, future studies involving females with ASD, children with high-functioning autism, and adult patients are needed. Although our findings

suggest that significant between-groups differences were not dependent on IQ and chronological age, it could be worth training the computer algorithm also with data from age-matched typically developing participants and, most importantly, with a control group of participants with intellectual disabilities but not ASD. Unfortunately, it has not been possible to collect ADOS scores from the entire clinical sample; for this reason we could not perform a correlation analysis between kinematic data and the clinical characteristics of children with ASD. Moreover, further extensions of this study should also consider other neurodevelopmental conditions (e.g., intellectual disability without ASD, developmental delays without intellectual disability, or developmental coordination disorders) to verify if the classifier is specific to ASD, or if it identifies a neurodevelopmental disorder in general. On this purpose, some works have recently indicated the specificity of motor difficulties in older high-functioning children with ASD compared to children with ADHD (Izawa et al., 2012; Ament et al., 2014) and to healthy children matched by nonverbal IQ and receptive language (Whyatt & Craig, 2013). Finally, it should be noted that the predictive values of classification methods are conditioned by the percentage of neurodevelopmental disorder in the population (Bishop, 2010; Heneghan, 2010; Yerys & Pennington, 2011). Therefore, caution is needed when comparing classification-based accuracy values to the accuracy values of traditional diagnostic measures.

In conclusion, this study represents a "proof-of-concept" that kinematic analysis of simple upper-limb movement can reliably identify preschool-aged, low-functioning children with ASD. The significant predictive value of our SVM classification approach might be valuable to enhance the clinical practice of diagnosing ASD, even fostering a computer-aided diagnosis perspective. Moreover, our findings offer insight on a possible motor signature of ASD that is potentially helpful to identify a well-defined subset of patients, thus decreasing the heterogeneity within the broad behavioral phenotype; this may guide further exploration of neuropathology of the disorder with neuroimaging techniques or genetic analysis.

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CHAPTER 3:
EXAMINATION OF ANTERIOR-POSTERIOR CONNECTIVITY IN CHILDREN WITH AUTISM SPECTRUM DISORDER[5]

Aims of the chapter

- Previous studies have provided evidence of underconnectivity in ASD, with particular respect to reduced long-range connectivity between frontal lobes and posterior brain regions.
- This reduction in brain connectivity may contribute to motor abnormalities in children with ASD, including the above-described atypicalities in gait and reaching, and might provide useful insight into the mechanisms of social communication.
- The present study aimed to investigate whether a well-characterized group of high functioning children with ASD would demonstrate reduced white matter integrity in inferior fronto-occipital fasciculus, inferior and superior longitudinal fasciculus.
- Further, we aimed to explore the relationship between potential differences in diffusion properties and core features of ASD, as well as measures of motor function and praxis.

[5] The study presented in this chapter was carried out at Center for Neurodevelopmental and Imaging Research, Kennedy Krieger Institute (Baltimore, MD, USA), in collaboration with Deana Crocetti, Kathryn Hirabayashi, and Dr. Stewart Mostofsky. A previous version of the present study has been presented as a poster at the 2017 International Meeting for Autism Research (IMFAR), San Francisco, USA, and at the 2017 Meeting of Organization for Human Brain Mapping (OHBM), Vancouver, Canada.

Introduction

Despite the fact that high heritability estimates indicate a crucial role for genetic factors in Autism Spectrum Disorder (ASD) (Jeste & Geschwind, 2014), the etiology of this complex condition is generally considered multifactorial. It has been suggested that the heterogeneity of the ASD clinical manifestations could more likely implicate alterations in the connectivity between different neural networks rather than abnormalities in a specific cerebral area (Just et al., 2012; Kana et al., 2014; Maximo et al., 2014; Vasa et al., 2016). Brain network connectivity can be assessed *in vivo* using different neuroimaging techniques, such as diffusion tensor imaging, functional magnetic resonance imaging, and resting-state imaging. Among these, diffusion tensor imaging (DTI) has been found to effectively measure interregional structural connectivity (Basser & Pierpaoli, 1996). This non-invasive technique provides indirect quantitative measures of white matter integrity, such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), by measuring water diffusion in the underlying tissue microstructure (Pierpaoli & Basser, 1996). Mean diffusivity is the average of the diffusion in the different directions of the space, and its values are related to the presence of barriers or obstacles, like cellular membranes and axons, which can interfere with the free water displacement within a voxel. When diffusion of water molecules is not the same along the three axes of the space (as in axons), it is called anisotropic, which means it has a preferential direction of displacement. AD and RD measure the entity of displacement along the principal and its perpendicular axis. FA values, which range between 0 and 1, are also a measure of anisotropy that seem to be related with myelination, axon diameter, and fiber coherence (Beaulieu, 2002). High FA values denote well organized and normally myelinated axons that provide natural barriers to water movement within tissue. Lower

FA values, in contrast, may reflect axonal loss and/or demyelination (Beaulieu, 2002) as well as areas of crossing fibers. Thus, DTI enables reconstruction of structural connections.

In subject with ASD, previous DTI studies have described underconnectivity in ASD with particular respect to the long-range connectivity of frontal-posterior networks (see, for reviews, Travers et al. 2012, Vissers et al. 2012, and Ameis & Catani, 2015; for meta-analysis, Aoki et al., 2013). More precisely, evidence from DTI studies highlighted reduced white matter integrity of the long-distance tracts of the brain network in children with ASD, as indicated by reduced FA and increased MD. However, these findings have not been always consistent across studies because of variability in the inclusion criteria, limited sample sizes, and broad age ranges (see Travers et al., 2012). Furthermore, it is still not clear which of the major seven long-distance tracts ─namely, uncinate fasciculus, cingulum, superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior frontal occipital fasciculus, fornix, and corpus callosum─ is anomalous in ASD.

The present study aimed to investigate whether a well-characterized group of high-functioning children with ASD would demonstrate reduced white matter integrity, denoted by reduced fractional anisotropy (FA) and increased mean, axial or radial diffusivity (MD, AD, RD), in three long-distance frontal-posterior tracts: inferior fronto-occipital fasciculus (IFOF), inferior and superior longitudinal fasciculus (ILF and SLF, respectively). To achieve this goal, we used Automated Fiber Quantification (AFQ), a relatively novel tool that measures FA, MD, RD, and AD along the trajectories of major white matter tracts of the brain network (Yeatman et al. 2012). This technique allows to quantifies diffusion properties at multiple locations along a given fiber tract instead of providing a single mean value for the entire bundle; in doing so, AFQ creates a ‘‘Tract Profile’’ of diffusion measurements. It has been demonstrated that AFQ can generate tract

profiles consistent across subjects and concurrently compute the actual white matter bundles within the single participant's brain (Yeatman et al. 2012). We expected children with ASD to have reduced white matter integrity, denoted by reduced fractional anisotropy (FA) and increased mean diffusivity (MD) (see Travers et al., 2012, and Aoki et al., 2014, for review and meta-analysis) along these tracts. On the basis of established findings for impairments in language and praxis in ASD, we further hypothesized that the findings would be more prominent in the left hemisphere. Last, we aimed to explore the relationship between potential differences in diffusion properties and core features of ASD, as well as measures of motor function and praxis. Exploring the hypothesis of an anterior-posterior underconnectivity in ASD might provide useful insight into the mechanisms of social communication, given the key role of these pathways for language (see, for example, Just et al., 2004; Catani & Bambini, 2014), praxis, imitation, and basic motor coordination (see, for example, Villalobos et al., 2005; Mostofsky et al., 2009; Nebel et al., 2016).

Methods

Participants

One hundred four children, ages 8 through 12 years, participated in the study: fifty-two children with ASD (ASD; age 10.3 ± 1.4; 41 males, 11 females) and fifty-two typically developing children (TD; age 10.5 ± 1.2; 42 males, 10 females). All participants with ASD fulfilled DSM-5 (American Psychiatric Association, 2013) criteria for Autism Spectrum Disorder. Diagnoses were confirmed using the Autism Diagnostic Interview-Revised (Lord, Rutter, & Le Couteur, 1994) and Autism Diagnostic Observation Schedule-Generic (Lord et al., 2000) administered by research-reliable masters-level or higher psychologists, as well as the clinical judgment of a pediatric neurologist with expertise on ASD. The presence of comorbid psychiatric disorders was explored

using the Diagnostic Interview for Children and Adolescents, fourth edition (DICA-IV; Reich et al., 1997). Children with ASD received the following additional DICA-IV diagnoses: attention-deficit/hyperactivity disorder (ADHD) (23), obsessive-compulsive disorder (OCD) (3), specific phobia (10), generalized anxiety disorder (GAD) (7) and oppositional defiant disorder (ODD) (7). Twenty-four children with ASD were actively taking psychoactive medications, including stimulants (13), selective serotonin reuptake inhibitors (SSRI) (6), clonidine (2), risperidone (2) and lithium (2). Stimulant medications were discontinued the day prior to testing; all other medications were taken as prescribed. None of the participants had a history of seizures, other neurological disorders or diagnosed genetic disorders. The TD children had no previous history of social/communicative disorders or other developmental abnormalities. Further, we also excluded diagnosis or family history of ASD in the typically developing control group. Participants were recruited through advertisements within community-wide service groups, schools, and hospitals, and outpatient clinics at Kennedy Krieger Institute. This study was approved by the Johns Hopkins Medical Institutional Review Board, with written informed consent and assent obtained from parents and children, respectively. Intelligence quotient (IQ) was assessed by the Wechsler Intellectual Scale for Children 4th edition (WISC-IV, Wechsler, 2003). All participants were required to have full-scale IQ of 80 or above on the WISC-IV, or in cases where the FSIQ was less than 80, participants were still included if their verbal comprehension index (VCI) or perceptual reasoning index (PRI) was higher than 80. Being a measure of nonverbal abilities, PRI may better estimate cognitive abilities in subject with ASD than the FSIQ (Mottron, 2004). Therefore, PRI was used in the present study as the measure of intellectual reasoning ability, as previously done by our group (e.g., Peterson et al., 2015). Parents completed the Social Responsiveness Scales Second Edition (SRS-2) (Constantino & Gruber, 2012) to measure general autistic symptoms and

behavior difficulties, and to check for ASD symptoms in the TD participants. The participants filled out the Edinburgh Handedness Inventory (EHI; Oldfield, 1971), a questionnaire for determining hand preference. The sample comprised only right-handed individuals with EHI scores >40. Last, data on parental employment were used as a measure of socioeconomic status (SES) and coded according to the Hollingshead 9-point scale for parental occupation (Hollingshead, 1975).

Motor Assessment

Motor skills of the participants were assessed using the Physical and Neurological Examination of Subtle Signs (PANESS; Denckla, 1985), and the Movement Assessment Battery for Children 2 (MABC 2: Henderson, Sugden, & Barnett, 2007). The PANESS is a standardized battery of task that evaluates several components of motor function, such as lateral preference, gaits, balance, motor persistence, coordination, overflow, dysrhythmia, and timed movements. In the present work, the total PANESS score was examined, having better performance associated with lower scores. The MABC 2 consists of eight subtests that evaluate three components of motor proficiency: manual dexterity, ball skills, and static and dynamic balance. For MABC 2, higher scores are indicative of better motor performance. Lastly, a version of the Florida Apraxia Battery (Rothi et al., 1997), modified for children (Mostofsky et al., 2006), was used to assess ability to perform skilled gestures (Praxis). Children were asked to execute skilled gestures in the response to three different standardized verbal prompts: verbal command, imitation of the examiner performing the gesture, and actual tool use in response to the tool being placed on the table. Each participant's performance was video recorded and scored off-line independently by two raters, both blinded to diagnosis. At least 80% concurrence between raters was achieved for each

assessment to ensure reliability of scoring. For the present study, the percentage of correct responses was considered.

Imaging acquisition and preprocessing

All subjects participated in a mock scan training session wherein an inactive, life-size scanner was used to simulate the actual scanning environment. No sedation was used during this process. MRI data were acquired on a 3T 'Achieva' scanner (Philips, Best, the Netherlands) at Center for Neurodevelopmental and Imaging Research, Kennedy Krieger Institute. A high-resolution T1-weighted MP-RAGE was acquired for each subject using either an eight-channel head coil (42 children with ASD, 42 TD children; TR = 7.99 ms, TE = 3.76 ms, flip angle = 8°, voxel size = $1mm^3$ isotropic) or a 32-channel head coil (10 children with ASD, 10 TD children). DTI images were acquired with single-shot EPI (SENSE factor 2.5) with either an eight-channel or a 32-channel head coil. Two runs were collected in each subject, with 32 gradient directions (b = 700 s/mm3) and one b0 in each run. Sixty 2.2-mm axial slices were acquired for each volume, with 0.9 mm in-plane reconstructed resolution. The T1 images were first skull-stripped using FreeSurfer (http://freesurfer.net, Athinoula A. Martinos Center for Biomedical Imaging, Harvard-MIT, Boston, USA). Then, a trained graduate student identified manually the anterior and posterior commissures on the midsagittal plane using *mrAnatomy* package (Stanford VISTA Lab), and these points were used to align the anatomical image to a canonical ac-pc orientation. Diffusion images were preprocessed using the Functional Magnetic Resonance Imaging of the Brain Software Library (http://fsl.fmrib.ox.ac.uk/fsl/, FSL; Oxford, UK). The two scans were first averaged to improve contrast. Next, diffusion data were corrected for motion and eddy-current induced distortions (FSL *"eddy"*). Non-diffusion weighted image (i.e., b = 0) was extracted removing skull and any other non-brain tissue (FSL *"BET"*) and used as a mask for all diffusion-weighted images

(Smith, 2002). Maps of fractional anisotropy (FA), and mean, radial, and axial diffusivity (MD, RD, and AD, respectively) were calculated at each voxel by fitting a diffusion tensor model to the raw diffusion data using weighted least squares (FSL *"dti-fit"*). Last, the non-diffusion weighted image was aligned to T1 image using *mrDiffusion* package (Stanford VISTA Lab), to obtain the appropriate MATLAB format file needed for the following analysis.

Head motion control and motion matching

First, quality-checking control was performed consisting of visual inspection for signal dropout, the amount of noise in the scan, and the quantity of movement artifacts or any other artifact in the images. Since motion can significantly affect the reliability of diffusion MRI data (Yendiki et al., 2014: Solders et al., 2017), the head displacement for each participant was estimated (FSL *"rmsdiff"*) to examine possible between groups' differences in head motion. These estimates were used to compute the mean absolute root mean squared displacement and mean relative root mean squared displacement (Jenkinson et al., 2002).

DTI tractography

Tractography was performed using the Automated Fiber Quantification (AFQ, Yeatman et al., 2012) package to identify inferior fronto-occipital fasciculus (IFOF), inferior and superior longitudinal fasciculus (ILF and SLF, respectively), and compute diffusion metrics along the tract trajectory. AFQ uses a three-step procedure to identify 24 major tracts from an individual's diffusion data. As first step, AFQ performed whole-brain tractography using a deterministic streamlines tracking algorithm (Mori et al., 1999). This was followed by the segmentation of the fiber tracts using a waypoint region of interest (ROI) method (Wakana et al., 2007). According to this approach, pairs of waypoint ROIs labeled on the Montreal Neurological Institute (MNI) space were non-linearly warped to each subject's brain in order to identify the fibers passing through

both waypoints. Last, a cleaning and refinement step was performed comparing each candidate fiber with a fiber tract probability map created by Hua and colleagues (2008), that specifies the probability that a voxel is associated with a given fiber tract. This procedure discardede candidate fibers that are unlikely to be part of the segmented tract. Fiber tracts were further cleaned by iteratively removing outlier fibers as defined by their distance from fiber tract core or length compared to the average fiber.

Diffusion parameters (FA, MD, RD, and AD) were then calculated at 30 equidistant sample-points, –nodes–, along the core of each fiber tract defined by the same two ROIs used for tract segmentation. Tract portions beyond these ROIs were not included in the analysis. The contribution of the single fibers to the core estimate is weighted based on the likelihood that the each fiber belongs to the given fiber tract, computed as the Mahalanobis distance. The result of this process is a "tract profile", which describes the variations in diffusion properties along the core of each tract on an individual subject basis.

Statistical analysis

Group differences in age, socio-economic status, PRI, and scores of motor performance were examined using univariate analyses of variance. Chi square analysis was used to examine group differences in gender. To compare FA, MD, RD and AD between groups, t-tests were conducted point-wise along each fiber tract for 30 points. A permutation based, cluster size multiple comparison correction was applied to determine statistical significance (Nichols and Holmes, 2002), $p < 0.05$. For fiber tracts that showed significant differences between groups, a Pearson's correlation analysis was performed between mean diffusivity values for the significant cluster and age, and ASD symptom measures (ADOS, ADI-R, and SRS), and measures of motor functioning.

Results

As shown in Table 3.1, the ASD and TD groups were balanced on sex, age, PRI, handedness, and SES; the groups also did not significantly differ in average framewise displacement. There was a trend toward significance for the between-group difference in FSIQ, with TD children having higher scores, although all participants had the FSIQ in the average range. As expected, children with ASD have higher scores on Social Responsiveness Scale compared to healthy controls. With respect of motor skills and praxis, in comparison to the TD group, the ASD group had lower scores on PANESS ($p < 0.001$), on MABC2 manual dexterity, aiming and catching, balance, and total score (all $p < 0.001$), and lower praxis score ($p < 0.001$).

Table 3.1. Participant Characteristics

	ASD (*n* = 52)	**TD (*n* = 52)**	
Sex	41M/11F	42M/10F	
Handedness	52R	52R	
	Mean ± standard deviation	**Mean ± standard deviation**	**ASD vs TD *P*-value**
Age	10.3 ± 1.4	10.5 ± 1.2	0.457
FSIQ	104.8 ± 15.8	109.9 ± 10.1	0.055
PRI	107.3 ± 14.1	106.3 ± 10.6	0.687
SES family	52.7 ± 10.6	52.9 ± 8.6	0.926
Mean framewise displacement	0.26 ± 0.09	0.24 ± 0.10	0.142
ADOS total score	13.8 ±3.7	-	
ADI social	19.9 ± 5.4	-	
ADI communication	15.7 ± 4.3	-	
ADI restricted interests	6.1 ± 1.8	-	
SRS total raw score	96.6 ± 28.5	15.5 ± 8.4	0.000

ADOS, Autism Diagnostic Observation Schedule; ADI, Autism Diagnostic Inventory; FSIQ, full-scale intelligence quotient; ASD, high-functioning autism spectrum disorder; PRI, Perceptual Reasoning Index; SES, socio-economic status; SRS, Social Responsiveness Scale; TD, typically developing.

The main result of the present study was a significant between-group difference in white matter diffusion of the left SLF, with children with ASD showing larger MD and AD values compared to typically developing participants ($p < 0.05$, corrected). Significant differences in MD and AD spread approximately half of the left SLF, with both clusters surviving the correction for multiple comparisons located in the anterior portion of the tract (from node 1 to node 18 for MD, and from node 1 to node 16 for AD; see Figure 3.1 and Figure 3.2, respectively).

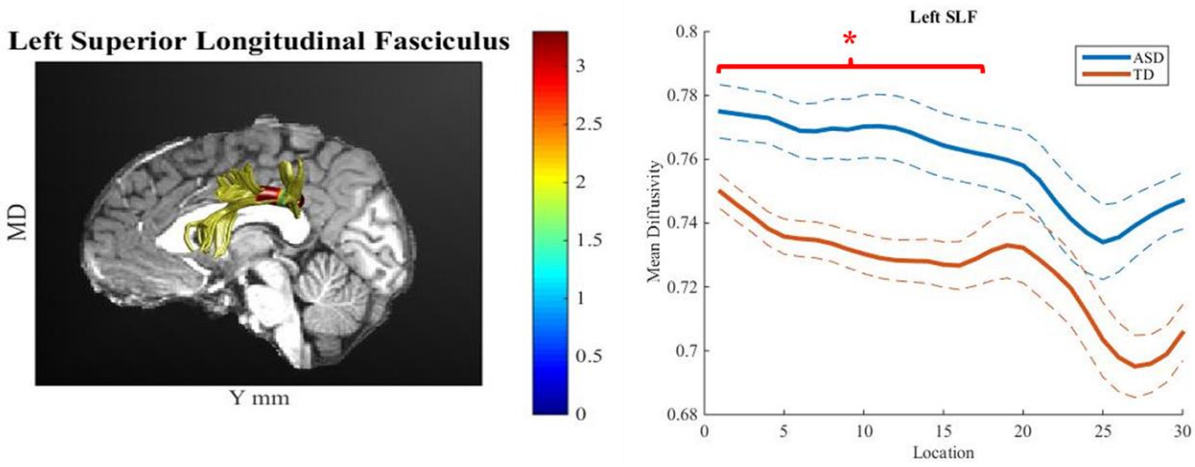

Fig 3.1. Between-group differences in MD measurement of the left SLF. The color bar indicates T-statistic values from cluster-corrected t-tests. The right graph shows the tract profile of MD values calculated at each of 30 equidistant points (x-axis) along the tract for children with ASD (represented in blue), and typical peers (represented in red). The plot depicts the mean tract profile ±1 standard error of the mean for each group. The cluster with a significant increase in MD in the ASD group ($p<0.05$, corrected) is indicated with an asterisk.

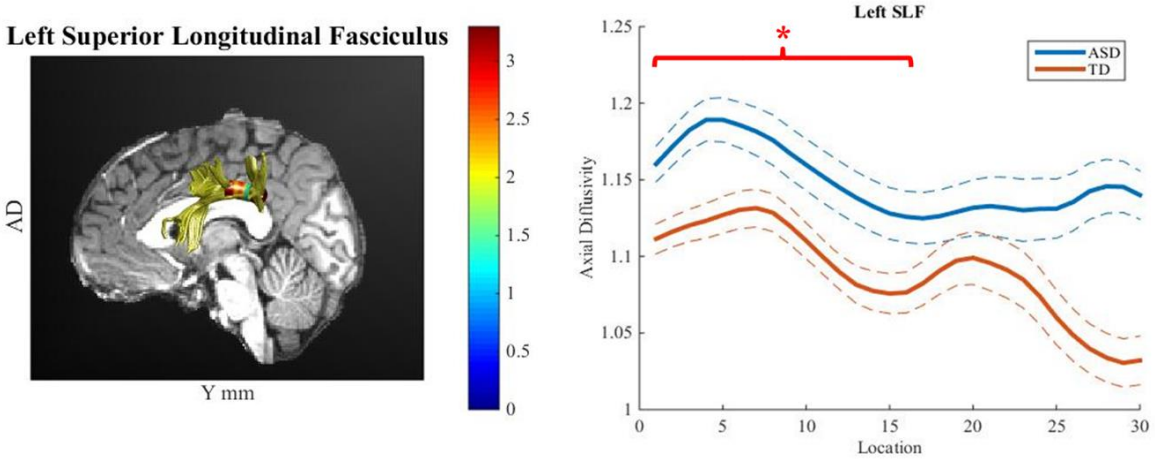

Fig 3.2. Between-group differences in AD measurement of the left SLF. The color bar indicates T-statistic values from cluster-corrected t-tests. The right graph shows the tract profile of AD values calculated at each of 30 equidistant points (x-axis) along the tract for children with ASD (represented in blue), and typical

peers (represented in red). The plot depicts the mean tract profile ±1 standard error of the mean for each group. The cluster with a significant increase in AD in the ASD group ($p<0.05$, corrected) is indicated with an asterisk.

Similar differences were also apparent for MD and RD values in the left IFOF and in the left ILF, as well as for RD values in the left SLF. However, these between-group differences did not survive correction for multiple comparisons. Next, the possible relationship between diffusion measures and demographics of participants, autism symptomatology, and motor skills was explored. A significant negative correlation between mean MD value for the left SLF cluster (nodes 1 to 18) and the total raw score on Social Responsiveness Scale was found only in children with ASD ($r = -.354$, $p = .0170$; Figure 3.3). No other statistically significant correlation emerged for any of the other characteristics considered.

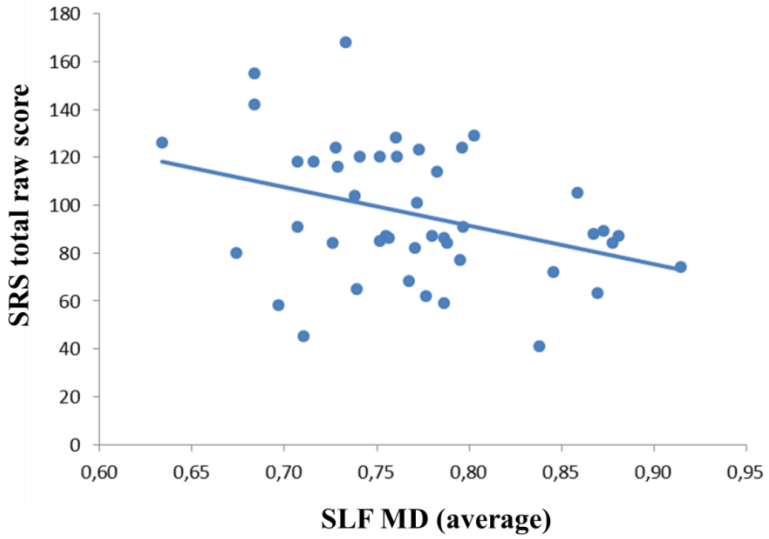

Fig 3.3. Plot showing the correlation between mean MD values for children with ASD for the significant cluster on the left SLF and the total raw score on Social Responsiveness Scale ($r = -.354$, $p = .0170$).

Discussion

The goal of the present study was to examine the white matter integrity in three long-distance frontal-posterior tracts ─namely, IFOF, ILF, and SLF─ of a well-characterized group of high-functioning children with ASD in a homogeneous age range of 8 through 12 years, compared to a cohort of typically developing children. To achieve this purpose, we used Automated Fiber Quantification (AFQ; Yeatman et al., 2012), a relatively novel technique, to analyze DTI data. The current work did not find any significant differences in FA along the tracts studied. In contrast, robust differences were found for MD and AD in the left SLF, with children with ASD having greater values of these diffusivity measures in comparison to participants with typical development. Therefore, our study provides evidence for alterations of diffusion in the left SLF in children with ASD. This result extends the findings of a previous AFQ application to DTI data (Libero et al., 2016), showing a significant reduction in FA and a significant increase in RD in the LSLF in a mixed group of children and adults with ASD (age range of 8 through 40 years), to a more homogeneous, school-aged cohort. Increased diffusivity values (even though RD) along the same tract was also observed in a few other previous studies in ASD (Jeong et al. 2011; Shukla et al. 2011). These results, together with the findings of increased MD and AD observed in the present study, may indicate microstructural abnormalities in autistic brain, such as alterations in size and spacing of minicolumns, particularly in the frontal and temporal cortices, as described in postmortem studies (Casanova, 2006; McKavanagh et al., 2015). The SLF is a major association fiber tract that connects the temporoparietal junction to the superior temporal gyrus and with the frontal lobe (more precisely, with the inferior frontal gyrus) (Wakana et al. 2004; Makris et al., 2005; Wang et al., 2016). The SLF has been known to play a role in movement selection (including that crucial to praxis and imitation) (Hartwigsen et al. 2012), language (Nagae et al., 2012; Catani

& Bambini, 2014), and visuo-spatial attention (Thiebaut de Schotten et al., 2011). Specifically, the present findings suggest that abnormalities in white matter connectivity may not affect in ASD the entire left SLF bundle but, in our case, the most anterior part. The anterior section of SLF has been found to project to precental gyrus with terminations in frontal cortex (Martino et al., 2013; Wang et al., 2016). Therefore, atypical connectivity in this portion of left SLF could have a significant impact on many ASD manifestations, including the socio-communicative features of the disorder. Accordingly, and consistent with our hypothesis, our findings (both significant and subthreshold differences) indicate a pattern of left-lateralized white matter abnormalities, in line with "left hemispheric dysfunction theory" in ASD proposed by a number of studies throughout the years (Dawson, 1983; Hier et al., 1979; Peterson et al., 2015; Floris et al., 2016).

The current study also found, surprisingly, a positive correlation between increased MD values and better social skills in children with ASD, as measured by Social Responsiveness Scale. This might suggest the presence of compensatory mechanisms in high-functioning children with ASD, moderating the effect of white matter alterations on observed behavior. No significant correlations were found between the significant cluster on the left SLF and praxis or any motor measures. This is not surprisingly considering the fact that the anterior portion of SLF has been more prominently related to language (Nagae et al., 2012; Catani & Bambini, 2014).

Despite our promising results, this study did have some limitations. A first limitation was that study was restricted to a group of children with high-functioning ASD, and the findings may not be directly generalizable to children with ASD and intellectual disability or to adult patients. Future investigations should also extend the present method of analysis also to younger populations, so that an accurate longitudinal pattern of the neurodevelopmental trajectories in ASD can be identified. Unfortunately, the number of female participants in this work was not large

enough to investigate possible gender differences in the white matter tracts studied. Another potential limitation was that many participants with ASD presented also comorbid disorder and were taking psychotropic medications; it is unclear whether these may have impacted the study findings. The study also had limitations related to the neuroimaging method. Indeed, DTI is unable to resolve crossing fibers with differing trajectories within a single voxel. This may limit the reliability of diffusivity measures quantification, especially in regions with multiple intersecting white matter bundles. Finally, although participants of this study did not differ in terms of head motion, future extension of the present work should include subjects more closely matched for head displacement during the scan, since motion has been reported to significantly affect the reliability of diffusion MRI data (Yendiki et al., 2014: Solders et al., 2017).

In conclusion, the present study provides evidence for alterations in white matter diffusivity of the left SLF in a well-characterized group of high-functioning children with ASD. Consistent with our hypothesis, our findings indicate a pattern of left-lateralized white matter abnormalities. As demonstrated by this study, AFQ, a tool for analysis of DTI data, may be useful in delineating these anomalies in school-aged children with ASD.

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GENERAL CONCLUSIONS

Autism spectrum disorder (ASD) is a highly heterogeneous neurodevelopmental disorder characterized by a dyad of persistent social-communication abnormalities and restricted and repetitive behaviors. ASD manifests with broad phenotypic variability and evolves along multiple possible developmental courses. This notable heterogeneity of the clinical manifestations represents a major obstacle to identification of pathophysiological processes, risk factors, and causes underlying the disorder. Therefore, in the last years, the need for developing biomarkers, that stratify patients according to distinct biological "forms" of the disorder, has become imperative.

The purpose of the present project was to provide further evidence supporting the use of motor impairments as a bio-behavioral marker of ASD. Indeed, atypicalities in motor behavior are one of the features most frequently associated to the disorder and have a significant impact on quality of life and social development. In this project, we aimed to expand and deepen current knowledge about a possible "motor signature" of ASD, approaching this topic at three different levels of analysis (i.e., behavioral, computational, imaging) and using either innovative tools (Chapter 1 and Chapter 3) or new methodology (Chapter 2).

In the experiment presented in Chapter 1, we investigated the gait pattern and the motor adaptation to discrete gait perturbations of drug naïve, school-aged children with ASD compared to typically developing peers. We were interested in studying gait since locomotor activity represents a milestone for the progressive development of the children's communication. Further, we additionally focused on motor adaptation during a split-belt walking task because we know that

this split-belt adaptation rely on cerebellum, one of the anatomical structure primarily implicated in ASD. To achieve these goals, we used the Gait Real-time Analysis Interactive Lab (GRAIL), an innovative multi-sensor platform based on immersive virtual reality. To date, this was the first study that makes use, in a cohort of patients with ASD, of this innovative and motivating environment dedicated to gait analysis. In line with previous literature, results highlighted an altered gait pattern in children with ASD. Slower rates of adaptation to the perturbation were also found in the clinical group. Interestingly, diminished learning adaptation was significantly related with more severe autistic traits.

In Chapter 2, we presented a proof-of-concept study that we have undertaken to investigate whether a simple upper-limb movement could be useful to accurately classify low-functioning children with ASD who are between the ages of two to four. In order to answer this question, we developed a supervised machine-learning method to identify and correctly discriminate preschool children with ASD from typically developing children by means of kinematic analysis of a simple reach, grasp and drop task. Our machine-learning method reached a good mean individual classification in the comparisons between children with ASD and healthy controls (overall mean accuracy = 84.9%), with a maximum accuracy up to 96.7% using only seven kinematic features related to the second, more goal-oriented, part of the movement. Thus, we demonstrated that machine-learning classification approach might be helpful for supporting the clinical practice of diagnosing ASD, even fostering a computer-aided diagnosis perspective.

Finally, in the study described in Chapter 3, we investigated whether a well-characterized group of high-functioning children with ASD would demonstrate reduced white matter integrity, as indirectly measured by diffusion tensor imaging, in three long-distance frontal-posterior tracts —namely, inferior fronto-occipital fasciculus, inferior and superior longitudinal fasciculus. We

were interested in exploring the hypothesis of an anterior-posterior underconnectivity in ASD because it might provide useful insight into the mechanisms of social communication, given the key role of these pathways for language, praxis, imitation, and basic motor coordination. To this end, we used Automated Fiber Quantification, a relatively novel tool that measures diffusivity measures along the trajectories of major white matter tracts of the brain network. Our DTI study revealed alterations in white matter diffusivity of the left superior longitudinal fasciculus in a well-characterized group of high-functioning children with ASD.

Overall, these data contributed to highlight the central role of motor impairments in children with ASD and supported the usefulness to take into account motor difficulties as a potential bio-behavioral marker of the condition. The results from first two chapters depicted two motor paradigms potentially useful to identify a well-characterized subset of patients, homogeneous for difficulties in motor domain. The walking adaptation paradigm was an effective task to individuate differences in motor adaptation of ASD that were intriguingly related to the severity of the clinical manifestations. Moreover, based on previous physiological studies on split-belt walking adaptation, the present thorough paradigm could be a valuable starting point for future research to investigate the cerebellar contribution to motor abnormalities in ASD by brain-mapping techniques. The results derived from our machine-learning approach might potentially represent new methodology for a computational identification of ASD based on a simple reach, grasp and drop task. Because of the easiness of the task, this paradigm could be feasible for studying study motor functioning, also in a challenging clinical population, such as young or low-functioning individuals with ASD. Finally, the results from the third study shed more light on neural correlated of the motor difficulties.

A main limitation of the present project was that our findings could be highly specific to the cohorts we recruited, namely preschool-aged and school-aged children with ASD. Future extensions of this project should therefore include other neurodevelopmental conditions without ASD comorbidity (e.g., intellectual disability, developmental delays without intellectual disability, or developmental coordination disorders) in order to verify the specificity of both our walking paradigm and classifier to ASD, rather than a neurodevelopmental disorder in general. Future extension of this work should also investigate the predictive value of the putative motor markers here described. Specifically, the reach, grasp and drop task is particularly suitable for evaluating high-risk infants (i.e., siblings of ASD patients) before the clinical diagnosis. If also replicated in a pre-clinical population, the present findings could even foster the perspective of employing such a paradigm as screening device for health and educational services. Given these considerations, we have recently started Medea two new projects at IRCCS Eugenio that employ the reach, grasp and drop task presented in Chapter 2. The first study is currently recruiting, as "control" groups, a sample of children with intellectual disabilities without ASD and a sample of children with a clinical diagnosis of attention deficit/hyperactivity disorder, to test the specificity to ASD of our classifier. The second ongoing project is enrolling high-risk infants with an older sibling with ASD and low-risk infants from the general population at 18 months. The aim of this latter study is to investigate the potential usefulness of our reach and drop paradigm as screening tool for a pre-clinical population.

All in all, our findings offered insight on a possible, multi-domain (i.e., behavioral, computational, and imaging) motor signature of ASD that is potentially useful to identify a well-defined subset of patients, thus reducing the clinical heterogeneity within the broad behavioral phenotype. This may guide, on one hand, further exploration of pathophysiology of the disorder,

adding power to neuroimaging investigations by means of coupling both structural and functional imaging with experimental neurobehavioral paradigms proposed here. On the other hand, the detection of a well-characterized subset of patients, homogeneous for difficulties in motor domain, could potentially facilitate identification of common genetic syndrome.

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