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"Corpus callosum in preschoolers with Autism Spectrum Disorder: an imaging study"

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Introduction

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The term " autism " describes a spectrum of heterogeneous neurodevelopmental disorders characterized by early-onset abnormalities in social communication, and atypically restricted and repetitive behaviors and interests.

Since the first description of the 11 children with autism performed by Leo Kanner in 1943 , to date, autism had different definitions and nosographic descriptions (Asperger, 1944; Rutter, 1978; DSM III, 1980). In 1980, autism was first included in the Diagnostic and Statistical Manual of Mental Disorders, in its third edition (DSM -III), that described the diagnostic category of Pervasive Developmental Disorders (PDD, Pervasive Developmental Disorders). In 2000, DSM -IV (Diagnostic and Statistical Manual of Mental Disorders, 2000) included Autistic Disorder in the nosographic category of PDD , with other four entities similar to autism. According to the DSM -IV PDD were characterized by " severe and pervasive impairment in several areas of development : reciprocal social interaction skills , communication skills , or the presence of stereotyped behaviors, interests and activities " and included: Autistic Disorder , Rett Syndrome, childhood disintegrative disorder , Asperger Syndrome , and Pervasive Developmental Disorder Not Otherwise Specified. The ' International Classification of Diseases, in its tenth edition (ICD-10, 1993 the World Health Organization) classified the PDD in a manner comparable to the DSM -IV (except for the division of the Pervasive Developmental Disorder Not Otherwise specified in 3 sub-categories: atypical autism , pervasive developmental disorder not specified and other pervasive developmental disorder) . ICD-10 also included in the DPS category the hyperactive syndrome associated with mental retardation and stereotyped movements. Within the category of PDD in the DSM -IV and ICD -10 , Autistic Disorder is the most important disorder , because it is the most frequent, and because it covers fully and complete description of the DPS . It fact, as outlined in the DSM -IV, is characterized by the “ presence of a significantly abnormal or deficient development of social interaction and communication and a markedly restricted repertoire of activities and interests.”

Among the PDD described by the DSM -IV, Rett Syndrome , first described in 1966 , is a rare neurodegenerative condition that affects mostly females, characterized by an apparently normal early development (during the first 6-18 months) following then by a deterioration or a slowing of cognitive , social- communicative and motor development: children with Rett Syndrome show a regression or retardation of motor skills , a decline of social interactions , a regression of cognitive skills and communication skills learned, and the loss of use of hands, replaced by stereotyped hands behaviors. Originally called " infantile dementia " (Heller,

1908) , the Childhood Disintegrative Disorder is a rare and severe developmental regression , which occurs in 3-4 years old children, with an earlier apparently normal development . The onset is usually gradual, but rapid (weeks or months) , and sometimes associated with psycho-social stress conditions . The disorder has the same symptoms of autism, with a clinically significant loss of previously acquired skills and social communication. Asperger Syndrome is a developmental disorder characterized by marked abnormalities in social interaction , despite adequate cognitive and verbal ability . Social withdrawn can remember the autistic isolation, however, children with Asperger Syndrome can often be hungry for relationships with other people . The social approaches , however, are inappropriate , often characterized by strict formal rules, and impaired social integration. Although verbal skills are formally adequate in children with Asperger Syndrome , language is often characterized by abnormalities in the pragmatic component . Also the restricted interests and repetitive tasks may resemble the stereotyped behavior of autistic disorder. The Pervasive Developmental Disorder Not Otherwise Specified (PDD -NOS) , although it represents a diagnosis of DSM-IV , is not a uniform clinical entity . PDD-NOS includes those PDD whose clinical features are not fully described by another diagnosis of DSM- IV or ICD -10. Sometimes the PDD-NOS is a sort of "wildcard" , a label for diagnostic unfavorable conditions , when available information are inadequate, PDD-NOS diagnosis can be a temporary diagnosis. Other times, it is taken into account for children who , despite symptoms of the autistic spectrum, are at the edge of a more normal functioning , or without impairment in one of the 3 areas of the disorder is mild or absent.

One of the most expected changes in the fifth edition of the DSM (Diagnostic and Statistical Manual of Mental Disorders) , in May 2013 (DSM -5), was the revision of the diagnostic criteria for disorders related to autism, an important change in clinical practice of psychiatry. According to the American Psychiatric Association (2013) in fact, the new revision of diagnostic criteria provide a new, more accurate and useful tool to diagnosis individuals with autism. The DSM-5 eliminates previous 5 possible diagnostic labels (Autistic Disorder , Rett Syndrome , Childhood Disintegrative Disorder , Asperger Syndrome, and Pervasive Developmental Disorder Not Otherwise Specified) and it introduces the concept of spectrum for disorders related to autism , with the creation of the new diagnostic category of Autism Spectrum Disorders (ASD) . Subjects previously diagnosed according to DSM -IV, with a diagnosis of PDD, today receive a unique diagnosis of ASD. The use of autism spectrum concept has solved some of the concerns about the appropriateness of term "pervasive" in the description of the morbid condition , the validity and applicability of certain diagnostic labels sometimes used in different ways (ie. PDD -NOS) , the differentiation of clinical conditions often very similar and overlapping (ie. high functioning Autism and Asperger Syndrome) , the inclusion within a psychiatric category of a neurological disorder (ie . Rett Syndrome) . The use of a single , large " spectrum " of clinical conditions related to autism therefore better reflects the variability of clinical presentation and the phenotypic variability in time and reduces potential variability arising from the use of previous diagnostic labels.

DSM -5 recommend the use of the diagnostic category of ASD, without a definition of subtypes, in all those subjects with " persistent deficits in social communication and social interaction in different contexts " and " patterns of restricted, repetitive behavior , interests or

activities ", then the previous diagnostic labels of Autistic Disorder, Asperger Syndrome, and Pervasive Developmental Disorder Not Otherwise Specified are unified in the diagnosis of Autism Spectrum Disorder . Another change in DSM-5 is the elimination of diagnostic labels of Rett Syndrome and Childhood Disintegrative Disorder , given their different clinical characteristics and etiological relation to other ASD. Another change is on diagnostic criteria: the new "socio-communicative" domain of DSM- 5 combines "social" and "communicative" domains, separated in DSM-IV. This is because, according to the Authors, communication deficits are intimately related to deficit in social skills, so the two clinical domains are actually different manifestations of a single socio- communicative domain. Third domain of restricted and repetitive interests and activities , otherwise remains unchanged , with the exception of the inclusion of the stereotyped or idiosyncratic language criteria, previously included in the communication domain. Atypical language development (historically linked to an autism diagnosis) was removed from the criteria, and is now classified as a co-occurring condition, even though large variation in language is characteristic of autism. Other changes introduced in the new DSM edition is the inclusion of Specifiers: With or without accompanying intellectual impairment; With or without accompanying language impairment; Associated with a known medical or genetic condition or environmental factor; Associated with another neurodevelopmental, mental, or behavioral disorder; With catatonia; current Severity.

Current diagnostic criteria for ASD are:

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history (examples are illustrative, not exhaustive, see text):

1. Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interests, emotions, or affect; to failure to initiate or respond to social interactions.

2. Deficits in nonverbal communicative behaviors used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expressions and nonverbal communication.

3. Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behavior to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.

B. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history (examples are illustrative, not exhaustive; see text):

1. Stereotyped or repetitive motor movements, use of objects, or speech (e.g., simple motor stereotypies, lining up toys or flipping objects, echolalia, idiosyncratic phrases).

2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns or verbal nonverbal behavior (e.g., extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat food every day).

3. Highly restricted, fixated interests that are abnormal in intensity or focus (e.g, strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interest).

4. Hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment (e.g., apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement).

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

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

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

Epidemiology:

For several decades after its first definition, autism was considered a rare disorder (2-4 out of 10,000 children). In recent years, the prevalence of ASD is gradually increased : about 60 per 10,000 (Fombonne et al., 2003). The Centers for Disease Control and Prevention (CDC) in 2007 indicated that in Europe and the U.S., about one in 150 children was suffering from autism: an alarming statistics that led to talk of " epidemic autism ". This concern has been steadily growing even for the significant prevalence increase reported by the CDC, in 2012, when prevalence of autism was estimated about 1 in 88 children (1 : 54 in males and 1 : 252 in females) . Other data on the prevalence of autism in the U.S. have recently been published (Blenner, 2014) by the same group, that currently estimates 1 child every 68 (14.7 in 1000) is suffering from an ASD (1 : 42 males and 1 : 189 females).

Hypotheses formulated to explain this increase of diagnoses number are varied (Fombonne et al., 2001; Wing et al., 2002; Baird et al., 2003; Prior et al., 2003; Wazana et al., 2007; Bryson et al., 2008) and include: an increase in the number of diagnoses (greater definition in diagnostic criteria, development of the spectrum concept, use of different study methods , increased awareness and knowledge of the disorder among parents and operators, and an increase of diagnostic possibility) and of course the real increase of autism cases. Evidence

from several studies suggests that the growth of incidence and prevalence was due to greater diagnostic potential and enlargement of the definition of ASD, and consequently to a wider "recruitment". If there is a real increase in the number of cases is still debated , and some Authors believe that actual number of cases of ASD is even higher than estimated by CDC , because the presence of many mild cases undiagnosed.

Males are significantly more affected than females , with a ratio of about 4-4.5 / 1 , although this difference tends to decrease in subjects associated with intellectual disability (Fombonne et al., 2011) . However, some Authors (Baron-Cohen et al., 2011) argue that diagnosis in females may be underestimated because the diagnosis is later in life and is frequently due to the presence of major cognitive and / or behavioral problems.

Nevertheless, a male predominance is a consistent epidemiological finding that has aetiological implications (Lai et al., 2014). It could imply female-specific protective effects, such that females would have to have a greater aetiological (genetic or environmental) load than would males to reach the diagnostic threshold. Alternatively, male-specific risks could heighten susceptibility (Werling et al., 2013).

Some Authors suggest that the existence of sex-linked aetiological load and susceptibility emphasises the importance of stratification by sex, and of comparisons between males and females to disentangle the aetiological role of sex-linked factors at genetic, endocrine, anatomical, epigenetic, and environmental levels.

Clinical features in preschoolers:

Autism (from the Greek "autùs") is an early-onset disorder characterized by abnormal development or deficit of social interaction, communication and interests.

Since the first description by Kanner, early age of onset represents one of the most consistent features of ASD (in DSM-IV included age of onset in the diagnosis criteria of autism, with onset no more later 36 months of age). In DSM-5 Authors underlined that symptoms must be present in early childhood but may not become fully manifest until social demands exceed limited capacities.

Signs of autism apparently are not present at birth, but emerge through a process of diminishing, delayed, or atypical development of social-communication behaviors, starting between the ages of 6 and 12 months (Ozonoff et al., 2010).

Some years ago, children with autism were often identified when older than 4-5 years, but toddlers are now frequently diagnosed because their atypical development is earlier recognized, and it allows early intervention.

Early signs of autism are deficits or delays in the emergence of joint attention (ie. shared focus on an object), poor attention to social scenes or human faces (by 6 months of age), decreased response to own name, deficits in reciprocal affective behavior and little infant–parent interaction at age 12 months (ie. reduced dyadic mutuality, including shared attention, infant acceptance of parental involvement, playing together, interactive flow, and shared body orientation; infant positive affect; and attentiveness to parent), and atypical implicit perspective taking. Pretend play is poor or delayed, imitation is decreased, verbal and nonverbal communication is delayed or atypical. Children with ASD also show motor delay, unusually repetitive behaviors, atypical visuomotor exploration, inflexibility in disengaging visual attention, and frequently extreme variation in temperament (Zwaigenbaum et al., 2009).

These symptoms contribute to early detection of toddlers with ASD. However, identification of high-functioning children is still frequently later than it should be, particularly for females.

Deficits in social communication and social interaction

ASD are characterized by persistent deficits in social communication and social interaction across multiple contexts. Core elements of autism are deficits in social-emotional reciprocity, in non-verbal communicative behaviors used for social interaction, in developing, maintaining, and understanding relationships.

The social communication disability is due to a lack or deficit or atypicalities of inter-subjectivity and verbal and non-verbal communication.

ASD are characterized by deficits in social-emotional reciprocity (Carpenter, 2013), ranging from abnormal social approach (eg., unusual social initiations, intrusive touching; licking of others or use of others as tools), failure of normal back and forth conversation (eg., poor pragmatic/social use of language, failure to respond when name called or when spoken directly to, does not initiate conversation, or one-sided conversations/monologues/tangential speech), through reduced sharing of interests (eg., doesn't share, lack of showing, bringing, or pointing out objects of interest to other people or impairments in joint attention, both initiating and responding), reduced sharing of emotions/affect (eg., lack of responsive social smile, failure to share enjoyment, excitement, or achievements with others, failure to respond to praise, does not show pleasure in social interactions, failure to offer comfort to others, indifference/aversion to physical contact and affection) or total lack of initiation of social interaction (eg., only initiates to get help, has limited social initiations, has poor social imitation and failure to engage in simple social games).

Deficits in nonverbal communicative behaviors used for social interaction are characterized by impairments in social use of eye contact, in the use and understanding of body postures (e.g. facing away from a listener) or gestures (e.g. pointing, waving, nodding/shaking head), abnormal volume, pitch, intonation, rate, rhythm, stress, prosody or volume in speech, abnormalities in use and understanding of affect, impairment in the use of facial expressions (may be limited or exaggerated), lack of warm, joyful expressions directed at others, limited communication of own affect (inability to convey a range of emotions via words, expressions, tone of voice, gestures), inability to recognize or interpret other's nonverbal expressions, lack of coordinated verbal and nonverbal communication (e.g. inability to coordinate eye contact or body language with words), or lack of coordinated non-verbal communication (e.g. inability to coordinate eye contact with gestures).

Subjects with ASD have deficits in developing and maintaining relationships, appropriate to developmental level (beyond those with caregivers): lack of "theory of mind"; inability to take another person's perspective, difficulties adjusting behavior to suit social contexts (eg., does not notice another person's lack of interest in an activity, lack of response to contextual cues or inappropriate expressions of emotion, such as laughing or smiling out of context, unaware of social conventions/appropriate social behavior; asks socially inappropriate questions or makes socially inappropriate statements, does not notice another's distress or disinterest and does not recognize when not welcome in a play or conversational setting, limited recognition of social emotions). They show difficulties in sharing imaginative play

with peers, including social role playing (>4 years developmental age), difficulties in making friends (eg., does not try to establish friendships, does not have preferred friends, lack of cooperative play over 24 months developmental age; parallel play only, unaware of being teased or ridiculed by other children, does not play in groups of children, does not play with children his/her age or developmental level, has an interest in friendship but lacks understanding of the conventions of social interaction or does not respond to the social approaches of other children), absence of interest in others (eg., withdrawn; aloof; in own world, does not try to attract the attention of others, limited interest in others; unaware or oblivious to children or adults, limited interaction with others and prefers solitary activities).

The impaired social interaction and communication although permanent, is expressed by symptoms that can vary over time, during development.

Infants can show gaze avoidance, lack of anticipatory behavior (such as stretching arms when try to pick him up), absence of social smile in response to a face or voice, that are early stages of social communication behavior's development. Gaze contact is an important step, in early months of life, to development of social interaction and social adjustment during entire life (Jones et al., 2008). Children with autism, aged about 2 years, have a significantly reduced eye contact . This deficit in visual interaction involves critical consequences for social communication development, so that the avoidance of the gaze is a good predictor for future social disability.

In older children, symptoms will be more explicit, especially in interaction with peers: subjects with ASD show lack of shared attention, lack of attention to social environment, difficulties in interaction with peers, tendency to isolation, inability to develop relationships appropriate to child's age, apparent emotional indifference. Sometimes these children seem to not understand that other people have thoughts and feelings, knowing that usually develops in the first few years of life and is essential for a social life (Volkmar et al., 1997). Frequently children with ASD does not participate and does not require the participation of others in activities, does not show the objects of interest to others for the sake of sharing, their gestures are poor, they often use others as a "tool" to reach object. Frequently interpersonal relationships seem to be predominantly "requestive" and purposive rather than marked on the pleasure for interaction. For example, pointing sometimes is used by children with ADS, but often only with demand function, and not to sharing with others. The presence of communicative gestures correlates inversely with ASD severity and seems to be a good predictor of subsequent communication and language development (Mundy et al. , 2003; Camaioni et al. , 2003). Even physical contact, often rejected when not required, is designed to meet their own needs or to seek physical comfort.

At school age, thanks to a social adaptation, interaction with others often can improve, although a poor involvement in social relations is frequent. The lack of understanding social rules can lead, especially in older children and adolescents, to inappropriate behavior, such as temper tantrums, often apparently unprovoked, aggressions, destructiveness, screaming, attempts to escape. Often children with ASD show lack of social reciprocity and empathy.

Impaired communication can be related to all expression's and reception's codes of language. Frequently children with ASD have a delay or absence of verbal language and non-verbal communication abilities. Infants with autism can have a delay or absence of vocalism and babbling (usually gained around 4-8 months). Subsequently autism can manifest itself more

explicitly with an impairment in language development: the toddler does not speak, does not call parents, does not turn when called by name (the criterion of language delay, present in DSM -IV diagnostic criteria, has been eliminated in the current DSM-5 criteria). Some of these children remain mute lifelong: about 33%-50% of children with ASD never acquires any form of finalized language (Bailey et al., 1996; Bryson et al.,1996; Marans et al.,1997).
When language is present children with ASD show anomalies in various aspects of speech. Preschoolers with ASD frequently show deviant and delayed in comprehension and two-thirds have difficulty with expressive phonology and grammar. In these children language is sometimes very fluent but often they still have a typical production: verbal stereotypies, bizarre or idiosyncratic phrases, echolalia, reverse pronouns, abnormal prosody (speech-song, monotonous or emphatic, not adequate to the meaning of the sentence), use of concrete language and literal meanings of words. Some children with ASD have a rich vocabulary, with long and sometimes bombastic words, in other cases sentences are telegraphic and uncorrected. Often even non-verbal components of language and communication are affected, such as intonation, pauses, gestures, facial expressions. Abnormalities in phonetic - phonological aspect and phonological difficulties are frequent and also in language programming and lexical access, with impairment in the morpho-syntactic phrasal structure. Even lexical and morpho-syntactic comprehension are often impaired.
Older children with ASD usually show deviant pragmatics, semantics, and morphology, with relatively intact articulation and syntax (ie, early difficulties are resolved).
Children have typically a difficulty in understanding "symbolic" language, and an inability to understand puns, metaphors, proverbs and figurative language. So communication , when present, is inadequate especially in the "pragmatic skills", eg. in defining social rules of language in relation to the purpose and the participants in dialogue. The communication is often not aimed to social interaction and enjoyment of interpersonal relationship, but frequently it is merely a tool to have what the child wants, or to talk about his interests, often expressed with repetitive and restricted content. The impairment in the pragmatic component of language is sometimes the only sign of ASD in children with high-functioning disorder.
Other symptoms of this domain frequently shown by children with ASD are deficits in: emotion perception, face processing, biological motion perception, social attention and orienting, social motivation, social reward processing, imitation, affective empathy and sympathy, joint attention, theory of mind or mental perspective taking, self-referential cognition, and alexithymia.

Restricted, repetitive patterns of behavior, interests, or activities

ASD are characterized by restricted, repetitive patterns of behavior, interests, or activities. These children could show stereotyped or repetitive speech (eg. pedantic speech or unusually formal language, child can speaks like an adult or “little professor”, echolalia; repetitive vocalizations such as repetitive guttural sounds, intonational noise-making, unusual squealing, repetitive humming, may include repetition of words, phrases, or more extensive songs or dialog, gibberish, idiosyncratic or metaphorical language, neologisms, pronoun reversal, refers to self by own name and does not use “I”), stereotyped or repetitive motor movements (eg., repetitive hand movements, stereotyped or complex whole body movements, abnormalities of posture such as toe walking or full body posturing, unusual facial grimacing,

excessive teeth grinding, perseverative or repetitive action / play / behavior) and stereotyped or repetitive use of objects (eg. nonfunctional play with objects such as lines up toys or objects, repetitively opens and closes doors or repetitively turns lights on and off).
They could have excessive adherence to unusual routines, ritualized patterns of verbal or nonverbal behavior (eg., repetitive questioning about a particular topic, verbal rituals or compulsions), or excessive resistance to change (eg., difficulty with transitions, insistence on same route or food, or extreme distress at small changes) and rigid thinking (eg., inability to understand humor and nonliteral aspects of speech such as irony or implied meaning, excessively rigid, inflexible, or rule-bound in behavior or thought).
Highly restricted, fixated interests that are abnormal in intensity or focus (such as strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interests, that are abnormal in intensity or focus, preoccupation with numbers, letters, symbols, or focus on nonrelevant or nonfunctional parts of objects, being overly perfectionistic, attachment to unusual inanimate object and having to carry around or hold specific or unusual objects).
ASD frequently have hyper-or hypo-reactivity to sensory input or unusual interest in sensory aspects of environment (such as apparent indifference to pain/heat/cold, adverse response to specific sounds or textures, unusual sensory exploration with objects such as excessive smelling or touching of objects, fascination with lights or spinning objects, unusual visual exploration / activity such as close visual inspection of objects or self for no clear purpose or looks at objects and people out of corner of eye or extreme interest or fascination with watching movement of other things).
A typical feature of children with autism is the inability to pretend play (Baron-Cohen et al.,1996; Charman et al., 1997, Rogers et al., 2003), a must step in typical development, it is characterized by a progressively increasing complexity during typical growth (eg, by pretending to drink from an empty cup to 1 year, to pretend to give the bottle to a doll to 1 year and a half, to pretend to be a doctor in later life), and it represents the acquisition of symbolic thought. In children with ASD the beginning of these simulations is later than typical development and play tend to be poor and repetitive. The interests and the activities of children with autism are usually restricted, repetitive and stereotyped and play is usually solitary.
Children with ASD can show stereotyped or repetitive motor movements, use of objects, or speech.
Typically they are involved in motor mannerisms, such as twist, look or bite hands, rocking, repeated head movements, or bizarre postures. During growth these simple motor stereotypes tend to disappear, giving way to more complex repetitive behaviors, such collection, alignment objects, etc.
Highly restricted, fixated interests, that are abnormal in intensity or focus, attention to detail or parts of objects or rotations are typical in children with ASD; objects and toys often are not used with the proper function and as representative elements in pretend play but are observed, handled, rotated.
The involvement in specific and narrow topics is sometimes overly invested (eg, children with ASD know everything about dinosaurs, or recite the scenes of a movie). Children can spend their time on repetitive tasks that absorb them fully.

They can show insistence on sameness, inflexible adherence to routines, or ritualised patterns of verbal or non-verbal behaviour. Behaviors are often monotonous and ritualistic, daily activities follow rigid and immutable sequences (eg, individuals with ASD want to always eat the same limited number of foods, always sit in the same chair, always follow the same paths, etc). Often children with autism show increased perception, endurance, and discomfort to changes (eg. sometimes they place objects in an order that must remain unchanged, they can notice if placement of the objects was minimally altered and have a strong discomfort reaction). Stereotypic behaviors and rituals, in older children with ASD, can be less clear, but often they are very abitudinary and they have circumscribed interests, in topics that typically require a significant investment of mechanical memory (eg. dates, numbers, etc.).

Other symptoms, co-occurring conditions and prognosis:

Another characteristic symptoms of ASD is the hypo-reactivity or hyper-reactivity to sensory stimuli. In fact children with ASD often show an abnormal reactivity to sensory input or unusual interest in sensory aspects of the environment, they can show abnormal reaction to certain auditory stimuli (eg. bells, loud noises), visual (eg. flash, special colors, repetitive visual patterns), tactile (eg. roughness of surfaces or clothing, reduced sensitivity to pain), olfactory, gustatory or vestibular - proprioceptive, which can trigger unusual and idiosyncratic responses, such as fear or, conversely, charm. Atypicalities include too much attention to the "sensory characteristics" of objects or paradoxical responses to sensory stimuli (eg. children with ASD may react covering eyes in response to an auditory stimulus).

Children with ASD also can show motor abnormalities, like motor delay, hypotonia, catatonia, deficits in coordination, movement preparation and planning, praxis, gait, and balance.

Hyperactivity is also a common symptom. Hypotonia and joint laxity are found, according to some Authors (Ming et al., 2006), in 51% of children with ASD. Sometimes it can be detected several neuromotor non-specific symptoms or signs (eg. residual of primitive reflexes, delayed hand dominance). Apraxia is frequently identified in children with autism (34 %), with prevalent impairment in manual motility. Some individuals may show echopraxia gestures.

Children with ASD may have a tendency to walk on their toes (19 % of children with autism). Reduced mobility is also reported in the literature, but it is rare.

The low prevalence of motor deficits in older children suggests an improvement over time, probably due both to the natural history of disease, both to therapy, although clumsiness and handling difficulty may remain well into adulthood. The severity of motor deficits seems to be proportional to intellectual deficits.

More than 70% of subjects with ASD have concurrent medical, developmental, or psychiatric conditions (Lai et al., 2014) and childhood co-occurring conditions tend to persist into adolescence, instead some co-occurring conditions, such as epilepsy and depression, can first develop in adolescence or adulthood. Generally, the more co-occurring conditions, the greater the individual's disability.

The high frequency of comorbidity could be a result of shared pathophysiology of disorders, of secondary effects of growing up with a developmental disorder, of shared symptom domains and associated mechanisms, or caused by overlapping diagnostic criteria.

Approximately 45-50% of children with ASD has an intellectual deficit (Volkmar et al., 2004 Fombonne et al., 2011); also current data from the Centers for Disease Control and Prevention (2014) tend to confirm the percentages reported in the literature (46% of children with ASD at age 8 have standard or higher cognitive abilities). The relationship between autism and intellectual disability has been widely discussed by Authors and in clinical practice, because in children with severe cognitive impairment often is not easy to assess behavioral and socio-communicative disorder, because atypicalities may be related to a coexistence of an ASD, rather than to the intellectual disability per se; conversely, in children with autism is difficult to determine whether the low functional level is due to ASD or to an associated intellectual deficit (Lord et al., 1989).

Cognitive profiles presented by children with ASD are very heterogeneous. Many subjects in fact have non-verbal intelligence levels (nvIQ), higher than verbal cognitive skills (vIQ). Often the relationship between non-verbal and verbal cognitive skills is dependent on the disorder severity.

Deficits in executive functions are typical in ASD and skill levels often show deficit in tasks requiring fluid reasoning processes, interpretation, planning, integration or abstraction. Instead generally in non-verbal cognitive performance, children with ASD have best results in visual- perceptual tests, in which these subjects often demonstrate good skills. Other high-functioning subjects, especially in children previously diagnosed with a diagnosis of Asperger Syndrome, conversely, show lower peaks in non-verbal skills, in particular in visual-perceptual tasks, and better verbal skills. In general, individuals with ASD are characterized by atypical information processing skills (social, linguistic, sensory), and these anomalies raised the question of how best assess intellectual abilities of these subjects. This led some Authors to speculate that, because of its unusual nature and the lack of appropriate assessment test, the intelligence of subjects with ASD in some cases may be underestimated (Soulières et al., 2011).

Children with ASD can also show low cognitive flexibility, and deficits in planning, inhibitory control, attention shifting, monitoring, generativity and working memory

A small minority of children with autism (approximately 6%, O'Connor et al., 1988) show extraordinary abilities, for example they can have strong musical sensibility, exceptional computational skills, memory for numbers or dates, or unexpected talents as in drawing, reciting or playing music.

In addition to core symptoms, children with ASD often (70%) have comorbidity with other psychiatric disorders: more frequently anxiety disorders (42-56 %), depression (12 -70 %) , obsessive-compulsive disorder (7-24 %) , psychosis (12-17 %) , attention deficit disorder and hyperactivity disorder (28-44 %) and oppositional defiant disorder (16-28 %) , substance use (<16 %) and eating disorders (4-5 %) (Simonoff et al., 2008; Lai et al., 2014; Matson et al., 2014). In particular, 43 % of children with autism has at least one anxiety disorder (Sukhodolsky et al., 2008). The higher levels of anxiety are associated with higher levels of IQ, language, and with the presence of higher levels of stereotyped behaviors. In general, high-functioning subjects seem to be more susceptible (or symptoms are more detectable). In children with higher cognitive level, anxiety is associated with a greater impact on social relations (the most common are the social anxiety disorder and generalized anxiety disorder). Depression is more common in adults and less in children, particularly in high-functioning

individuals with less social impairment. The obsessive-compulsive disorder shares with autism the presence of ritualistic behaviors and poses problems of differential diagnosis, although the presence of anxiety-causing and intrusive thoughts or obsessions, typically present in obsessive-compulsive disorder, is not detected in autism. The opponents behaviors frequently found in children with ASD may be a manifestation of anxiety, resistance to change, difficulty to put themselves into other people's shoes and to acquire the point of view of other people, lack of awareness of their own behavior and difficulties, lack of empathy and lack of interest in social compliance. Aggressive behaviours (<68%) are often directed towards caregivers rather than non-caregivers and could be a result of empathy difficulties, anxiety, sensory overload, disruption of routines, and difficulties with communication; self-injurious behaviours (<50%) instead are associated with impulsivity and hyperactivity, negative affect, and lower levels of ability and speech; they could signal frustration in individuals with reduced communication, as well as anxiety, sensory overload, or disruption of routines and could also become a repetitive habit.

The presence of disruptive behaviors or avoidant behaviors and stereotyped activity seems to be associated with lower IQ scores (Tureck et al., 2014). In addition, children with autism can often show apparently unprovoked and extreme mood changes and major difficulties in emotional control. In particular, a recent study (Mazefsky et al., 2014) reports that subjects with ASD more often use emotional control strategies in involuntary forms, which are typically maladaptive (eg. rumination and emotional arousal, or emotional paralysis) and those subjects most frequently have higher levels of psychopathology.

Epilepsy has an increased incidence in individuals with ASD than the general population and it is estimated that up to 1 /3 (8-30 %) of subjects with autism have seizures during life. Age of epilepsy's onset follows two peaks of incidence in the early years of life and in adolescence, and critical events can be of various types (complex partial seizures, absence or generalized tonic-clonic seizures). Comorbidity with epilepsy is more common in individuals with intellectual disability, especially among females (Jokiranta et al., 2014) or in individuals with genetic abnormalities, and often is associated with a poor outcome. The relationship between autism and epilepsy is examined in several studies, as it is considered by some Authors (Yunta-Muñoz et al., 2008) as a key to the common etiopathogenic hypothesis (eg. neuronal damage could be a cause of developmental impairment, and a epileptogenic focus; seizures or epileptic phenomena can disrupt the central nervous system's development and impact on subsequent functioning). In support of this hypothesis, some data confirm the presence of autistic traits in adults with epilepsy, especially in those suffering from temporal lobe epilepsy (Wakeford et al., 2013). In addition to the increased risk of epilepsy, the detection of abnormal EEG activity is not uncommon in individuals with autism.

Some individuals with ASD may be subject to disorders of physiological rhythms, such as sleep disorders (50-80 %, most often insomnia), food, bowel rhythm, altered sense of thirst, or autonomic dysfunction, such as excessive sweating, tachycardia, or irregular breathing. Other symptoms frequently found in individuals with ASD are gastrointestinal symptoms (9-70 %, such as chronic constipation, abdominal pain, diarrhea, gastroesophageal reflux, gastritis, esophagitis, celiac disease and inflammatory colitis), and immune dysregulation (< 38%, an altered immune function, which interacts with neurodevelopment, could be a crucial

biological pathway underpinning autism, that is frequently associated with allergic and autoimmune disorders).

Autism is a lifelong disability but its expression can be variable over time.
Severity and symptomatology of ASD vary greatly from individual to individual and in most cases tend to improve with age. Social interaction, in general, improves with age, while still maintaining atypical traits: high-functioning children tend to develop into adulthood an artificial and conventional form of social interaction (Wing et al., 1992), that can allow good results in the socio-occupational field, but creates major difficulties in the most intimate relationships. A small number of children with autism becomes even more detached with growth. Despite any improvements during development, several follow-up studies suggest that the diagnosis of ASD remains stable over years to adulthood (Lord et al., 2006; Moore et al., 2003; McGovern et al., 2005; Kocovska et al., 2013). There is agreement that the disorder's pervasiveness in adulthood leads to a limitation in autonomy and social life, although over years is detected increasing variability of psychopathology (Billstedt et al., 2007). In addition, many difficulties may arise during adolescence or early adulthood, and other psychiatric conditions can overlap. Co-occoring conditions, most commonly anxiety, depression, obesity, and drugs use, are common in adults with autistic disorder (Eaves et al., 2008). Although in recent years the autism prognosis has improved and some adults, particularly high-functioning subjects, acquire a state of independence, most of them are not self-sufficient, their communication is poor, and the presence of stereotypes behaviors or interests persists into adulthood (Howlin et al., 2004), so 58–78% of adults with autism have poor or very poor outcomes in terms of independent living, educational attainment, employment, and peer relationships. The mean proportion of adults with autism in employment (regular, supported, or sheltered) or full-time education is 46%. A recent meta-analysis (Woolfenden et al., 2012) showed that subjects with ASD have a mortality risk that is 2-8 times higher than that of unaffected general population, of the same age and sex. This difference is mostly related to co-occurring medical conditions.

Clinical assessment

Diagnostic evaluation should be multidisciplinary and should use a developmental framework composed by an interview with parents or caregivers to collect information about behaviour in community settings (ie., home, school, etc), direct assessment of the child and of his play, interaction and communication skills, cognitive assessments, and a medical examination (Ozonoff et al., 2005). Co-occurring conditions should be carefully screened. The interview with parents should cover all history's step of the child (gestational, birth, developmental, and health history) and family medical and psychiatric history. Clinician should assess specific foci of the disorder: the development of social, emotional, language and communication, cognitive, motor skills, the sensory profile and unusual behaviours and interests. A standardised structured interview should be incorporated into the investigation process (eg, ADI-R). Adaptive skills also should be evaluated by standardised instruments (eg, Vineland adaptive behaviour scales). In direct observation is also useful the parent-child interaction assessment, because parents are relevant for the planning of interventions. The direct assessment of the child should be interactive and engaging to enable evaluation of social-

communication skills, in both structured and unstructured contexts and in a peer environment. Informations should be acquired with standardised instruments (eg, ADOS-G, CARS). Cognitive assessments of intelligence and language are essential; standardised, age-appropriate, and development-appropriate instruments should be used to measure both verbal and non-verbal ability. Neuropsychological assessments are also helpful for individualized diagnosis and service planning.
A medical and instrumental examination is important in view of the high frequency of comorbidity. Physical and neurological evaluation (eg, head circumference, minor physical anomalies and skin lesions, and neuro-motor functions) and genetic analyses (eg, karyotype analysis, FMR1 testing, and chromosomal microarray analysis) should be done. Other laboratory tests can be done as necessary (eg, EEG, neuroimaging, thyroid hormones testing, celiac disease testing and neurometabolic profiling).

Brain development and atypicalities

Neurobiological investigations about autism aetiology have helped to create an impressive number of studies in literature.
Studies have identified several atypicalities in brain of subjects with ASD and possible neuroanatomical, cellular, and molecular underpinnings aetiological basis of autism have been identified. Since MRI is the method of choice for in vivo and non-invasively investigating human brain morphology, this neuroimaging tool has been used for a lot of studies about development of brain in children and adolescents with ASD.
An old paper by Brambilla et al. (2003) reviews all structural MRI studies that investigated brain anatomy in autistic patients from 1966 to 2003, in order to elucidate brain anatomy and development of autism. Another review of neuroimaging literature in autism, from 1985 to 2008, by Verhoeven et al. (2009), confirms some of evidences in brain anatomy in autistic patients, particularly in size of the cerebellum, caudate nucleus, thalamus, amygdala and of the corpus callosum.
Several structural abnormalities, involving total brain volume, the cerebellum and corpus callosum have been consistently replicated, suggesting the existence of morphometric abnormalities in several brain structures in autism, even though some findings have often been controversial. However the available evidence suggests in subjects with ASD the existence of a disturbed neural network, involving cortical and subcortical areas, temporo-parietal cortex, limbic system, cerebellar, and prefrontal regions. In according to Brambilla et al. (2003), hypothetically, abnormalities of these structures might be relevant underlying neuroanatomical basis of some of impaired abilities in ASD, such as altered responses to emotional clues, information processing, social and higher cognitive functions.
Increased total brain, parieto-temporal lobe, and cerebellar hemisphere volumes were the most replicated abnormalities in autism, instead others studies suggested that the size of amygdala, hippocampus, and corpus callosum may also be abnormal.
A meta-analysis by Stanfield (2008) reported, although the considerable heterogeneity of results across the studies included, findings about enlarged total brain volume, hemispheres,

cerebellum, and caudate in ASD, and decreased volumes in other brain regions including midbrain regions, regions of the cerebellar vermis, and area of the corpus callosum (gray and white matter volumes were not reported separately in this meta-analysis).

ASD display significant heterogeneity, both in clinical and neurobiological manifestations. Although most neuroimaging studies in ASD have been designed to identify commonalities among affected individuals, rather than differences, some studies have explored variation within ASD (Lenroot et al., 2013). Findings have implicated many regions with prominent roles in social cognition, such as the superior temporal sulcus, amygdala, and insula. About age-related differences, volumetric and functional atypicalities appear to be more pronounced in younger individuals, with a tendency toward larger volumes earlier in life. With maturation these differences decrease in magnitude, such that by adolescence summative measures such as total brain volume are not significantly different than controls. The few structural imaging studies explicitly designed for gender comparison have generally not found significant differences in the pattern of abnormalities, except for the likelihood of females to show more pronounced brain differences than males.
Clinically defined categories in neuroanatomical studies have included subgroups such as Aspergers's versus narrowly defined Autism, ASD with and without significant language impairment, and low-functioning versus high-functioning autism. None of these comparisons have provided a strong case for a neurobiologically robust and distinct subtype, which is not to say variation along these clinical dimensions is not of ongoing interest. The relationship of intellectual disability to the pathophysiology of ASD has continued to be a challenging issue, complicated by the fact that brain imaging studies of individuals with significant cognitive impairment are very difficult to carry out, and so samples including these subjects are often underpowered. This has created a situation where despite the predominance of intellectual deficits in ASD, most imaging studies, particularly those with the sample sizes necessary for multivariate analyses, are carried out in ASD individuals with normal or near-normal IQ.

Increased brain volume

Enlarged total brain area and volume have consistently been reported in individuals with autism, after adjusting for height, IQ, and intra-cranial volume (ICV). Also Kanner, in his original paper in 1943, described "large head" in some of the children diagnosed with autism. This observation in combination with increased fronto-occipital head circumference appears to be one of the most consistent neurobiologic findings in autism (see as example Bailey et al., 1993; Aylward et al., 2002). Up to 15% of patients with ASD show macrocephaly (defined as head circumference greater than the 97th percentile).
Brain overgrowth correlates with the measureable increase in rate of growth of head circumference during the first few years of life (Hazlett et al., 2005). But, although macrocephaly is common in children and adults with autism, it isn't common at birth: it appears to develop after birth in 90% of cases, as an abnormal postnatal brain overgrowth, both in white and gray matter (Courchesne et al., 2001).

Courchesne indicated that by age 2–3 years over 90 % of autistic toddlers exhibited abnormally larger total brain, and they had more cerebral (18 %) and cerebellar (39 %) WM, and more cerebral cortical GM (12 %) than normal boys.

In two reviews by Courchesne (2004) was shown that abnormal brain overgrowth occurs during the first 2 years of life in children with autism. Head circumference, an accurate indicator of brain size in children, was reported to jump from normal or below normal size in the first postnatal months in autistic infants, to the 84 th percentile by about 1 year of age; this abnormally accelerated growth was concluded by 2 years of age. Infants with extreme head growth fell into the severe end of the clinical spectrum and had more extreme neuroanatomical abnormalities.

In a quite recent longitudinal study of brain growth in toddlers (beginning at 1.5 years up to 5 years of age) with autism compared with neurotypical toddlers, Schumann (2010) reported that by 2.5 years of age, both cerebral GM and WM was significantly enlarged in toddlers with autism, with the most severe enlargement occurring in frontal, temporal and cingulate cortices. In the longitudinal analyses, all regions (cerebral gray, cerebral white, frontal gray, temporal gray, cingulate gray, and parietal gray) except occipital gray, developed at an abnormal growth rate in toddlers with ASD. Females with ASD displayed a more pronounced abnormal growth profile in more brain regions than males with the disorder.

Before 2 years of age brains of subjects with ASD show an abnormal growth trajectory. In a recent paper by Shen et al. (2013), was reported significantly larger total cerebral volumes in infants who developed autism spectrum disorder before 24 months of age, when compared both to children with typical development and to those with developmental delays. Age, gender and weight all seem to had significant effects on total cerebral volume: total cerebral volume increased with age from 6-9 months of age to 18-24 months of age, was higher in males than females and was higher for children who weighed more. ASD group shows a significantly faster growth trajectory of total cerebral volume than the other groups, and by 12–15 months, had significantly larger total cerebral volume. Infants who developed ASD had 7% larger total cerebral volume than low-risk typical infants by 12–15 months and 8% larger total cerebral volume at 18–24 months. There were no interactions with gender. Compared to children with typical developmental and those with developmental delays, infants who developed autism had also shown significantly greater extra-axial fluid at early ages (6–9 months, 12–15 and 18–24 months). Extra-axial fluid is characterized by excessive cerebrospinal fluid in the subarachnoid space, particularly over the frontal lobes. The amount of extra-axial fluid detected as early as 6 months was predictive of more severe ASD symptoms at the time of outcome.

Some Authors (Nordahl et al., 2011) examined the relationship between TBV and autism onset status in a sample of 2-4 years old children with ASD, with and without regression, compared with age-matched typically developing controls. An abnormal brain enlargement was most commonly found in males with regressive autism, instead brain size in boys without regression did not differ from controls. Retrospective head circumference measurements indicate that head circumference in boys with regressive form of autism is normal at birth but diverges from the other groups around 4–6 months of age. There were no differences in brain size in girls with autism. These results suggest that there may be distinct neural phenotypes associated with different onset forms of autism.

For boys with regressive autism, divergence in brain size occurs well before loss of skills is commonly reported, thus, a rapid head growth may be a risk factor for regressive autism.
By 2-4 years of age, the most deviant overgrowth is in cerebral, cerebellar, and limbic structures that underlie higher-order cognitive, social, emotional, and language functions. Excessive growth is followed by abnormally slow or arrested growth. From middle childhood (about 6-8 years) onwards, in fact, brain growing speed seems to fall below normal, so that in later childhood and adolescence, head circumference in subject with ASD appears similar (or smaller) than tipically developed subjects.
Subsequent longitudinal studies of hundreds of children and adults with ASD in fact documented volume enlargement during preschool years, most prominently in the anterior regions, followed by possible growth arrest or exaggerated losses later in childhood (see as examples Courchesne et al., 2011). Schumann et al. (2010), for example, reported that children with ASD show 10% greater white matter volume, 6% greater frontal gray matter volume, and 9% greater temporal gray matter volume at 2 years of age.
However a recent systematic review by Raznahan et al. (2013) suggests that differences in head circumference in ASD may be much more subtle than previously thought because of exaggerated differences to biased normative data in the database of general population head circumference growth curves, to the selection of control groups, as well as to a failure to control for head circumference confounders such as weight and ethnicity.
The abnormal development of brain growth in subjects with ASD thus might be characterized by increased rate of brain growth from early infancy (2-3 years old) through preschool period (particularly in frontal, temporal and parietal lobes, and cerebellum), followed by an abnormally slow cerebral and cerebellar volume increase during late childhood, puberty and adolescence.
Early brain over growth tends to be reported more in boys who have developmental regression than in other subgroups and might be a result of generalized physical overgrowth.
Several developmental processes may be contributing to brain abnormalities in autism, and hypothetically, brain enlargement in autistic children can occur as a result of several atypical developmental processes: increased neurogenesis and/or myelination, decreased neuronal apoptosis and/or increased growth of non-neural tissues (ie., glial cells or blood vessels).
These developmental abnormalities could be the result of gene mutations, inappropriate levels of neurotrophines, and environmental factors which, together or independently, are affecting brain development and leading to pathological states.

Neuroanatomical findings

In the posterior fossa, enlarged total volumes in cerebellum (also in gray and white matters) have been shown by several well-designed controlled MRI studies in children and young adult individuals with autism; cerebellar vermis is one of the most structures involved in autism aetiology: particularly in lobules VI–VII areas, where 87% of the patients show hypoplasia and 13% hyperplasia. A meta-analysis by Stanfield et al. (2007) confirmed the reduction in size of the lobules VI-VII of the vermis in subject with ASD. Cortical dysgenesis, with thickened cortices, high neuronal density, irregular laminar patterns, poor gray matter boundaries and decreased number and size of Purkinje cells have been reported in several studies about cerebellum abnormalies in brain of autistic subjects.

In the literature about neuroanatomy in ASD there is significant heterogeneity with respect to cortical thickness and cortical morphology, and sometimes data seem to show contradictory results depending on the age, IQ, and clinical severity of the study population. In ASD some studies support a pattern of very early overgrowth in cortical surface area and volume (<2 years of age), followed, throughout childhood and adolescence, by cortical dysmaturation, with evidence suggesting both exaggerated and impaired cortical thinning.
In a group of infants with ASD was shown, at the age of 2 years, an increased cortical volume and surface area (but not thickness) compared to controls (Hazlett et al., 2011). The rate of cortical growth between ages 2 and 5 years, instead did not differ between groups. These data suggest the implication of the prenatal and early postnatal periods as central to disease pathogenesis. In older age groups, many Authors have observed evidence of exaggerated cortical thinning in ASD: children ages 8–13 years show increased cortical thickness, particularly in the temporal lobe, as compared to age-matched controls. In a longitudinal study imaging, 2-years later on the same groups, children with ASD were found to show an exaggerated cortical thinning compared to controls (non-significant after controlling for multiple comparisons and variation in IQ) and that the degree of thinning correlated with the severity of symptoms (Hardan et al., 2006 and 2009).
Another study by Mak-Fan et al. (2012), confirms these data: it showed a similar pattern of increased brain volumes (cortical thickness, surface area, and gray matter volume) in children with ASD at 6–10 years, that then underwent exaggerated losses compared to controls, such that by 12–13 years of age, controls show greater volumes in all three measures.
Brain cortical regions proposed to play a role in social-communication skills have been a focus of a lot of investigations in ASD and several anomalies were found in any cortical regions.
For example thinning of several areas in the temporo-parietal region, particularly on the left side, has been shown in subjects with ASD at all ages (McAlonan et al., 2005; Scheel et al., 2011; Greimel et al., 2013). This region is thought to be central to the integration of social information and empathy, as well as selective attention to salient stimuli.
Boys with ASD show a significant asymmetry reversal in the inferior lateral frontal language cortex (wich in these subjects is 27% larger on the right side compared to controls, who have larger cortical language regions in the left hemisphere) (Herbert et al., 2002).
The orbital frontal cortex (in the ventromedial prefrontal region) is thought to play a role in sensory processing, goal directed behavior, adaptive learning, and attachment formation. Despite increased overall cortical thickness in the frontal region, subjects with ASD have specific reduction in cortical thickness, volume, and surface area in the orbital frontal cortex, which correlated with symptoms severity. Other frontal lobe structures showing reduced cortical thickness in ASD include the inferior and middle frontal gyri and the prefrontal cortex (Jiao et al., 2010; Hadjikhani et al., 2006).
The anterior cingulate cortex is a highly connected part of the social brain network, situated along the medial side of the frontal cortex, and has a role in self-perception, social processing, error monitoring, and reward based learning. Studies have shown both increases and decreases in volume and thickness of the anterior cingulate cortex in ASD (given that cortical regions may grow at different rates between individuals with ASD and controls, variation in

the age of groups may account for these inconsistencies) (Doyle-Thomas et al., 2013; Ecker et al., 2013).

Post-mortem studies on adults and adolescents with ASD, have shown a reduction in neuron number in the amygdala, fusiform gyrus, and cerebellum, and signs of persistent neuroinflammation, but no data are available on early development. One exception is a study on young children that showed significant increases (rather than decreases) in neuron number in the prefrontal cortex (Courchesne et al., 2011).

A recent study by Zielinski (2014), analyzed longitudinal changes of cortical thickness in autism compared to typical development. Authors assessed MRI of 97 subjects with ASD (3-36 years old), and found differences in some cortical regions thickness (bilateral inferior frontal gyrus, pars opercularis and pars triangularis, right caudal middle frontal and left rostral middle frontal regions, and left frontal pole) when compared to controls. Group differences in cortical thickness varied by developmental stage and were influenced by IQ. Differences in age-related trajectories emerged in bilateral parietal and occipital regions (postcentral gyrus, cuneus, lingual gyrus, pericalcarine cortex), left frontal regions (pars opercularis, rostral middle frontal and frontal pole), left supramarginal gyrus, and right transverse temporal gyrus, superior parietal lobule, and paracentral, lateral orbitofrontal, and lateral occipital regions. Authors suggest that abnormal cortical development in ASD undergoes three distinct phases: accelerated expansion in early childhood, accelerated thinning in later childhood and adolescence, and decelerated thinning in early adulthood. Moreover, cortical thickness abnormalities in ASD are region-specific, vary with age, and may remain dynamic well into adulthood. This hypothesis could be endorsed by another recent study on cortical thickness of subjects with ADS ranging from 7 to 25 years of age (Ecker et al., 2014). Authors reported that, when controlling for the effects of age, individuals with ASD show reductions in cortical thickness relative to controls, particularly in fronto-temporal regions, and also showed significantly reduced surface area in the prefrontal cortex and the anterior temporal lobe (a significant group × age interactions for both measures was observed also). Cortical thickness has been also related to ASD symptoms in a recent study by Doyle-Thomas (2013), who assessed effects of age and symptomatology on cortical thickness in individuals with ASD, between the ages of 7 and 39 years, in comparison to typically developing controls. An increased thickness in the rostral anterior cingulate cortex was associated with poorer social scores at Autism Diagnostic Interview-Revised (ADI-R). Additionally, a significant interaction between age and social impairment was found in the orbitofrontal cortex, with more impaired younger children having decreased thickness in this region. These results suggest that differential neurodevelopmental trajectories are present in individuals with ASD and some differences are associated with diagnostic behaviours.

About subcortical structures, the caudate nucleus has been shown to be enlarged in ASD, in a recent volumetric meta-analysis (Duerden et al., 2012), and interestingly, caudate volumes significantly correlated with ritualistic-repetitive behaviors (Sears et al., 1999), suggesting that it may be part of an abnormal neural network sustaining stereotyped behaviors.

Instead, a volume loss in the putamen has been shown in adults with ASD, but enlargement of the putamen has also been observed in younger populations (Hua et al., 2013).

Decreased hippocampal measures and enlarged amygdala volumes have been found in patients with autism (mostly highfunctioning), even after total brain adjustment, but not in all

studies. In fact, volume losses emerge in meta-analytic approaches (Nickl-Jockschat et al., 2012), but volume increase was noted in younger patient groups (Bellani et al., 2013). From a functional perspective, amygdala anomalies may account for impaired emotional perception and regulation.

Abnormally reduced areas of lenticular nucleus has been reported in subjects with autism, instead no abnormalities in size of thalamus, and globus pallidus were reported. Several studies in brains of subjects with ASD reported no findings for size abnormalities in some regions, such as brainstem, some basal ganglia, and ventricles, suggesting that these structures are anatomically preserved; however, the absence of volumetric abnormalities does not exclude the existence of functional impairments sustaining a possible role in the pathophysiology of the illness.

White matter findings

In subjects with ASD, some meta-analyses suggest no differences in overall white matter volume in adults, although early white matter volumetric overgrowth may occur in younger patient samples.

Earlier volumetric analyses in fact suggested a pattern of accelerated white matter growth and increased white matter volume in younger children, particularly in the frontal regions, instead in adolescents with ASD similar or reduced white matter volume compared to controls was found (Courchesne et al., 2004). In the frontal and temporal lobes, in 2-4 years old children with autism, there have been reports of abnormal increases in gray and white matter, reduced metabolic measures and deviant diffusion tensor imaging results in white matter.

A meta-analysis of studies on white matter volume in older children (>6 years), adolescent and adults groups reported that, while global white matter volumes were not different, evidence of increased volumes in regions relevant to language and social cognition were found (Radua et al., 2011).

Looking at specific white matter regions, volume losses have been noted in the corpus callosum and cingulum.

In a quite recent investigation about the neurodevelopment of GM and WM in autism, a combined protocol of voxel-based morphometry (VBM) and diffusion-weighted imaging (DWI) was applied in 20 children (age range: 4-14 years) with autism and in matched controls (Mengotti et al., 2011). Compared to normal children, those with autism had significantly: (1) increased WM volumes in the right inferior frontal gyrus, the right fusiform gyrus, the left precentral and supplementary motor area and the left hippocampus, (2) increased GM volumes in the inferior temporal gyri bilaterally, the right inferior parietal cortex, the right superior occipital lobe and the left superior parietal lobule, and (3) decreased GM volumes in the right inferior frontal gyrus and the left supplementary motor area. Abnormally decreased apparent diffusion coefficients (ADC) of water molecules values in the bilateral frontal cortex and in the left side of the genu of the CC were also reported in autism. Finally, age correlated negatively with lobar and callosal ADC measurements in individuals with autism, but not in children with normal development. Authors concluded that these findings support cerebral dysconnectivity theory in autism, coupled with an altered WM maturation trajectory during childhood, potentially taking place in the frontal and parietal lobes, which may represent a

neurodevelopmental marker of the disorder, possibly accounting for the cognitive and social deficits.
Regarding diffusion tensor imaging studies, a recent systematic review (Aoki et al., 2013), about DTI data from 14 studies, from 1980 to 2012, including both children and adults with ASD, summarized some areas of consensus in the literature: decreased FA was most consistently demonstrated in the corpus callosum, left uncinate fasciculus, and left superior longitudinal fasciculus of individuals with ASD. Mean diffusivity was increased in the corpus callosum, and bilaterally in the superior longitudinal fasciculus. These data emphasize important roles of the superior longitudinal fasciculus, uncinate fasciculus, and corpus callosum in the pathophysiology of autism spectrum disorders.
However few studies have been conducted in very young children, and in this age range less consistency emerges in the data available in literature.
Contrary to data in older populations, some Authors reported that FA was greater for children with ASD ages < 6 years compared to controls in the areas of the corpus callosum, superior longitudinal fasciculus, and cingulum (Weinstein et al., 2011), and in this age range an accelerated white matter maturation (marked by increased FA and reduced displacement values) was found, most prominently in frontal regions, in a sample of children with ASD (Ben Bashat et al., 2007).

Evidences from electrophysiology, functional neuroimaging, structural neuroimaging, molecular genetics and information processing have given rise to the idea that autism is characterized by atypical neural connectivity, rather than by anomalies in different brain regions. So, in recent years, there is an abundance of literature on functional connectivity in ASD.
The synchronization is used in functional MRI (fMRI) studies as a measure of the degree of functional connectivity between brain regions and is defined by the correlation of activation levels in two activated areas over a time period. Anomalies in the synchronization between the frontal and parietal regions have been observed in autism in fMRI studies. This has led to the theory of antero-posterior underconnectivity or long-distance dysconnection of autism (Just et al., 2007).
Ideas about the precise way in which connectivity is atypical vary in studies, from decreased fronto-posterior and enhanced parietal-occipital connectivity, reduced long-range and increased short-range connectivity, to temporal binding deficits.
Although yet consistency about connectivity is low, (findings depend on the definition of connectivity, the developmental stage of the individual, the spatial and temporal scales, task vs no-task conditions, how motion artifacts are handled, and specific neural systems of concern), data support the hypothesis that neural networks in autism are atypical in various ways.
An emerging hypothesis based on data from fMRI, suggests that in ASD brains are characterized by an abnormal functional connectivity between cortical areas, with increased local connectivity but decreased global connectivity.
Several studies in adults have reported that functional connectivity between brain areas engaged during cognitive tasks is weaker in ASD, leading to the "under-connectivity theory" of autism (Just et al., 2012). fMRI data reveal hyper-connectivity in subcortical networks and

hypo-connectivity in cortical networks in adult males with ASD. Regional inter-connection circuits are damaged in individuals with ASD (especially connections of coordination and integration between the anterior and posterior regions, that are more susceptible). In fact, in functional MRI of individuals with ASD, the activation of frontal areas and posterior areas is much less synchronous than in controls.

Under-connectivity however has not been the only finding, because some fMRI studies show evidence of increased connectivity or altered developmental trajectories with respect to integrated neural networks. For example, a recent meta-analysis of fMRI studies (Dickstein et al., 2013) found that children and adolescents with ASD (< 18 years) versus adults with ASD (>18 years) had significantly greater hyperactivation in the left post-central gyrus (and hypoactivation in the right hippocampus and right superior temporal gyrus) in response to a social task, suggesting that the neural alterations associated with ASD are not static, occurring only in early childhood. Instead, children with ASD have an altered neural activity compared to adults during both social and nonsocial tasks, especially in fronto-temporal structures.

However despite the early developmental origins of this disorder and its variable developmental trajectory, almost all of the current literature on brain connectivity has focused on adolescents and adults with ASD, rather than children. Smaller studies in younger age groups suggest important age effects regarding the connectivity hypothesis as well, with younger children with ASD seemingly showing more "over-connectedness" than adults.

While hypoconnectivity seems most prevalent in the literature for adults subjects, Uddin et al. (2013) observed long-range hyperconnectivity across remote regions in children ages 7–12 years with autism compared to controls. Hyperconnectivity was noted to involve the default mode network, fronto-temporal, motor, visual, and salience networks. Hyperconnectivity of the salience network (which involves the anterior cingulate and insula) was most predictive of the diagnosis of ASD and was able to discriminate between cases and controls with 83% accuracy, a finding that was reproduced in a separate image dataset.

In a recent study (Superkar et al., 2013), in which results are replicated in 3 independent cohorts (110 children, age 7-13 years), Authors show that brains of children with ASD it is widely hyperconnected. Using neuroimaging techniques ("task-free" fMRI), Authors in fact report an increased connectivity in brains of children with ASD compared to typically developing children. Hyperconnectivity was detected between both near and distant anatomical regions, thus the increased connectivity in children with ASD involves both short fibers circuits and long fibers circuits. Results of this study suggest that in children with autism, in contrast to findings from adults, there is evidence of a hyperconnectivity rather than a functional hypoconnectivity, suggesting the hypothesis about the existence of possible developmental trajectories altered in subjects with autism compared to typical neurodevelopment.

The literature in very young patients with ASD is relatively sparse but seems to suggest altered developmental trajectories for affected children beginning at very young ages.

A recent publication (Keehn et al., 2013) reported increased functional connectivity at 3 months, which disappeared by 12 months in high risk infants. Alternatively, Redcay (2008) found increased connectivity between hemispheres in 2–3 year old children with ASD compared to chronological age matched controls (however the opposite pattern emerged when they were compared to mental age matched controls).

One of the earliest signs of autism is enlarged head circumference or macrocephaly and infants and young children with ASD show signs of early brain overgrowth; also postmortem studies of children with ASD show that they have an overabundance or excess numbers of neurons in the prefrontal cortex and animal models of autism have provided evidence for hyper-connectivity at very early time points in development (Testa-Silva et al., 2011). These findings of macrocephaly and hyper-connectivity have yet to be reconciled with human neuroimaging studies in early ages children with autism.

Hypothetically neocortical dysgenesis, marked by atypical patterning of cortical minicolumns (reduction in size, increased neuronal density, and increase in cell dispersion) is potentially associated with atypical synaptogenesis and an imbalanced excitatory-to-inhibitory ratio, both of which are important for neural connectivity (Lai et al., 2014).

Interaction between the immune and the nervous systems is substantial throughout life and frequency of immunological anomalies is increased in individuals with autism and their families. Thus in autism, altered immune processes could affect several neurodevelopmental processes (eg, neurogenesis, proliferation, apoptosis, synaptogenesis, and synaptic pruning), with persistent active neuroinflammation, increased concentrations of pro-inflammatory cytokines in serum and cerebrospinal fluid, and altered cellular immune functions. Neuroimmune mechanisms could have key roles in some aspects of the pathophysiology of autism, but the exact biology awaits clarification (Lai et al., 2014).

A brief review on structural connectivity, recently performed by Zikopoulos (2013), highlights at multiple levels (on macro and micro scales), the atypical brain connectivity in autism, that affects in distinct ways short- and long-range cortical pathways, disrupting neural communication and the balance of excitation and inhibition. Converging evidence from genetic, functional, and structural studies suggests that there are changes in excitatory and inhibitory neural communication in ASD and in the structure of the underlying cortical circuits or networks. At the microscopic and synaptic level, numerous genetic studies have highlighted a large variety of polymorphisms and epigenetic factors that primarily affect axons growth, synapse formation, and synaptic transmission of excitatory and inhibitory neurons. At the level of the network, most imaging studies have also focused on affected brain systems by identifying abnormalities in the GM and WM, primarily in frontal and temporal lobes, or in their major pathways. Studies performed at cellular level, have described changes in the cytoarchitecture, density and neurochemical features of excitatory and inhibitory neurons in frontal and temporal areas in autism. Imaging studies in children and adults with autism, show decreased functional connectivity between frontal and other brain areas, and gross changes in the structural integrity of GM and WM, particularly in frontal lobes. Typical findings in the WM include lower fractional anisotropy (FA) and higher radial diffusivity in ASD groups than in controls, which may come about by a reduction of diffusion barriers between axons. These findings suggest decreased axon diameter and/or decreased myelination that reduce axon volume, and may result in changes in the density of axons.

A wide range of data sources (MRI, EEG, etc) reported the presence, in ASD, of relatively high levels of short-range (functional and structural) brain connectivity, concomitant with low levels of long-range connectivity, and this theory represent one of the best replicated and best-supported findings in the study of autism (Crespi, 2013). The causes of relatively reduced long-range connectivity in autism remain the subject of intense study, but appear to include,

among other causes, larger overall brain size, especially in early childhood, alterations to cortical minicolumns, increased dendritic spine density, and genetically-based reductions in development of long-range connections. In typical development, from infancy to early adulthood, there is a functional and structural shift from short-range connectivity to long-range connectivity, in association with early overproduction of neurons and synapses, differential pruning of relatively short-range connections, and increasing myelinization. Findings on connectivity in autism in comparison with that one in typical development, concord with a model of "heterochronic development" in ASD, representing, at least in part, the result of slower or incomplete connectivity-pattern maturation. Crespi proposed a developmental heterochronic model, with regard to changes in short-range structural and functional brain connectivity. In this specific model, autism involves a slower rate of pruning for short-range connections. Neurodevelopmental variation salient to this process may also involve cortical volume and early short-range connectivity, that are greater in autism than in typically developing individuals.

Sex differences

The male-to-female ratio is about 4:1 (Fombonne, 2003). The female-to-male ratio is skewed even more in individuals with the highest levels of intellectual capacity, to about 11:1 (Gillberg et al., 2006). Among individuals diagnosed with an ASD without any physical or cerebral abnormalities as measured by MRI the ratio has been estimated to be as high as 23:1 (Miles and Hillman, 2000).

Mechanisms for the sex difference are ascribed either to a genetic (eg., overexpression of sex chromosomal genes due to mono- or polysomy, sex-specific Y-linked genes, sex-specific expression of X-linked genes, mosaicism and skewed X-inactivation, aberrant X chromosome imprinting), environmental mechanisms (eg., related to maternal immune activation, or parental age) or to a sex-related hormones etiology (eg., due to prenatal gonadal hormones).

In typical development there is an early sexual dimorphism of brain volumes (Gilmore et al., 2007), characterized by a neonatal enlargement of intracranial volume, cortical and subcortical GM, cortical WM in males as compared to females and by a prefrontal and occipital asymmetry (left hemisphere-greater than-right hemisphere) more pronounced for females than males.

A recent meta-analysis quantified current literature regarding sex differences in typical human brain morphology (Ruigrok et al., 2014). In a wide age range, from newborns to individuals over 80 years old, differences in overall brain volumes were found between males and females. On average males have larger intracranial volume (12%), total brain volume (11%), cerebrum (10%), GM (9%), WM (13%), cerebrospinal fluid (11.5%) and cerebellum (9%) absolute volumes than females. At a regional level, males on average have larger volumes and higher tissue densities in the left amygdala, hippocampus, insular cortex, putamen; higher densities in the right VI lobe of the cerebellum and in the left claustrum; and larger volumes in the bilateral anterior parahippocampal gyri, posterior cingulate gyri, precuneus, temporal poles, and cerebellum, areas in the left posterior and anterior cingulate gyri, and in right amygdala, hippocampus, and putamen. Females have on average higher density in the left frontal pole, and larger volumes in the right frontal pole, inferior and middle frontal gyri, pars

triangularis, planum temporale/parietal operculum, anterior cingulate gyrus, insular cortex, and Heschl's gyrus; bilateral thalami and precuneus; the left parahippocampal gyrus and lateral occipital cortex (superior division). The results from the regional volume and density analysis mostly include areas that are part of the limbic and language systems.

Biological sex may contribute significantly to the heterogeneity in autism and sex differences in the behavioral presentation of autism in boys and girls were reported. Growing evidence suggests in fact that females with autism differ from males at multiple levels. Some studies report no sex differences in cardinal autistic behavioral characteristics after controlling for IQ and cognition, but data are highly inconsistent (Solomon et al., 2012; Lai et al., 2011; Lai et al., 2012; Mandy et al., 2012).
While no sex differences were found in the broad social criteria presented in the DSM-IV-TR or DSM-5, numerous differences were evident in how boys and girls came to meet each criterion and in the absence of intellectual impairment ASD is diagnosed both less and later in females. Behaviorally, females may go undetected due to a 'non-male-typical' presentation or a greater ability to camouflage their difficulties (Baron-Cohen et al., 2011; Kopp and Gillberg, 2011). For example, according to data recently published (Hiller et al., 2014), girls were more likely to show an ability to integrate non-verbal and verbal behaviors, maintain a reciprocal conversation, and be able to initiate, but not maintain friendships. Moreover, girls presented with both less and different restricted interests. Teachers also reported substantially fewer concerns for girls than boys, including for externalising behaviors and social skills. Results suggest that girls with ASD may present a clinical manifestation a little bit different from the classic presentation of ASD.

Few studies analyzed developmental sex differences in brains of ASD subjects, especially in the infant population.
Females with autism have been found to differ from males with autism at the levels of early brain overgrowth (Sparks et al., 2002; Bloss and Courchesne, 2007; Schumann et al., 2010; Nordahl et al., 2011).
In the first longitudinal MRI study of brain volume growth during early ASD childhood, Schumann (2010) accounted for the presence of a gender effect on brain volumes. His analysis reveals that ASD females present an abnormal growth of whole brain and specific anterior regions when compared to typical females and that this finding is more prominent than in ASD males versus typical males. An abnormal brain enlargement was most commonly found in males, particularly with regressive autism, instead there were no differences in brain size in girls with autism (Nordahl et al., 2011). Shen et al. (2013), reported that age, gender and weight all seem to had significant effects on total cerebral volume: total cerebral volume was higher in males than females and was higher for children who weighed more.
Recently, Lai (2013) reported neuroanatomical differences between males and females, in a high-functioning adults sample group. Regarding GM, males had larger volume than females in several brain regions, (distributed across the bilateral frontal and occipital poles, dorsomedial prefrontal cortices, sensori-motor cortices, superior temporal gyri, Heschl gyri, lingual and calcarine gyri, temporo-occipital and lateral temporal regions, precuneus, posterior cingulate cortices, superior cerebellar hemispheres and brainstem), instead females

had larger volume than males in other regions (involving left dorsolateral prefrontal cortex, supplementary motor area, primary somatosensory cortex, and bilateral orbitofrontal cortices, caudate, thalamus, fusiform, hippocampal and parahippocampal gyri, cerebellar vermis and hemispheres). For WM, males were larger than females bilaterally in the frontal, occipital and temporo-parieto-occipital junction regions, whereas females were larger than males in the cerebellum and brainstem, and bilaterally in posterior frontal lobe involving internal capsule and fibres from the body of CC. These findings suggest that aspects of the neuroanatomy of autism in high-functioning adults are sex-dependent and males and females may have different structural neurophenotypes.

Corpus Callosum

Anatomy, Embryogenesis and Development

Corpus callosum is the largest white matter structure in human brain and it is the main connection and information transfer structure. In fact it connects the left and right cerebral hemispheres and facilitates interhemispheric communication. It is the largest white matter structure in the brain, consisting of 200–300 million contralateral axonal projections.

The CC is the largest commissural interemispheric formations, which include the anterior commissure, (that connects the main structures of limbic system), and the hippocampal commissure, (that connects the temporal lobes, particularly the hippocampus). The CC, even though its size may vary considerably, has approximately 300 million fibers connecting several cortical areas of both hemispheres (Barr et al , 1995; Paul L. et al, 2007 ; G. Lanza , 1993; Osborn AG, 2007); according to other AA the number of fibers in the CC is around 190 million (Tomasch J., 1954). The CC is ~10cm in length and is C-shaped, in a gentle upwardly convex arch.

The CC is a massive structure located at the base of the interemispheric fissure, in sagittal orientation, curved at the front end and at rear end. The CC , in a median sagittal section, appears as a quadrilateral sheet of white color, composed by myelinated fibers oriented in almost horizontal direction, transversely stretched between the two hemispheres. Its fibers, penetrating within the hemispheres, are involved in the formation of the lateral ventricles roof, and then fibers radiate mostly in the semi-oval centers.

The upper or dorsal callosal surface is convex in sagittal section, in the front section instead appears concave. Immediately above the body of the CC, lies the interhemispheric fissure in which runs the falx cerebri, the anterior cerebral vessels. The dorsal surface of the CC is covered by a relief called median raphe and is covered with a thin layer of gray matter known as indusium griseum, which incorporates two bundles of fibers (medial and lateral longitudinal striae of Lancisi) that constitute part of the dorsal hippocampus and the limbic system. On either sides, he dorsal surface of the CC is separated from the cingulate gyrus by a sulcus (callosal sulcus) in which passes the pericallosal artery, branch of the anterior cerebral artery.

The CC ventral surface (or bottom) is concave in a sagittal view, and attached to its concave undersurface there is the septum pellucidum, anteriorly, and the fornix and its commissure, posteriorly. The ventral surface of the CC becomes part of the lateral ventricles of which form the roof. The splenium of the CC lies on the quadrigeminal plate of the midbrain and takes relationships with superiors tubercles and epiphysis.

The corpus callosum (CC) has a rich blood supply, relatively constant and is uncommonly involved by infarcts. The majority of the CC is supplied by the pericallosal arteries (the small branches and accompanying veins forming the pericallosal moustache) and the posterior

pericallosal arteries, branches from the anterior and posterior cerebral respectively. In 80% of patients additional supply comes from the anterior communicating artery, via either subcallosal artery or median callosal artery.

Fibers and subdivisions:

The CC is the largest white matter fiber bundle in the brain with more than 300 million axons connecting the corresponding areas of the 2 hemispheres. Although many of these fibers connect homologous/mirror image areas of cortex, there is a significant proportion of asymmetry and a number of heterotopic fibers also asymmetrically link functionally different cortical areas. The CC fibers form the so-called radiation of the CC. These consist of all the fibers originating from various areas of the telencephalic cortex, that are directed medially to cross the midline and pass in the contralateral hemisphere. These fibers take on a characteristic curved course to the front and rear of the CC, giving rise to two arch formations called, respectively, of forceps minor or frontalis (anteriorly, given by the fibers that connect the frontal lobes) and forceps maior or occipitalis (posteriorly, given by the fibers that connect the occipital lobes). Some fibers form a thin white matter layer, the tapetum, above the temporal horns of the lateral ventricles, which connects the ventral surfaces of the temporal lobes.

Proceeding in antero-posterior direction, thus in the CC could be identified the following portions (Raybaud C. et a., 2010; Barr et al., 1995):
1. The rostrum, so named for its resemblance to a bird's beak, is localized in the anterior inferior part of the CC, continues in the terminal lamina (that delimits anteriorly the third ventricle), and its fibers connect the frontal cortex;

2 . The genu, forming the frontal end of the CC, curves gently following the lower limit of the frontal lobe; it is the greater density of fibers portion of CC, and connects the prefrontal cortex and the area of the anterior cingulate;

3 . The body, constitutes the main portion and is the compact part of the CC; changes in the size of the body may be related to the preferential use of one hand, and its fibers, larger and less dense, connect the pre-central motor cortex, insula and cingulate gyrus;

4 . The isthmus, whose fibers connect the cortex of the pre-central and post-central gyri (motor and somatosensory areas) and the primary auditory area;

5 . The splenium, lying on the quadrigeminal plate, marks the posterior end of the CC, appears in the sagittal section, generally larger in women than in men; its fibers connect the posterior parietal, occipital medial and temporal medial cortex.

Due to the fact that there are no macroscopic anatomical landmarks that clearly delimit distinct callosal areas in a midsagittal cross-section, several geometric partitioning schemes have been designed to subdivide the CC and one of the most well-known is the Witelson subdivision (Witelson, 1989) that is the conventional partitioning schemes used to divide the CC into functionally significant regions. In fact, most studies rely on Witelson's classification, although the underlying data predominantly originates from non-human primates. In

particular, the CC is subdivided by Witelson into five regions, based on arithmetic fractions of major antero-posterior axis. Subdivisions include the anterior third, the anterior and posterior midbody, the posterior third, and the posterior one-fifth. Fibers of the anterior third (including the rostrum, genu, and rostral body) are assigned to prefrontal, premotor, and supplementary motor cortical areas. Fibers originating in the motor cortex are assumed to cross the CC through the anterior midbody, whereas somaesthetic and posterior parietal fiber bundles cross the CC through the posterior midbody, and fibers of the posterior third (including the isthmus and splenium) are assigned to temporal, parietal, and occipital cortical regions. It should be noted, however, that neither Witelson's classification nor other geometric partitioning schemes exactly mirror the texture of the CC at the cellular level.

The fibers of the anterior body are transversely oriented. Fibers projecting from the genu and splenium tend to arch more anteriorly and posteriorly, forming the forceps minor and forceps major, respectively. Projections from the splenium, which pass inferiorly along the lateral margin of the posterior horn of the lateral ventricle to the temporal lobes, are easily identifiable in midsagittal plane by their right–left orientation. Near the cortex, callosal fibers interdigitate with the association and projection fibers and are difficult to delineate.

Conventional MRI of the human CC does not reveal morphologically discernable structures to distinguish subregions, although microscopy techniques help to identify myelinated axons with a relatively small diameter in the anterior and posterior third of the CC as opposed to thick fibers in the midbody and posterior splenium. Recently, knowledge about the anatomo-functional topography of the human CC has been revolutionized by the technique called tractography by diffusion tensor imaging (DTI), that allows to measure the location, orientation, and anisotropy of particular tracts within the white matter fibers.
In 2006 Hofer and Fahm applied DTI technique in conjunction with a tract-tracing algorithm to gain cortical connectivity information of the CC in individual subjects. With DTI-based tractography, they distinguished five vertical segments of the CC, containing fibers projecting into prefrontal, premotor (and supplementary motor), primary motor, and primary sensory areas as well as into parietal, temporal, and occipital cortical areas. Thus they proposed a modification of the widely accepted Witelson scheme and a new classification of vertical CC partitions. Similar to Witelson, they defined a geometrical baseline connecting the anterior and posterior borders of the CC, then, in accordance with DTI fiber tractography, they distinguished five vertical partitions of the CC:
- Region I, as the most anterior segment covers the first sixth of the CC and contains fibers projecting into the prefrontal region.
- Region II, that is the rest of the anterior half of the CC, contains fibers projecting to premotor and supplementary motor cortical areas. Together, these callosal fibers occupy the largest subdivision of the CC, which extends far more posteriorly as compared to Witelson's scheme.
- Region III was defined as the posterior half minus the posterior third and comprises fibers projecting into the primary motor cortex. This finding is in clear contrast to Witelson's scheme, which postulates that primary motor fibers cross the CC in the anterior half.
- Region IV, the posterior one-third minus posterior one-fourth, refers to primary sensory fibers.

- Region V is cossed by callosal parietal, temporal, and occipital fibers, and is defined as the posterior one-fourth.

In summary, the new segmentation proposed by Hofer-Frahm, differs from Witelson's scheme mainly at the anterior tip and the broad midbody area. It has already been used as a template for several anatomical and MRI studies, especially when studying callosal fibers connecting to primary motor and sensory cortical areas (ie., Blumenthal et al., 2013; Wade et al., 2012; Ozalay et al., 2013, etc).

The highest density of fibers of large size (3-5 μm) is located in the posterior body, in the isthmus and splenius, and project to the motor and somatosensory areas; the highest density of small fibers instead is located in the genu and the rostrum, and connect to the prefrontal cortex and temporo-parietal associative cortex. The highly density thin fibers of genu and rostrum have a poor myelination and have a slower conduction speed, but their high density allows an highly refined topography mapping. In contrast, the large fibers of the posterior part of the body and splenius, have high conduction speed and are highly myelinated. These fibers make up a small percentage of all fibers of the CC (< 1%), and they have higher density in regions connecting auditory cortices, possibly because them role to facilitate the inter-aural comparison for auditory localization.

The area of the CC is positively correlated with the density of small fibers but not with that of the large fibers (Aboitiz et al. 1992). Thus, measures of the CC reflect axonal density in the genu and the anterior splenium, portions where fibers are more densely packed and connecting associative cortical areas. However, the area of the CC is not a meaningful indicator of axonal density of the posterior body and the posterior splenium, where fibers are less dense, and connected respectively to the primary motor cortex and the occipital lobe cortex. Although some of the CC connections are inhibitory (useful to hemispheres to inhibit each other, to facilitate the functioning of some independent functions), the majority of axons passing through the CC are excitatory, and facilitate the inter-hemispheric transfer (IHT) and the integration of information between the hemispheres.

Embryogenesis and development

Embryogenesis of the CC is a quick process that begins on the midline, between the 12-13th and the 18th gestation weeks, but first differentiation as a commissural plate within the lamina terminalis starts at 39 embryonic days (Sarnat et al., 1991).

According to a recent review by Paul (2011) there have been two main theories regarding the progression of callosal development in utero. For many years, the prevalent theory maintained that callosal axons first cross the midline toward the anterior end and callosal development proceeds posteriorly, with the rostrum added last. However, evidence from both earlier neuroanatomic literature and recent neuroimaging studies of human embryology indicates that callosal connections begin more centrally in the hippocampal primordium and the subsequent growth progresses bidirectionally both anterior and posterior, with more prominent anterior growth.

Callosal development involves a previous exuberant axon growth, followed by a period of axonal pruning, that extends from late in gestation through the first 2 postnatal months (Innocenti and Price 2005).

In the human embryo, the earliest callosal axons appear at 74 days, the genu and the splenium begins to be recognizable at 84 days, and adult morphology is achieved by 115 days (Loser et al., 1968).
The plate acts as a passive bed for axonal passage and provides a preformed glial pathway to guide decussating growth cones of commissural axons (Silver et al., 1982). Rudimentary CC fibers, during the proliferation stage, cross the midline to the other hemisphere by the 12th gestational week. They move along a connective tissue bridge, the lamina terminalis (Korkmaz - Njiokiktjien, 2013). This process is complete by 4 or 5 months, but without lamina terminalis the CC fibers do not cross the midline and the CC fails to form.
Growth of anterior sections is clear in the 14th–15th gestational week and development of posterior sections is clear by the 18th–19th gestational week. By birth, cross-sectional area of the splenium and genu are uniform (Barkovich,1988) and the splenial fibers have developed greater directional organization than the genu.
The anterior and posterior callosal sectors are among the most rapidly developing white matter structures in humans. Increases in callosal fiber direction and external axonal structures (for example myelin) are visible in neuroimaging by 4 months, with the most significant increase in external axonal structures appearing between 13 and 18 months of age (Morriss et al. 1999).
During the 3 months after birth, the size decreases, as a large proportion of the huge population of callosal axons is eliminated.
Throughout postnatal development, white matter maturation of the splenium generally precedes genu maturation. Thus splenium myelinates first and increases in thickness by 40% in the first year of age, with a further 110% increase in subsequent years. Around the 6th month of life, when the cerebellum and the internal capsule genu complete the myelination process, the CC is yet partially myelinated. Around the 8th month of life the CC genu myelinates; until the end of the first year of life, the CC does not acquire its classic signal intensity on MRI (hyperintensity on T1-weighted images and hypointensity on T2-weighted images). However the splenium begins to work at approximately 3.5 motnhs and parts of the body of the CC at about 5 months.
While total brain weight increases by 35% after the second year of age, the medial callosal area increases by 115%; CC in fact continues to increase in relative size between 6-10 years and 11-15 years (Mukherjee et al., 2001). Until age of 4 years of age CC grows in the rostrocaudal direction, and between 3 and 6 years there is a growth of the frontal fibers. At a later age one sees growth in temporal-parietal connections (isthmus).
By 11 years of age, both the anterior and posterior regions of CC, have reached 90% of their maximum fiber directionality, and by 20 years they have 90% of their maximum external axonal structures maturation. The final volume is achieved at around 6-9 years of life.

CC formation involves multiple steps, including correct midline patterning, formation of hemispheres, birth and specification of commissural neurons and axon guidance across the midline, and their final connection in the contralateral hemisphere. Much of data about the stages of callosal development comes from animal models. Several mechanisms have been proposed to regulate callosal formation and development, such as guidance by pre-existing axons and support by midline glial structures.

The first axons to cross the midline arise from neurons in the cingulate cortex. In mice, these pioneer axons cross the rostral midline, providing a path for the fasciculation of later-arriving neocortical axons. In humans, pioneer axons express the guidance receptor neuropilin 1, which can guide the axons themselves or the later-arriving callosal neurons from the neocortex.

Cingulate cortex neurons also project axons into the rostrolateral cortex, perhaps to initially guide neocortical axons towards the midline. In more caudal regions of the corpus callosum, the hippocampal commissure, which is formed earlier than the corpus callosum, may provide a growth substrate.

Multiple glial structures includingthe glial wedge, midline zipper glia and indusium griseum are present at the developing midline and are probably required for corpus callosum formation.

Guidance by the glial wedge occurs through both SLIT–ROBO and WNT–RYK signalling.

The indusium griseum glia expresses SLIT2 and guide callosal axons across the midline; in knockout mice, when the receptors of growth factors, fibroblast / glial fibrillary acidic protein (FGFR1/GFAP) is eliminated from glial cells, the CC fails to form; also when genes NFIA and NFIB are mutated the CC does not form. FGFR1 is also important for the migration and development of the midline glial structure and at different stages of formation and development of the CC.

After crossing the midline, callosal axons grow into the contralateral hemisphere towards their designated target region, usually homotopic to their region of origin, and then innervate the appropriate cortical layer. Such processes probably involve both molecular recognition of the appropriate target region and activity-dependent mechanisms that regulate axon targeting to the correct layer and the subsequent refinement of the projection. It is not yet clear whether the defects in axonal pruning modify the CC size and contribute to some callosal anomalies (eg, hypoplasia).

The adult form of the CC is already present between the 16th and 18th gestational week and is known to be highly genetically determinated, as studies have shown that its shape and size are very similar in twins. Differences in the size and form of the CC in adults have also been shown to be related to differences in hemispheric representation of cognitive abilities.

The fetal CC serves as a sensitive indicator for normal brain development and maturation (Achiron et al., 2001). A comprehensive evaluation of CC development during normal human fetal gestation is essential to detect and understand the congenital abnormalities within the fetal brain. As the CC is part of the highest order, latest maturing mental network of the brain, its measurements are important to assess normal brain development and to locate structural changes that may disturb cognitive skill development. Although prenatal detection of CC abnormalities has been widely reported, its normal in utero growth and development are scarcely documented (Pilu et al., 1993; Chasen et al., 1997).

Callosal cells are located in both supra- and infra-granular layers, depending on the species and area; most callosal neurons lie only in the column in which callosal axons from the other side terminate, thus a strong reciprocity seems to exist in each column. Many cells in the superficial layers send callosal projections to the homotopic site in the contralateral hemisphere, whereas most cells in the deep layers project mainly to heterotopic sites.

The CC is present from lower animals to highest primates and the phylogenetic increase in the size of the neocortex is followed by a corresponding increase in the size of the CC, thus the CC develops in proportion to the neocortex and reaches its highest development in the human brain. Some AA suggest that a loss of callosal connections might accompany the development of an enlarged brain in higher primates, as the macaque monkey.

Function

After 1970 we see an increase in interest in the CC. CC function in humans was classically investigated in classic studies of 'split-brain' patients, whose callosum is severed surgically for the treatment of epilepsy, and secondly studies involved individuals with developmental absence (agenesis) of the CC. Subsequently CC research entered the field of psychiatry, mostly focused on schizophrenia.

The main role of CC is to transfer information between hemispheric cortical regions, mostly homologous, allowing information filed in the cortex of one hemisphere is also available for the corresponding cortical area of the opposite hemisphere. Thus CC allows the hemispheric functional cooperation. It is the indispensable link for integrating the specialized functional activities of the right and left cortices. As example, a motor learning exercise with one hand, can be performed efficiently even with the other hand, because memory traces learned are transferred from one hemisphere to another thanks to the CC integrity. When CC is damaged, a new exercise learned with one hand can not be transferred, so it can not be executed by the contralateral hand.

Depending on its location within the CC, focal callosal damage can cause unique combinations of functional impairments and spared functional capabilities. The impaired functions can thus be ascribed to the cortical regions disconnected by the callosal damage, and the spared capabilities can thus be ascribed to the cortical regions with callosal connections spared by the damage.

CC injury determine mental disorders, with ideation incoordination, changes in the character and especially alteration in the execution of movements, due to the lack of information transfer from one hemisphere to the other (Krupa K. et al, 2013).

The interhemispheric connections of the cortical areas of the human brain are distributed within the CC according to a topographic order which is being studied in detail by novel imaging techniques. Total section of the CC is followed by a variety of interhemispheric disconnection symptoms each of which can be attributed to the interruption of fibers in a specific callosal sector. Disconnection symptoms deriving from posterior sections (disconnecting parietal, temporal and occipital lobes across the midline) are more apparent than those following anterior callosal sections (disconnecting the frontal lobes). In spite of the massive bulk of the frontal callosal connections in human brain, consequences of their interruption are limited to disorders of motor control, with particular regard to bimanual coordination (Berlucchi, 2012). An example of disorders related to CC lesions is given by the so-called "alien hand syndrome": in case of a frontal CC damage (eg., caused by stroke, trauma or surgical callosotomy), and therefore loss of interemispheric connection, it results in an intermanual conflict, characterized by involuntary movements in the hand "anarchist"

contrasting the voluntary activity of the agent hand. Frontal callosal disconnection causes the inability to learn movement patterns that require simultaneous, mutually adjusted movements of both upper limbs, such that in the movement of each limb, the action of the contralateral limb has to be continuously taken into account.
Another example of CC role in interemispheric information transfer is the spread of electrical activity and the effectiveness of surgical callosotomy in drug-resistant epilepsy: patients undergoing a callosotomy suffer a "disconnection syndrome" (or split-brain syndrome), characterized by sensory and neuropsychological deficits. After callosotomy patients will no longer be able to verbally describe an object, with eyes closed, held in the left hand, or if the object is kept in the left visual hemicampo (sensory information reaching the right hemisphere are not transferred to the left hemisphere where the function resides linguistics). This difficulty does not occur if sensory information come from the right side (and then reach the left hemisphere).
Section of the CC anterior portion causes memory impairment, disturbance in executive and cognitive functioning, behavioral disturbances and increase in response time to a stimulus (Peltier J. et al, 2012).
Although splenium correlates with language skills in typical subjects, in developmentally impaired populations both over- and under-development of this callosal subregion results in impairments of visuospatial skills, attention, and motor coordination (Paul et al., 2011). In addition to deficits in those skills, individuals with reduced posterior callosal connections also have subtle diminished processing speed during complex tasks, and social-skill impairments. This suggests that in addition to the general cognitive effects, callosal reduction has also a behavioral impact on social skills and other forms of rapid problem solving.
There is empirical evidence that not only callosal disconnection but also subtle degradation of the CC can influence the transfer of information and integration between the hemispheres. The reviewed studies on patients with callosal degradation with and without disconnection, indicate a dissociation of callosal functions: while anterior callosal regions were associated with interhemispheric inhibition in situations of semantic (eg., Stroop interference test) and visuospatial (hierarchical letters stimuli test) competition, posterior callosal areas were associated with interhemispheric facilitation from redundant information at visuomotor and cognitive levels.
Together, the reviewed research on selective cognitive functions provides evidence that the CC contributes to the integration of perception and action within a subcortico-cortical network promoting a unified experience of the way we perceive the visual world and prepare our actions (Schulte et al., 2010).

CC can be affected by various morbid events, congenital or acquired. Both genetic and environmentally caused birth defects often involve callosal malformations, with particular vulnerability in the posterior callosum.
Among congenital disorders have particular relevance the agenesis and the hypoplasia of the CC, characterized by absent or incomplete development of the callosal structure. Unlike patients who have undergone a callosotomy, individuals with agenesis of the CC show few symptoms of disconnection syndrome due to neuronal plasticity, that allows the construction of alternative pathways for information transfer. Rarely agenesis of the CC is an isolated

anomaly, instead often it is associated with agenesis or hypoplasia of other commissures, and in 80% of cases with other diseases of the CNS (eg., interhemispheric cysts, cortical malformations, meningeal dysplasia, holoprosencephaly, Chiari II malformation, Dandy-Walker syndrome, microcephaly, etc.). CC isolated congenital abnormalities are generally asymptomatic and highlighted only by neuropsychological tests. Other morbid events that can affect the CC are phacomatosis (eg., neurofibromatosis NF1, where you can find the so-called UBO, unidentified bright objects), or neurometabolic demyelinating diseases (eg., Alexander's disease, X-linked adrenoleukodystrophy, Menkes disease, Pelizaeus-Merzbacher disease, etc.), mitochondrial diseases (eg., MERRF syndrome), acquired demyelinating diseases (eg., multiple sclerosis, Marchiafava-Bignami disease), infectious-inflammatory diseases (eg., subacute sclerosing panencephalitis, streptococcal meningitis, Lyme disease), vascular lesions (eg., ischemia, vascular malformations), tumors (eg., glioblastoma, lymphoma, metastasis), and iatrogenic or traumatic events.

Several studies have examined and compared symptoms of the agenesis of the CC with ASD (Paul et al., 2007; Lau et al., 2012; Booth et al., 2011).

Agenesis and hypoplasia of the CC (AgCC) are congenital conditions (Paul et al., 2007), with genetic etiology, often found in many genetic syndromes, with known or unknown causative gene, or with environmental etiology (eg., alcohol exposure, hypothyroidism, prematurity, environmental deprivation). The phenotypic consequences of callosal agenesis are highly variable and include cognitive, neuropsychological, neurological and behavioral problems. Isolated AgCC does not appear to have a direct or dramatic impact on general cognitive ability. IQ scores frequently remain within the average range, even though full-scale IQ may be lower than expected based on family history and there is an unusual tendency for significant discrepancy between performance IQ and verbal IQ (in either direction). Among individuals with AgCC and normal range IQ, linguistic impairments and social impairments are common. Deficits in communication and social interaction in patients with AgCC frequently overlap with the diagnostic criteria for ASD. Subjects with agenesis of the CC show a wide variety of social symptoms, attentional and behavioral characteristics, that resemble those of some psychiatric disorders.
Psychiatric diagnoses are based on complexes symptom cluster, which probably involve multiple neurobiological mechanisms. Also data on atypical structural brain connectivity are present in almost all psychiatric disorders. For example, many studies reported alterations of the CC morphology in schizophrenia (both in the shape and in the size of the CC) and microstructural abnormalities in some callosal regions, detected by MRI diffusion. Some studies reported also the presence of complete agenesis of the CC in patients with schizophrenia.

In a study, 8,5% of subjects with AgCC received a diagnosis of autism (Doherty et al., 2006). Another recent study shows that 45% of 4-11 year old children with agenesis of the CC reaches clinical cutoff in screening tools for autism (Lau, 2012). Some Authors (Booth et al., 2011), comparing the "fractionable autism triad" with callosal agenesis symptoms, reported that in 10% of cases callosal agenesis symptomatology was "autism-like" and characterized by social immaturity, difficulty in establishing friendships, ingenuity, hyperfamiliarity,

difficulty in sustaining a conversation, atypia in the use and understanding of non-verbal communication, slight degradation of performance in cognitive tasks, emotional distress, alexithymia, poor humor. Symptoms attributed to the "third area" of autism instead were absent (ie., stereotypies, attention to detail, sameness, etc.). Communication deficits are also evident in comprehension of syntax and linguistic pragmatics (including idioms, proverbs and vocal prosody) and in phonological processing and rhyming.

Differences in the midsagittal area of the CC have been associated with a number of cognitive and behavioral phenotypes, including obsessive-compulsive disorders, psychopathy (often characterized by a larger callosal area; Park et al., 2011; Raine et al., 2003) and suicidal tendencies, bipolar disorder, schizophrenia, autism, and attention deficit hyperactivity disorder (often characterized by smaller callosal area; Cyprien et al., 2011, Arnone et al., 2008, Frazier and Hardan, 2009; Cao et al., 2010).

A general observation is that callosum is positively correlated with attention, regardless of how other comorbid conditions may impact callosal structure. Microstructural abnormalities of the CC were detected in patients with Tourette's syndrome and patients with attention deficit hyperactivity disorder (ADHD). Some studies have reported splenium reduction in children and adolescents with ADHD. In several clinical populations callosal size was compared in those subjects with and without comorbid ADHD (e.g., dyslexia, NF-1, and 22q deletion syndrome). Across conditions, the findings indicate that subjects with comorbid ADHD are likely to have diminished callosal size.

According to recent studies, several neuropsychiatric disorders would result from an abnormality in the connectivity development, which could be increased or decreased. Further studies are needed to define the pathophysiological mechanisms, to shed light on the cognitive and behavioral consequences of abnormal connectivity during development, and also to highlight the potential compensation due to the treatment. In most cases, individuals with autism, schizophrenia or ADHD do not have gross abnormalities of the CC. However, these disorders, as well as AgCC, could provide a model for studying brain connectivity and identify conditions that contribute to the cognitive and behavioral symptoms of these psychiatric illnesses. Since these complex disorders, particularly autism, are likely to have not a single etiologic explanation, identification of genetic and neuroanatomical models underlying aspects of these disorders, might be useful to explain the origin and inter-individual diversity of symptoms.
Overall, functions of CC are thought to be involved in motor and sensory integration as well as in higher cognitive functions and behavioral skills, including information processing, abstract reasoning, problem solving, ability to generalize, processing speed, working memory, planning, social skills, attention, arousal, language comprehension and expression of syntax and pragmatics, emotion and memory (Paul et al., 2007).

Although there is evidence to suggest that CC size is heritable in normal human populations (Scamvougeras et al., 2003), there is surprisingly little evidence concerning the genetic modulation of this key neuroanatomical and functionally critical part of the brain.

Sex differences

The prevalence, age of onset, and symptomatology of many neuropsychiatric conditions differ between males and females.

Several studies reported the presence of a sexual dimorphism in the human CC (Luders et al., 2014). However it is not yet clear whether CC morphological differences described in literature are related to differences in brain size, typically greater in males than in females, or are themselves linked to sex dimorphism.

Although various observations suggest that sexual dimorphism in callosal morphology exists, findings have been non consistently replicated. For example, discrepancies concern the affected callosal region and some studies reported sex differences for the splenium, for the isthmus, for the genu or for the entire CC. Disagreement also exists with respect to the direction of the sexual differences, with some studies reporting larger CC in men and other studies reporting larger callosal regions in women. Several studies also failed to detect any significant sexual difference. Study-specific criteria for callosal measurements may account for these discrepancies in results (Luders et al., 2010).

Some literature data (meta-analysis of Bishop and Wahlsten, 1997) report that callosal size, unadjusted to total brain volume, are greater in males than in females, but these differences disappear when it is taken into account also the total brain size. Subsequent studies however (Sullivan et al, 2001) show that, when CC sizes were corrected for total intracranial volume, males have a larger midsagittal callosal area than females, suggesting that sexual dimorphism of the CC is not simply an artifact due to the different size of the brain. Conversely, Tepest (2010) reported that the difference of the ratio of CC to TBV can be convincingly explained as a function of brain size per se, but not of gender, thus rejecting the sexual dimorphism hypothesis of the CC. In this study males had a significantly larger TBV than females and there were no gender differences in CC raw data, but the CC/TBV ratio was significantly larger in females than in males. Thus, these results are in accordance with the hypothesis that brain size, per se, is the relevant factor and contradict the sexual dimorphism hypothesis of the human CC.

In a recent study (Luders et al., 2014), Authors examine the CC morphology in adult male and female brains well matched for total intracranial volume (TIV). This study shows that the CC is larger in males than in females. However, this sex difference is strongly related to variation in brain size: the larger the discrepancy between the male and female brain volume, the greater the difference in the thickness of the CC. For example, when males with greater TIV (M extreme XL) are compared to females with lower TIV (F extreme XS), CC thickness is greater in males than females in all its extension, except in small portions of the genu and isthmus-splenium, where no significant differences were identified. These data suggest that individual differences in brain size are associated with CC anatomical differences in both sexes, and in particular increased brain size in males is associated with larger CC.

In an old study (Allen et al.,1991) Authors observed a dramatic sex difference in the shape of the CC, instead there was no conclusive evidence of sexual dimorphism in the area of the CC or its subdivisions. Subjective and mathematical evaluation indicated that the posterior region of the CC, the splenium, was more bulbous shaped in females, and more tubular-shaped in

males. However, sex differences in bulbosity did not reach significance in children (aged 2-18 years).

Sexual differences in hemispheric asymmetry and interemispheric connectivity may be underpinned not only by CC macro-structural differences but also by differences in the microscopic structure.

In an old DTI study (Shin et al., 2005) was found an increased T1 signal intensity and decreased FA in the females CC, when compared to males one.

A fairly recent study (Westerhausen et al., 2011), based on analysis of DTI parameters, showed in males increased FA and lower MD than females, in the CC frontal regions, indicating sexual differences in the micro-architecture of the callosal interemispheric connections, particularly in the frontal lobes. In a more recent study (Takao et al., 2013), Authors conduct a microstructural analysis of all cerebral WM of adult males and females, placing it in relation to the brain size. Analysis without adjustment for TIV, showed several regions with a significant effect of sex on FA; these included the splenium of the CC (also bilateral superior corona radiata, and posterior limbs of the internal capsule, midbrain, and cerebellum). Significantly higher FA was seen in males compared with females in these regions. Analysis with adjustment for TIV, however, showed a greatly reduced number of such regions (remaining mostly differences in cerebellum). These data therefore emphasize the importance of considering gender differences and total brain volume in clinical trials studies on the CC.

Corpus callosum and ASD: Review of the literature

The increasing number of neuroimaging studies on autism has resulted in many new suggestions about the underlying brain abnormalities. However, diagnosis is still made from the observation of behaviour.

Since the early 90s CC has been one of the main focuses of imaging studies on autism. One of the firsts paper about CC in ASD was that of Egaas in 1995. It reported an overall size reduction of callosal midline area, concentrated in posterior subregions, in 51 subjects with ASD (age range 3-42 years). Thereafter many studies has been performed about CC size and, to date, we can find 191 papers about CC and autism on the main engine of scientific research.

Head circumference, postmortem brain weight, and brain volume from MRI studies are consistent in suggesting the brain overgrowth (in both gray and white matter) in early developmental stages of children with ASD. The period of most pronounced head/brain enlargement in ASD appears to occur in the 1–4-year age range, and by adulthood, brain size does not seem larger than norms. This has led Authors to suggest that the brain of subject with ASD prematurely undergoes to an arrested growth (Courchesne et al., 2007), and, later in development, it is characterized by a reduction in its tissue, reflected in thinner cortex (Wallace et al., 2010).

Some Authors suggested that brains of subjects with ASD are characterized by an aberrant connectivity (Frith, 2004). Connectivity is assessed in terms of the extent to which variations over time in one brain region are correlated with activity in another brain region. High correlations indicate that the two brain regions are interacting and are thus connected in terms of their function. Subjects with ASD had substantially reduced connectivity between brain regions (eg., language regions) compared with healthy volunteers (Just et al., 2004).

The reduced connectivity and the aberrant growth trajectory in brains of subjects with ASD, led Authors to speculate that an aberrant synaptogenesis could be the pathogenic mechanism of these atypicalities in autism: a lack of pruning during the early childhood brain development (when brain volume continues to increase), could result in the abundance of unnecessary connections, and increased brain size; subsequently, an overpruning process could result in cortical thinning. These processes would likely impact the connectivity between brain regions during phases of brain overgrowth and later periods. Thus, these abnormalities in brain growth have been suggested to contribute to the atypical neural connectivity in ASD.

Subsequently neural theories of ASD, like those of many disorders, moved from a lesion-based model, to a focus on disordered structural and/or functional connectivity and the most basic index of anatomical brain connectivity is the integrity of the corpus callosum.

A further boost to the study of the CC in autism has been given by detection of autistic symptoms in individuals with agenesis of the CC (AgCC) (Booth et al., 2011). In the light of the reduced connectivity theory in ASD, individuals with AgCC provide an interesting comparison condition. But while in many idiopathic cases of ASD, also with severe behavior and development impairment, little if any discernable abnormality are found in brain, in

AgCC individuals can be found with almost no behavioral difficulty, despite the absence of the major connective tract between the two hemispheres. Authors concluded that findings supported functional connectivity models of autism, but while impaired interhemispheric transfer, perhaps due to reduced callosal integrity, may be an important factor in ASD, it is not a sufficient cause in isolation. Thus, abnormal interhemispheric connectivity could be only part of the pathogenic basis of autism but additional neural atypicalities are maybe necessary.

Another comprehensive comparison between ASD and AgCC was recently made by Paul (2014), who compared 26 adults with AgCC to 28 matched adults with a diagnosis of ASD without any neurological abnormality. No one had intellectual disability. About a third of agenesis subjects presented autism symptoms detected with ADOS (Autism Diagnostic Observation Schedule). No relationship between intelligence quotient and autism symptomatology in callosal agenesis was found. These findings support the hypothesis that congenital disruption of the corpus callosum constitutes a major risk factor for developing autism.

Callosal abnormalities found in ASD

In a meta-analysis of 10 studies, from 1970 to 2008, that included in total 503 participants (average age 14.5 years, 86% males subjects), Frazier and Hardan (2009) found a general reduction in the area of the CC in ASD compared to controls. This reduction shows more pronounced effects for anterior than posterior regions. Those studies using Witelson subdivisions, reported that rostral body subdivision showed the largest effect, suggesting greatest reduction in the region of the CC containing premotor/supplementary motor neurons, that is crucial for motor planning and disruption of these regions may be the neural substrate for impairments in fine motor skills and imitation observed in autism. Additionally, these CC regions, while important for motor planning, have also been identified as supporting a subset of mirror neuron functions.

The greatest reduction of CC area was observed in anterior regions providing a neuroanatomical link to the prominent executive dysfunction in autism.

In this meta-analysis CC size reductions were moderated by two major factors, i.e. magnet strength and participant age. Larger CC reductions were found in advanced age and this data are consistent with previous white matter findings (Courchesne et al., 2001). Authors suggested that increasing reductions in CC size over time might be a consequence of early abnormal cortical development, a result of ongoing neurobiological disruptions, or due to other unknown processes. Early neural proliferation with abnormal cortical cytoarchitecture and densely-packed minicolumnar organization may lead to poor coordination with distal brain regions. Consequently, developmental alterations of these neural networks may result in reductions over time of long fiber neurons responsible for intra-hemispheric or inter-hemispheric regional communication.

This meta-analysis is mainly made with studies performed on adults and the only paper carried out with preschoolers was that by Boger-Mediddo (2006). In this paper, CC areas of 45 preschoolers with ASD (7 girls and 38 boys, age range 38-54 months) were compared to CC areas of 26 children with typical development (TD) and 14 children with developmental delay (DD). In this study, CC mid-sagittal area, although not smaller in an absolute measure

in ASD compared to TD, it does not show an increase proportional to brain volume enlargement: therefore 3-4 years old children with ASD, compared to TD, have a smaller CC when adjusted (co-varied or ratio) for cerebral volume (that is increased), even when considering gender differences. Size differences appear to be widely distributed in the CC, with no single subregion accounting for the overall size reduction in ASD. Comparison in ASD clinical group showed that structural abnormalities tend to be more accentuated in children with classic autism expression when compared to those with fewer symptoms. Since previous studies, performed on older samples, have generally found in absolute measures, CC to be smaller in ASD, Authors suggested that age-related changes could results in a relative decrease of CC size during childhood, becoming an absolute reduction in callosal size over time. Considering the larger TBV in ASD children, findings of a disproportionately smaller CC led Authors to speculate that increase in brain volume may be due to increase in non-neural tissues (such as astrocytes and intercellular tissue). Alternatively, Authors suggested an alteration in inter-hemispheric connectivity in the autistic brain.

More recent investigations have employed a 3-dimensional volumetric measures than areas measures.

A brief recent review by Bellani et al. (2013) examined MRI studies on CC in ASD subjects, since 2004, and analyzed clinical and demographic factors of samples. Findings of several studies in literature are not so consistent: recent studies report a reduction of the entire volume of the CC (Hardan et al. 2009; McAlonan et al. 2009; Duan et al. 2010; Anderson et al. 2011), other studies report a reduction of one or more parts of the CC, mainly in the anterior region (Alexander et al. 2007; Keary et al. 2009; Thomas et al. 2011), others mainly in the posterior region (Waiter et al. 2005) or simultaneously in the anterior and posterior regions (Vidal et al. 2006).

Volumetric reduction of CC size has been found both in adults and children, but studies performed on children, assessed mainly school-age subjects and adolescents, and only few studies are carried out on preschoolers (Duan et al., 2010; Riva et al., 2011; Calderoni et al., 2012).

Among these, Duan (2010) performed a study on 30 ASD subjects compared to 28 controls; the age range of participants was 3-30 years. In this wide age range sample Authors found a significant reduction in each sub-region of the CC in the patients. Besides the traditional volume test, they also conducted tests based on the length, width, and shape of the CC. Authors reported a significant reduction in the CC length in the patients, but the difference in the width is far from significant, suggesting that the decrease in the CC volume is caused by the decrease in the anterior–posterior length more than the top–bottom length.

In a voxel-based morphometry (VBM) study, Calderoni (2012) found in 38 females, preschool-age ASD no size differences in CC volume when compared to 38 age and IQ-matched female controls.

In 2011, Riva analyzed VBM data, covaried with IQ, age, and brain volume, of 21 developmentally delayed children with ASD (aged 3–10 years) and compared them with those of 21 controls matched for age, sex, and sociocultural background. Aside from a pattern of regional gray matter (GM) reduction affecting some brain regions (basal forebrain, accumbens nucleus, cerebellar hemispheres, and perisylvian regions, including insula and putamen), they found no regional white matter (WM) differences between the 2 groups and no

significant differences between patients and controls were found regarding total brain volume, total GM, and total WM. Particularly, no differences in CC volume were found.

More recently, Prigge (2013) examined CC mid-sagittal area from a developmental perspective, across a 30-year age range, in 68 individuals with ASD compared to a typically developing sample (47 subjcets). All subjects were males, age-range was 3-36 years in the ASD group and no individuals with intellectual deficits were included in the study. Increased variability in total CC area was found in the ASD group and after adjusting for TBV, the total CC and isthmus areas were found smaller in ASD subjects, but none of these results survive our correction for multiple comparisons ($p<0.007$). Neither performance-IQ nor verbal-IQ were significant predictors of CC area. In autism, increased midsagittal areas were associated with reduced severity of autism behaviors, higher intelligence, and faster speed of processing, suggesting that individuals with autism benefit functionally from increased CC area. In the total CC area, similar age-related changes were found in autism and controls, but a trend toward group differences in isthmus development was found, and Authors supported the hypothesis about potential maturational abnormalities in autism. Particularly, in ASD, a slower isthmus growth was found.

The first longitudinal study of the CC in autism was that by Frazier and Hardan group (2012). The study investigates the volumetric changes of the CC in 2 years, their correlations to behavioral phenotype, reported in the follow-up of 23 subjects with ASD and 23 controls, 7-13 years old. The volume of the CC increases with age in a similar measure between ASD and controls (3.1% vs 1.5%), especially at the genu, the anterior and posterior body and the splenium. This finding contrasts with the meta-analysis by the same group (2009), when there was reported a worsening of the reduction in the volume of the CC over time. In 2012 Frazier-Hardan reported that the CC is smaller in subjects with ASD compared to controls at all ages and in all its subregions. The only callosal portion that is not always smaller in ASD than in controls is the rostral region, that over time shows a different pattern of growth between ASD and controls: in control subjects, with the increase of age, rostral region is slightly reduced in volume (1.4%), while in the ASD children volume of this region increases significantly (4.7%). This growth is associated with clinically reduction of externalizing behaviors, with greater emotional modulation and control of motor behavior. The rostral region of the CC in individuals with ASD is the only one that reaches normal size in adolescence (and it is the smallest in the ASD compared to the other subdivisions). This area connects fibers of the pre-supplementary motor cortex (involved in fine motor coordination, motor planning and motor imitation, all impaired in ASD) and implicated in the functioning of mirror neurons (involved in synchronization with intent and motivation of others). These findings, as reported by Authors, support the hypothesis that individuals with ASD could have abnormalities in the process of axon myelination.

Recently, Xiao (2014) analyzed brain images of 50 toddlers with ASD and 28 age, gender, and developmental quotient matched toddlers with developmental delay, between ages 2 and 3 years, to assess overall GM and WM volumes, and regional alterations, by voxel-based morphometry. DTI was also used to investigate the WM tract integrity. Compared with developmental delayed controls, significant increases in global GM and WM volumes and in right superior temporal gyrus regional GM and WM volumes were observed in ASD. No differences were reported on callosal volume between ASD and controls.

Higher fractional anisotropy value was instead observed in the CC (and in the posterior cingulate cortex and limbic lobes) in the ASD toddlers group. These findings about structural and WM abnormalities in ASD, suggest that alterations in neural-anatomy of different brain regions may be involved in autism phenotype, especially in an early ages (Xiao et al., 2014).
The fractional anisotropy (FA) value, a measure derived from diffusion data, is a structural WM integrity index and provides a simple and robust means to assess the degree of anisotropic diffusion occurring within a region. It is sensitive to developmental changes and pathological differences in axonal density, size, myelination and coherence of organization of fibers within a WM voxel. Because FA reflects the degree of anisotropic diffusion, it will be high (ie, nearly one) in regions of higher fibers organization (eg, corpus callosum), intermediate in regions with a lower degree of organization (eg, WM regions with lower predominant axon fiber axis orientation), and low in tissues where the predominant cell shape, and therefore diffusion, is not specifically oriented (eg, GM) and approaching zero in free fluids (eg, CSF). Particularly, FA has proven to be highly sensitive to microstructural changes, but not very specific to the type of changes (e.g., radial or axial) (Alexander et al., Jul 2007).
FA of CC of subjects with ASD was reported to be reduced in an old paper by Alexander (Jan 2007), especially in the genu and splenium regions. This study examined CC volume and DTI analysis of callosal fibers of 43 subjects with high-functioning ASD and 34 controls (mean age 16 years). CC volume was reduced in ASD, with large variability between subjects, and the FA reduction correlates positively with CC volume, thus a reduction in CC volume was associated with a lower FA value. The radial mean diffusivity (MD) is increased in individuals with ASD, suggesting the possibility of an alteration of myelination, axonal diameter and density of axonal and glial cells in the CC of individuals with ASD. Authors found that 72% of ASD had normal callosal FA, but the ASD-Control group difference was driven by a subgroup of ASD subjects (28%) who had low FA of the CC. This subgroup also exhibited decreased performance IQ, increased MD, increased radial diffusivity, and decreased CC volume compared to their ASD peers. These data suggest that within-group differences in callosal WM integrity may be related to within-group differences in performance IQ.
Another study performed by Keller (2007), focused on the developmental changes in the organization of WM in autistic subjects aged 10-35 years, found lower FA values for the whole age range in long-range communication tracts (anterior corona radiate, right retrolenticular part of internal capsule) and in interemispheric connections (corpus callosum).
Many studies analyzed callosal microstructure.
A study, analyzing major frontal lobe tracts and CC of young children with autism (mean age: 5 years), nonautistic developmentally impaired children (DI) and typically developing children, using DTI tractography and tract-based spatial statistics, found that FA was lower in CC in ASD and DI children compared with TD children. The ASD group showed increased length and density of CC compared with the TD group and these callosal features correlate with communication impairment in ASD. Compared with DI group, instead ASD group had increased callosal length only (Kumar et al., 2010).
A quite recent review of literature (Travers et al., 2012) provide an overview of studies that investigate the integrity of white matter in subjects with autism. Among the 48 studies

analyzed, subjects with autism tend to have a reduced FA and increased MD in tracts crossing several brain areas, such as the CC, the cingulate and the temporal lobe. The FA reduction is often associated with an increase in radial diffusivity (RD). RD has been shown to be modulated and increased by myelin in models of dys- and de-myelination (see as example Harsan et al., 2006). Changes in axonal density, axonal diameter, cytoskeletal properties, swelling from neuro-inflammation and WM complexity (e.g., crossing, curving and branching fibers) are also plausible explanations for changes to RD and other DTI measures (including FA). Findings of an increased RD suggest that ASD subjects have a higher diffusivity of water perpendicular to axons. Taken together these findings suggest that the axons of the CC may be less myelinated in autism or, alternatively, the increase of the callosal RD may be due less dense and / or thinner axons.

Although several studies reported an FA reduction in the CC of subjects with autism, there are some exceptions. For example, some Authors (eg., Cheng et al., 2010; Thomas et al., 2011) did not detect significant differences in the FA of the CC of the diagnostic group. Furthermore, two studies with the youngest sample (Ben Bashat et al., 2007; Weinstein et al., 2011), performed on preschoolers, actually demonstrated increased FA of the CC in ASD. One possible explanation is that sample heterogeneity may have contributed to the variation in their findings, as certain characteristics (e.g., intellectual/cognitive ability, language ability, head circumference) may be more indicative of atypical WM microstructure in ASD.

A recent meta-analysis of DTI tractography studies (Aoki et al., 2013) selected DTI studies comparing individuals with ASD with typically developing individuals from 1980 through 2012. 14 studies were included in the meta-analysis, which demonstrated significant FA reductions in the CC and other brain regions (such as left uncinate fasciculus and left superior longitudinal fasciculus), and significant increases of MD in the CC and superior longitudinal fasciculus bilaterally in subjects with ASD compared with typically developing individuals with no significant publication bias. These findings emphasize important roles of these regions, and particularly of CC in the pathophysiology of autism and support the long-distance under-connectivity hypothesis.

Atypical brain connectivity has repeatedly been implicated in neuroimaging studies of people with ASD (Vissers et al., 2012). Some evidences from postmortem studies and structural and functional MRI studies provided the hypothesis that, in ASD, brain is characterized by long-distance under-connectivity. However, it remains unclear which of the major long-distance tracts that compose the cortical network in the human brain is disordered (these tracts include main association or intrahemispheric fibers, such as the uncinate fasciculus, cingulum, superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior frontal occipital fasciculus and fornix and one commissural or interhemispheric fiber tract, the CC).

Another recent diffusion weighted MRI study (Razek et al., 2014), performed on preschoolers with ASD (mean age 55 months), analyzes the ADC (apparent diffusion coefficient), in various brain regions and correlated with clinical data (CARS scores, social age as measured by the Vineland Social Maturity Scale and language skills). ADC provides a measures of the magnitude of diffusion of water molecules within tissues and can be helpful in assessment of WM integrity. Significant differences in the ADC between ASD and controls were found, in some WM regions, particularly in genu and splenium of the CC, the frontal and temporal WM, where the ADC value is increased in subjects with autism. All of these areas correlated

positively with autism severity. This finding is consistent with disruption, poorly organization and microstructural differences in the WM of ASD children, that allow unrestricted diffusion of water throughout the tissue. The increased intercellular space in autism may be due to a reduced number of fibers, which was supported by decreased fiber density found in these children. According to Authors, the volume and density increasing and the decreased functionality (connectivity) in WM of autistic children, may be because a large number of remaining disrupted axons leads to higher cross-connection and increased noise, which may lead to inefficient signal transmission.

In a quite recent investigation about neurodevelopment of WM in autism an abnormally decreased ADC values in the left side of the genu of the corpus callosum was reported in children with autism (4-14 years old). Age correlated negatively with callosal ADC measurements in individuals with autism, but not in children with normal development (Mengotti et al., 2011). The negative correlation between age and callosal ADCs in autism supports the role of the altered trajectory of WM growth during childhood. During normal development, in fact, ADC values of cortical WM decrease in the first 2–3 years of life, with subsequent stabilization, reflecting the process of WM maturation and its structural integrity. In contrast to normal development, in autism there is a constant decrement of ADC values.

A recent spectroscopy analysis (Goh et al., 2014) found that lactate doublets, a marker of mitochondrial dysfunction, were present at a significantly higher rate in a wide age-range (5-60 years) participants with ASD (13%) than controls (1%). In the ASD group, the presence of elevated lactate correlated significantly with age and was detected more often in adults (20%) than in children (6%), though it did not correlate with sex, ASD subtype, intellectual ability, or the Autism Diagnostic Observation Schedule total score or subscores. In those with ASD, lactate was detected within some regions, including CC (the cingulate gyrus most frequently and it was also present in the subcortical gray matter nuclei, superior temporal gyrus, and pre- and postcentral gyri). This study demonstrate evidence for mitochondrial dysfunction in vivo in the brains of individuals with ASD.

Recently the aberrant brain connectivity hypothesis was supported by studies reporting whole-brain hyper-connectivity in children with ASD (Supekar et al., 2013; Di Martino et al., 2014).

In particular, Di Martino focused on whole-brain intrinsic functional connectivity of a large sample > 6 years old high-functioning ASD males in the Autism Brain Imaging Data Exchange (ABIDE) consortium (360 males with ASDs compared to 403 age-matched typical males). Whole-brain analyses seems to conciliate findings of both hypo- and hyper-connectivity in the ASD literature, in fact both were detected, although with different degrees and with distinct topographies. Specifically, while findings of hyper-connectivity were limited and primarily associated with subcortical regions, hypoconnectivity dominated, particularly for cortico-cortical and interhemispheric functional connectivity. This result underscores the hypothesis of alterations in interhemispheric connectivity, but preschoolers were not recruited in this large analysis.

From the same cohort (ABIDE), recent data about CC analysis among 694 subjects (328 patients, 366 controls, 7-40 years old) were published (Lefebvre et al., 2014). No differences in CC size between groups were reported: the observed difference was < 7 mm^3 (larger in controls), not statistically significant, as were none of the differences in the 5 callosal

subregions analyzed. There was a small but statistically significant increase in mean CC size per year, and the increase was more pronounced in the Posterior and Anterior subregions, which are those that more strongly correlate with TBV. Mean CC size was statistically significantly smaller among females by 7.4% compared with males and a similar difference was observed in all subcallosal regions especially the Posterior and Anterior ones; however this difference is explained by the significant difference in TBV between females and males (as adding TBV as a covariate made the sex effect not statistically significant). The CC appeared to scale non-linearly with brain size, with large brains having a proportionally smaller corpus callosum. Additionally, intelligence scores correlated with brain volume among controls but the correlation was significantly weaker among patients.

Also in the Supekar study (2013) three different cohort of children with ASD were used, all older than 7 years. Connectivity was analyzed with preprocessed fMRI datasets that were parcellated into cortical and subcortical regions. Hyper-connectivity was observed in ASD, both at the whole-brain and subsystems level, and both across long- and short-range connections. Brain hyper-connectivity predicted symptom severity in ASD, such that children with greater functional connectivity exhibited more severe social deficits. These results reported that the brain in ASD is largely functionally hyper-connected and this anomaly contribute to social dysfunction.

Conversely to the wide consensus on whole-brain aberrant connectivity, recent data challenge the widely claimed general disruption of WM tracts in children with autism, instead implicating only one tract, the right inferior longitudinal fasciculus, in the ASD phenotype (Koldewyn et al., 2014).

Toddlers with autism exhibited significantly weaker inter-hemispheric synchronization (i.e. weak "functional connectivity" across the two hemispheres) in putative language areas, as shown by Dinstein (2011) in the spontaneous cortical activity of 72 naturally sleeping 1-3.5 years old toddlers with autism. The strength of synchronization was positively correlated with verbal ability and negatively correlated with autism severity.

Functional correlation of callosal abnormities

Structural morphometric and microstructural abnormalities in the CC have called attention to interhemispheric dysconnection in ASD (Just et al., 2004; Just et al., 2007; Dinstein et al., 2011; Anderson et al., 2011). While initial volumetric studies only found evidence of partial compromise of the CC, DTI studies of CC microstructure have suggested that abnormalities extend to its all subdivisions. Similarly, while initial fMRI studies reported abnormalities in interhemispheric functional connectivity only for sensori-motor and language areas, whole-brain analyses suggest broader compromises, affecting 30% of homotopic connections. Given that interhemispheric interactions are thought to facilitate high-load cognitive processes, findings of altered connectivity extending across systems, may be relevant to clear impairments of complex reasoning and information processing in ASDs.

Given that the CC has been considered as an index of interhemispheric connectivity, and brain is seen as an integrated system of regions of interest that must collaborate to achieve normal functioning, the poor connectivity between different cortical regions of autistic brain, results in impaired integrative processing and deficient higher order cognitive abilities. Decreased coordination between brain regions has been found during performance on tasks of sentence

comprehension (Kana et al., 2006) and executive planning (Just et al., 2007). Relationships have been found between smaller CC size in individuals with autism and decreased "functional connectivity" in specific CC subregions. Just, in its "theory of frontal-posterior underconnectivity" (2012), reported that callosal size has a relationship with functional connectivity of cortical regions, as a reduced degree of synchronization of the activation between frontal and posterior brain, during neuropsychological and social tasks (eg., language, social processing, executive functions, working memory, high-level inhibition, visuo-spatial processing). A study applied on a volumetric analysis (Keary et al., 2009), examined the size of the CC in non-mentally retarded individuals with autism (8-45 years old) and controls, to investigate the relationship between this structure and cognitive measures linked to interhemispheric functioning. Participants with autism displayed reductions in total CC volume and in several of its subdivisions (rostrum, genu, anterior body). The relationships between CC volumes and age and TBV were examined and no associations were found in the autism and control groups. Correlations were examined between CC structures and performance on neuropsychological tests (Wisconsin Card Sorting Test, Tower of Hanoi test, Tactile Finger Recognition test and the Tactual Performance Test) and relationships were observed only in the autism group. The largest difference in performance on the selected neuropsychological tests was observed in tests associated with frontal lobe function. Correlations between these neuropsychological test performance and CC measurements were observed and CC volume reductions were correlated with poor performance.

The neuroanatomical underpinning basis of ASD are still poorly understood, but theories surrounding atypical cerebral asymmetry as one possible factor influencing this disorder have received considerable attention. Many people with ASD show a pattern of deficits in skills ascribed to the left hemisphere, such as language, communication and symbol use, whilst appearing relatively unimpaired in right hemisphere functions such as visuospatial abilities. These observations have given rise to the hypothesis that autism might be related to atypical cerebral organization, with the left hemisphere being most affected. In typical individuals one of the most reliable findings is that consistently right-handed men have a smaller CC than non-consistently right-handed men (Witelson, 1989) and rightward asymmetry of anterior regions of the CC seems to be a normal pattern in right-handed men. An investigation about functional and neuroanatomical callosal asymmetry, in terms of handedness and CC measurements, in male adolescents with autism, and about their associations with executive dysfunction and symptom severity (Floris et al., 2013), found that adolescents with autism did not differ from controls in functional asymmetry (handedness), but when compared to controls ASD subjects neuroanatomically showed a reversed patterns of association between the posterior midbody and anterior midbody of the CC and handedness, and a reversed patterns of association between the isthmus and executive function also.

Most neuroanatomical asymmetry measures indicated that rightward lateralization was associated with stronger symptom severity. Measures of symptom severity were related to rightward asymmetry in three callosal subregions (splenium, posterior midbody and rostral body). Authors found the opposite pattern for the isthmus and rostrum with better cognitive and less severe clinical scores associated with rightward lateralization.

Because of its extensive connectivity, changes in CC structure and their correlation with several brain functions have been studied, also in ASD and its functional features.

Recently Prigge (2013) reported a relationship between callosal size and autism severity: increased midsagittal areas were associated with reduced severity of autism, above all ADOS-G social scores are related significantly to anterior midbody subregion of the CC. Lack of a significant interaction between age and CC area suggests the significant relationship between anterior midbody and severity of social features is similar across the broad age range (3-36 years) of the individuals with autism studied. No significant relationships between ADOS communication scores and CC subregions were found. Authors studied also relationship between callosal area and intelligence: relationship between IQ and rostral area was found to be different in the autism and control groups. A decreased rostral area was associated with increased VIQ in the typically developing group but decreased VIQ in the autism group. Authors concluded that a smaller CC area is associated with a greater social impairment and a lower intelligence in autism.

A similar correlation was also found by Alexander (2007), who described a subgroup of autistic patients with smaller CC volume, reduced WM integrity (as deduced by lower FA values and higher MD values) and poorer intellectual abilities and slower processing speed.

A DTI study revealed a correlation between fiber length and density of total CC and communication scores on the Vineland Adaptive Behavior Scales (Kumar et al., 2010).

Social and communication deficits have also been reported in patient with other type of callosal disruption, as in subjects with AgCC (Lau et al., 2013).

Anomalies on the callosal structure contribute to various motor deficits, including uni- and bi-manual movement disturbances (Beaulè et al., 2012), eye-hand coordination (Rademaker et al., 2004), and gait (Laat et al., 2011), all found in ASD. As example, larger CC size is associated with better motor performance in children: a poorer score on the Movement Assessment Battery for Children was related to a smaller CC and a larger CC was strongly associated with better Visual Motor Integration. Individuals with AgCC (Moes et al., 2009), exhibited a pattern of delayed motor development, difficulty with balance and bimanual movements, poor muscle tone, poor depth perception, reduced pain perception, and an increased proportion of left and mixed handedness.

A recent study was performed about callosal connectivity and its relationship with socio-communication deficits and motor deficits in children with autism (Hanaie et al., 2014). The ASD group (5-14 years old children) was compared to typically developed children and displayed abnormal macro and microstructure of the total CC and its subdivisions. ASD group had a significantly decreased callosal tracts volume and a shorter average fiber length. FA, axial diffusivity and radial diffusivity were similar in ASD and controls. Structural properties of CC in ASD were related to socio-communicative deficits but not to motor deficits of the clinical group.

The rostral body was identified, in the meta-analysis by Frazier and Hardan (2009), as the callosal portion showing the largest reductions of any CC sub-division in autistic subjects. Interestingly, this region of the CC connects fiber tracks originating in pre-supplementary motor cortical regions, which support fine motor coordination, motor planning, and motor imitation, all of which are impaired in autism; it has also been implicated in mirror neuron functions, that facilitate the tuning of individuals to others' intentions and motivations. Normalization of the rostral body size, in adolescence, is associated to improvements in some autistic features, usually observed in older children and adolescents, such as reciprocal social

interaction (Seltzer et al. 2003) and repetitive behavior observed (Esbensen et al. 2009) in individuals with autism. In the longitudinal study, proposed by Frazier (2012), decreases in externalizing behavior were associated with rostral body volume increases and Authors concluded that these results suggest that normalization of the rostral body size, in older children and adolescents (8-15 years old), is linked to improvements in motor coordination and emotion regulation, possibly indicating an ongoing developmental or compensatory process.
A recent EEG coherence study analyzed the current model proposed of ASD as a developmental disconnection syndrome (Peters et al., 2013). In ASD, both with and without a genetic syndrome (Tuberous Sclerosis Complex), decreased long- over short-range coherence and markedly increased network resilience were found. Authors concluded that the increased resilience in ASD may reflect an excessively degenerate network, with local over-connection and decreased functional specialization. Quite at the same time, also Boersma (2013) demonstrated aberrant functional brain networks in autistic toddlers. With the EEG recordings in 12 toddlers with autism (mean age 3.5 years) and 19 control subjects, Authors assessed interregional functional brain connectivity, with functional brain networks constructed at the level of temporal synchronization between brain regions underlying the EEG electrodes. Children with autism showed a significantly increased normalized path length and reduced normalized clustering, suggesting a reduced global communication capacity already during early brain development. In addition, whole brain connectivity was found to be significantly reduced in these young patients suggesting an overall under-connectivity of functional brain networks in autism. These findings support the hypothesis of abnormal neural communication in autism, with deviating effects already present at the early stages of brain development.

Sex differences

Recent studies continue to report in the prevalence of ASD a male bias, but also suggest some sex differences in phenotypic presentation, such as fewer restricted and repetitive behaviors and externalizing behavioral problems in females than males, that may contribute to this bias. Genetic studies demonstrate that females are protected from the effects of heritable and de-novo ASD risk variants, and other papers report that sex chromosomal genes and/or sex hormones, especially testosterone, may modulate the effects of genetic variation on the presentation of the phenotype (Werling et al., 2013).

The investigation of sex-related differences in brain structure is relevant to understanding the pathophysiology of ASD, but to date very limited neuroimaging data are available to evaluate this feature, mostly in children population. Particularly, to our knowledge, to date sex-differences in the CC structure in ASD have not been assessed enough.
Some data, although conflicting, suggest gender effects on CC size, in healthy subjects, but few data have been reported in the autistic population and no data, to our knowledge, are available in preschoolers with ASD.
In a high-functioning adults sample, the CC (splenium) showed a pattern of females with autism greater than typical females, but males with autism equal to typical males (Lai et al., 2013).

Recently, unpublished data about CC size in the ABIDE large sample (Lefebvre et al., 2014), reported that callosal volume was statistically significantly smaller among females by 7.4% compared with males, and a similar difference was observed in all subcallosal regions (especially the Posterior and Anterior ones); however this difference is explained by the significant difference in TBV between females and males (as adding TBV as a covariate made the sex effect not statistically significant).
In another high-functioning adults sample, with respect to gender, only TBV was significantly increased in males compared with females, resulting in a significantly decreased CC/TBV ratio in males (independently from gender and fully attributed to brain size). This finding is in accordance with the hypothesis that brain size, per se, is the relevant factor and contradicts the sexual dimorphism hypothesis of the human CC (Tepest et al., 2010).
Regarding microstructural organization, sexual dimorphism in the CC were reported, also in healthy subjects, but data are inconsistent. Men showed significantly higher values of FA, lower diffusion strength and lower radial diffusivity in this structure, when compared to women, based on differences in myelination (Menzler et al., 2011; Westerhausen et al., 2011). Some Authors underline a stronger inter-hemispheric connectivity between the frontal lobes in males than females, which might be related to sex differences in hemispheric asymmetry and brain size.
Diffusional measurements revealed, in the body of the CC of an adults sample, an higher FA for males than females in controls but not in the ASD group; furthermore, there was a trend toward lower FA in males with autism with respect to control males. No effects were observed for the genu and splenium of the CC. Authors concluded that autism-related changes in the CC microstructural organization, consistent with reduced axonal density or myelination, have been demonstrated selectively in males but not in females (Beachera et al.,2012).

Study design

Objectives

The hypothesis of abnormal neural connectivity, involving short- and long-distance connections, is one of the most sustained pathophysiological theories of ASD. Recently, whole-brain analyses reconciled seemingly disparate themes of both hypo- and hyperconnectivity in the ASD literature, because both were detected, although hypoconnectivity seems to dominate, particularly for corticocortical and interhemispheric functional connectivity.
CC is the largest WM structure in human brain and it is the main connection and information transfer structure involved in interhemispheric communication. A growing body of literature has identified size reductions of the CC in subjects with ASD, and CC size also appears to be inversely related to autism severity and the intelligence quotient (IQ). However to date very few studies have been conducted on preschool age, when the disorder show its higher clinical expression.

The main goal of our study is to compare the CC volume between preschoolers with ASD and controls subjects. We analyzed CC subregions volume in both groups also.

Then, callosal size relations to demographic and clinical variables of ASD and control group (gender, age, non-verbal IQ, and language) have been examined.

Lastly, in the ASD group we assessed callosal volume relationship with autism severity.

Methods:

Participants, Procedures, Clinical measures and Image processing

Participants

We selected a sample of 41 preschoolers diagnosed with an ASD not clearly due to organic pathology.
Each of them was assessed with a clinical and functional evaluation performed by multidisciplinary team, in a inpatient or outpatient condition. Autistic subjects were compared to 40 controls; control group was gender-, age-, and non-verbal IQ-matched with the sample.
All subjects of the ASD group satisfied the following inclusion/exclusion criteria.
Main inclusion criteria were: diagnosis of an ASD, age between 18 and 72 months and a non-verbal IQ≥30 (NVIQ).
Exclusion criteria, both for sample group and control group, were signs, symptom and information of a possible "organic" nature of the disorder, and included: neurological syndromes or focal neurological signs; dysmorphic features suggestive of a genetic syndrome; significant sensory impairment (e.g., blindness, deafness); anamnesis of birth asphyxia, premature birth, head injury or epilepsy; use of any psychotropic medication; presence or history of any other axis I mental disorder.

Exclusion and inclusion criteria (except diagnosis of ASD) were satisfied also for the control group.
Subjects with incomplete functional assessment and those with a not reliable evaluation (ie. for severe behavioral and/or cognitive impairment) were also eliminated from the sample. Instead subjects with complete functional assessment but without CBCL 1 ½ -5 behavioral assessment were included in the sample (7 subjects).
In the sample selection, subjects are chosen to be equally divided by gender, age and intellectual level.
Intellectual level is defined by non-verbal IQ (no development delay: NVIQ ≥70; developmental delay: NVIQ<70).
All subjects underwent a brain magnetic resonance imaging (MRI) examination for clinical goals (ie. to complete diagnostic protocol and etiopathogenic study), during the first admission or during subsequent hospitalization.
ASD group children also received blood tests aimed to exclude the "organic" nature of the disorder (dosage of thyroid hormones, screening for celiac disease, aminoacids dosage in plasma and urine, organic acids and mucopolysaccharides dosage in urine and creatine and metabolites dosage in plasma and urine, standard karyotype and fragile X research) and audiometric examination or auditory evoked potentials. Also controls children with developmental delay received the same assessment (except auditory evaluation).
In the control group, children without intellectual disability are healthy subjects observed by the child neuro-psychiatrist for the presence of non epileptic paroxysmal episodes (ie., headache, periodic syndromes, paroxysmal vertigo), developmental "immaturity", or affective problems (ie., elective mutism, separation anxiety disorder). In these subjects, ASD-related anomalies (eg., social and interaction atypicalities, communication difficulties, etc.) and others neurodevelopmental disorders were excluded.
All subjects were selected at the IRCCS Stella Maris Institute in Calambrone (Pisa - Italy).

Procedures

ASD diagnosis was made according to the DSM-IV-TR criteria (APA; 2000) and to the DSM-5 criteria (APA; 2013), by a multidisciplinary team during some days of extensive evaluation. Thus diagnosis of "Pervasive Developmental Disorder" or "Pervasive Developmental Disorder Not Otherwise Specified" according to the DSM-IV-TR or "Autism Spectrum Disorder" according to the DSM-5, was made. The diagnosis was processed by senior child psychiatrists with long experience in the neurodevelopmental disorders field, particularly autism, and has been supported by clinical neuropsychiatric evaluation, comprehensive psychological assessment by an experienced clinically trained research child psychologist, psycho-educational assessment and a speech-language evaluation. Each subject also received an intellectual skills assessment. All clinical parameters were assessed using standardized tests.

Even control subjects performed a clinical evaluation (neuropsychiatric assessment, psychological assessment, language evaluation and/or psycho-educational assessment).

Those children, in both sample and control group, who have not carried out the language assessment (eg., because expressive language was absent), however, have received a clinical estimation of language skills in the course of the functional observation.

For each subject (both in ASD and in control group), we analyzed demographic data (gender and age) and clinical data (NVIQ and expressive language level) in relation to brain imaging data. In the ASD group were also considered autism severity (assessed by ADOS-G and CBCL1½-5).

Given that many children in the sample has performed more than one functional assessment, during several hospitalizations, we selected clinical data (ADOS, cognitive, language and behavioral) closer to the date of MRI acquisition or otherwise within 1 year from that date.
Considering also that, during development, language can change very quickly over time, the empirical evaluation of expressive language level was made at the same time of MRI acquisition for all children.

Brain imaging were processed and volumetric segmentation was performed with an automated parcellation approach on T1-weighted MRI images. Previously, MRI images were assessed and, when required, a manual correction was made in the preprocessing phase. 4 different raters evaluated images and inter-rater reliability was analyzed on images of 10 subjects (equally distributed between ASD and controls, and males and females) assessed by all raters. All raters were blind to diagnoses and clinical-demographic features of subjects.

CC volume analysis was made in ASD and controls subjects. We analyzed CC subregions volume in both groups also. CC volume relationship with demographic and clinical features was assessed in ASD and control group, particularly were analyzed correlations with gender, age, non-verbal IQ, and language. To better explore age correlation we divided the sample, both ASD and controls, into two age groups using the mean age as watershed: $\leq$ 49 months of age and > 49 months of age. In the ASD group we analyzed CC volume relationship with autism severity.

Clinical measures

ADOS-G

The ASD diagnosis was confirmed by the Autism Diagnostic Observation Schedule-Generic (ADOS-G) in all patients.
ADOS-G is a semistructured, standardized assessment of social interaction, communication, play, and imaginative use of materials for individuals suspected of having ASD (Lord et al., 2000). The observational schedule consists of four 30-minute modules, each designed to be administered to different individuals according to their level of expressive language. It is still considered the gold standard instrument for diagnosing and assessing autism.
The modules provide social-communicative sequences that combine a series of unstructured and structured situations. Each situation provides a hierarchy of presses for particular social

behaviors. Module 1 is used for children who do not use spontaneous phrase speech consistently and consists of 10 activities with 29 accompanying ratings. Module 2 is intended for children with some flexible phrase speech who are not verbally fluent; it consists of 14 activities with 28 accompanying ratings. Module 3 provides 13 activities and 28 ratings and it is intended for verbally fluent children for whom playing with toys is age appropriate.
Module 4 contains the socio-emotional questions of the ADOS and it is intended for verbally fluent adults and for adolescents who are not interested in playing with toys such as action figures (usually over 12–16 years).
The operational definition of verbal fluency is the spontaneous, flexible use of sentences with multiple clauses that describe logical connections within a sentence. It requires the ability to talk about objects or events not immediately present.
Subsets of items in each module are used to generate separate diagnostic algorithms for each module in the ADOS-G. Items and the thresholds for classification of autism and of ASD differ for each module in the ADOS-G. However, the general principles and procedures for computation are the same across modules and similar to DSM-IV (American Psychiatric Association, 1994) and ICD-10 (World Health Organization, 1993). Classification is made on the basis of exceeding thresholds on each of two domains: social behavior and communication, and exceeding a threshold for a combined social-communication total.
The ADOS-G is intended to be one source of information used in making a diagnosis of ASD, but is not sufficient to do so on its own.
Because only a small window of time is considered, the ADOS-G does not offer an adequate opportunity to measure restricted and repetitive behaviors (though such behaviors are coded if they occur). Thus, ADOS-G algorithms include only items coding social behaviors and communication.
Because it consists of codings made from a single observation, the ADOS-G does not include information about history or functioning in other contexts. This means that the ADOS-G alone cannot be used to make complete standard diagnoses.
ADOS-G provides scores that are distinct in 3 domains: Language and Communication-scores, Reciprocal Social Interaction-scores and Total-scores. In this study the 3 of them all were analyzed.

Intellectual assessment tests

The cognitive assessment was performed for all children using standardized tests, specific to the chronological age, such as: Leiter International Performance Scale - Revised (Leiter-R), Griffiths Mental Development Scale (GMDS) and Wechsler Preschool and Primary Scale of Intelligence (WPPSI, Italian version).
Leiter-R scale is an intelligence test in the form of a strict performance scale. It was designed for children and adolescents ages 2 to 18, it can be administered completely without the use of oral language, including instructions, and requires no verbal response from the participant. The Leiter-R contains 20 subtests organized into four domains (Reasoning, Visualization, Memory and Attention). The test have game-like tasks, and its easy administration and quick, objective scoring make for an efficient assessment. Because the Leiter-R is non-verbal, it is especially suitable for children and adolescents who are cognitively delayed, non-verbal,

speech or hearing impaired, motor impaired, or suffering from neurodevelopmental disorders, like ASD.

The GMDS is used to measure the rate of development of young children from birth to 8 years. The six areas of development measured by the six sub-scales include: Locomotor (gross motor skills including the ability to balance and to co-ordinate and control movements); Personal-Social (proficiency in the activities of daily living, level of independence and interaction with other children); Language (receptive and expressive language); Eye and Hand Co-ordination (fine motor skills, manual dexterity and visual monitoring skills); Performance (visuospatial skills including speed of working and precision); Practical Reasoning (ability to solve practical problems, understanding of basic mathematical concepts and understanding of moral issues). A kit of standardised equipment is required to administer the items in the Griffiths scales.

The WPPSI is an intelligence test designed for children ages 2 years 6 months to 7 years 3 months. It consist of 14 subtests and composite scores that represent intellectual functioning in verbal and performance cognitive domains, as well as providing a composite score that represents a child's general intellectual ability (ie., Full Scale IQ). In addition, the Processing Speed Quotient can be derived for children aged 4 – 7 years 3 months, and a General Language Composite can be determined for children in both age bands (2 years 6 months – 3 years 11 months & 4–7 years 3 months). Children in the 2 years 6 months – 3 years 11 months age band are administered only five of the subtests: Receptive Vocabulary, Block Design, Information, Object Assembly, and Picture Naming.

To promote greater data homogeneity, in those cases where cognitive assessment test provided verbal IQs (VIQ) and performance IQs (QIP) (ie., WPPSI) or different quotients for various developmental areas (ie., GMDS), we selected for each subject the non-verbal IQ scores (or performance quotient).

Language

As a measures of expressive language development, we choose the framework for describing spoken non-echolalic language acquisition in preschoolers with ASD defined by Tager-Flusberg (2009).

Within a developmental framework, thus five key phases of expressive language acquisition were identified:

Phase or level 1: Preverbal Communication

Children in this phase communicate using preverbal intentional communication through vocal (babble) and gestural means. This phase generally covers the age range of 6–12 months in typically developing children.

Phase 2 or level: First Words

Children in this phase use non-imitated spontaneous single words referentially and symbolically to communicate about objects and events, including those outside the immediate context. At least some of their speech is intelligible and incorporates the most frequent consonant sounds heard in typical babble. Children in this phase use speech with a variety of

people in different settings to serve several functions, including, but not limited to, labeling, requesting, and commenting on (directing joint attention to) some objects or activities. This phase generally covers the age range of 12–18 months in typically developing children.

Phase 3 or level: Word Combinations

Children in this phase have a vocabulary that is rapidly increasing in size and includes a variety of parts of speech (nouns, verbs, descriptors). They are able to combine words creatively to refer to objects and events. Two- and three-word combinations are used for several different communicative functions. This phase generally covers the age range of 18–30 months in typically developing children.

Phase 4 or level: Sentences

Children in this phase combine words into clausal structures, or sentences, and use some morphological markers such as plurals, prepositions, and some verb endings. Their vocabulary is sufficiently large to serve their communicative needs in everyday situations. They communicate a wide range of functions in different settings with both familiar and unfamiliar people. The portion of this phase relevant for the proposed benchmarks defined here corresponds to typically developing children between the ages of 30–48 months.

Phase 5 or level: Complex Language

By the end of the preschool years, typically developing children have large and rich vocabularies that they use to communicate a wide range of topics (including abstract or hypothetical ideas) using complex grammatical constructions (e.g., relative clauses, sentential complements, anaphora) in different discourse contexts (e.g., conversation, narrative).

Thus, according to this assessment framework about productive language abilities, patients and controls were directly assessed and assigned to one of the conditions described above.

CBCL 1½-5

To complete the behavioral assessment was compiled by parents the Child Behavior Checklist 1½-5. It is a widely used method of identifying behavioral problems in children. It is a component in the Achenbach System of Empirically Based Assessment developed by M. Achenbach (2000).

Problems are identified by a respondent who knows the child well, usually a parent or other care giver. Alternative measures are available for teachers (the Teacher's Report Form) and the child (the Youth Self Report). There are two versions of the checklist: the preschool form (CBCL/1½-5) is intended for use with children aged 18 months to 5 years; the school-age version (CBCL/6-18) is for children aged 6 to 18 years. It is an important measure for children's emotional, behavioral and social aspects of life. It is used as a diagnostic tool for a variety of behavioral and emotional problems such as attention deficit hyperactive disorder, oppositional defiant disorder, conduct disorder, childhood depression, separation anxiety, childhood phobia, social phobia, specific phobia and a number of other childhood and adolescent issues. The checklists consists of a number of statements about the child's behavior. Responses are recorded on a Likert scale: 0 = Not True, 1 = Somewhat or Sometimes True, 2 = Very True or Often True. The preschool checklist contains 100 questions. Similar questions are grouped into a number of syndromes and their scores are summed to produce a score for that syndrome. Some syndromes are further summed to provide scores for Internalizing and Externalizing problem scales. A total score from all

questions is also derived. For each syndrome, problem scale and the total score, tables are given that determine whether the score represents normal, borderline, or clinical behavior.

In our sample 6 subjects were excluded in the CBCL/1½-5 analysis, because the questionnaire data were not available (questionnaire was not compiled by parents or caregivers).
Of all CBCL/1½-5 scales we selected those that best describe symptoms of ASD: Internalizing problems scale, Externalizing problems scale, Total problems scale, Withdrawn scale, Attention problems scale, and the DSM-oriented Pervasive developmental problems scale.
Our research group, some years ago, reported that CBCL/1½-5 has high sensitivity and specificity for Pervasive developmental problems scale and Withdrawn scales when preschoolers ASD were compared to age-, sex-, and IQ-matched control children (Muratori et al., 2011).

Image processing

Structural MRI of the brain were performed on a 1.5 T MR system (Signa Horizon LX, GE Medical System). Both ASD and controls were sedated with a general anaesthesia with a halogenated agent while spontaneously breathing. The written informed consent from a parent or guardian of children was obtained.
Volumetric segmentation was performed with the Freesurfer image analysis suite: CC volumes were quantified with an automated parcellation approach on T1-weighted MRI images. Preprocessing steps of each subject included: 1) extraction of the folder containing the DICOM images from cd-patient related to the acquisition sequence FSPGR for T1-weighted structural brain images; 2) using the SPM software, the sequence of DICOM images was imported into ANALYZE format, getting a metadata consists of two files:. Img and. Hdr; 3) file in ANALYZE format was imported in the Freesurfer format, using the command: recon-all-the-s1.hdr subjid s1 4) The subject 'was processed with Freesurfer using the command: recon-all-all-subjid s1; 5) brain imaging of each subject was subjected to manual correction when needed (eg., registration, skull stripping and/or white matter editing); 6) manual corrections are implemented by Freesurfer software using the command: recon-all-autorecon2-autorecon3-subjid s1.
Freesurfer software is documented and freely available for download online. The technical details of these procedures are described in prior publications (see publications by Fischl et al.). Briefly, this processing includes motion correction and averaging of multiple volumetric T1 weighted images (when more than one is available), removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. The

maps produced are not restricted to the voxel resolution of the original data thus are capable of detecting submillimeter differences between groups.

The CC was automatically identified and segmented by the FreeSurfer processing software.

The Freesurfer software, using the script mri_cc, automatically divides the CC into 5 segments, using as a reference the subdivision proposed by Hofer-Frahm (2006), considered to be less arbitrary than other existing subdivision schemes. A publication describing the process of automated segmentation of CC was performed by Rosas et al. (Neuroimage, 2010). The CC has been divided into 5 segments along its main axis (eigenaxis), thus 5 callosal subregions were identified: CC-Anterior (CC-Ant), CC-Mid Anterior (CC-Mid Ant), CC-Central (CC-Cen), CC-Mid Posterior (CC-Mid Post) and CC-Posterior (CC-Post). Total CC volume was calculated as the sum of these five segment volumes for each study participant.

Supratentorial brain volume (TBV) was estimated using Freesurfer, and includes everything except the cerebellum and the brain stem. In particular, it includes the volume of the ventricles, choroid plexus, and vessels.

Freesurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (Han et al., 2006; Reuter et al., 2012). They have been widely used for neuroimaging studies and, in particular, in some publication Freesurfer is used to examine the CC, even in the pediatric age and in subjects with autism (Johnson et al., 2012; Salat et al., 2005; Vatta et al., 2011; Francis et al., 2011; Lefebvre et al., 2014). Overall, segmentations performed by Freesurfer were judged to be of excellent quality and relatively accurate for brain parenchyma (i.e. GM + WM) volumetry, although it was noted that they tend to over-estimated WM volume when compared to manual segmentations (Klauschen et al., 2009).
To date, to our knowledge, Freesurfer is the only imaging analysis tool that automatically parcellates CC subregions. In his publication, Johnson (2012) underlined his confidence in this methodology, because he reported that unpublished data shows a high correlation (about r=.95) between Freesurfer segmentation of the CC and manual measurements in a group of young normals and OCD patients.

Statistical analysis

The statistical examination of the CC volumes was performed using the analysis of variance (ANOVA) test. Even if it can be used to compare the means of the distributions of more than two variables, it was implemented here to compare a single variable, such as the CC volume, between ASD and control groups.

In the ANOVA, the test statistic has a Fisher-Snedecor distribution (F-distribution) under the null hypothesis of equal means between the groups, which variables are assumed to be normally distributed and to have the same standard deviation. The probability (p-value) of observing a statistical value of F greater than that observed was evaluated, considering $\alpha=0.05$ as significance level to reject the null hypothesis when $p<\alpha$.

The statistical analyses were univariate because each test was performed on a single variable compared between the groups. When the ANOVA tests were applied on correlated variables, such as the volumes of CC subregions, covariate analysis was considered, and then the significance level was Bonferroni corrected to take into account the problem of multiple comparisons.

If there is a linear relationship between two variables, it is evaluated by Pearson's correlation index. It is an index between -1 and 1. When it is 0 there is no correlation. When the index is closer in absolute value to 1 or -1, the correlation increases. Correlation significance is evaluated by calculating the p-value and considering $\alpha=0.05$ as significance threshold.

Inter-rater reliability statistical analysis was made on the volumes taken from the file aseg.stats, using the Freesurfer asegstats2table. On each volume, was made a paired two-sample t-test considering $\alpha=0.05$ as significance level.

Results

Sample description

CC volume of 41 children with ASD were quantified and compared to 40 gender, age, and non-verbal IQ-matched control subjects. In the ASD group 21 are males and 20 females; controls are equally divided by gender (20 males and 20 females). Regarding intellectual level, the distribution of non-verbal cognitive abilities is sufficiently uniform within groups and sex: in the ASD group 20 children (9 males and 11 females) have developmental delay or intellectual disability (NVIQ <70), and 21 children (12 males and 9 females) have not developmental delay or intellectual disability (NVIQ ≥70); NVIQ range is 34-113 and mean NVIQ is 73±22. In the control group 20 children (10 males and 10 females) have developmental delay or intellectual disability, and 20 children have not developmental delay or intellectual disability (10 males and 10 females); NVIQ range is 31-123 and mean NVIQ is 73±23. Mean age of ASD group is 49 ± 12 months (age range is 28-70 months); chosen 49 months as age cut-off, in the ASD group 22 children are younger (≤49 months of age: 10 males and 12 females) and 19 children are older (>49 months of age: 11 males and 8 females). In the control group the mean age is 49 ± 14 months (age range is 22-72 months); 20 children are younger than 49 months (10 males and 10 females) and 20 older (10 males and 10 females).

Demographic and main clinical features of subjects are summarized in Table 1.

Variable	Subject group, mean ± std [range]							
	ASD (n=41)				**Controls** (n=40)			
	Males (n=21)		**Females** (n=20)		**Males** (n=20)		**Females** (n=20)	
	ID (n=9)	**no-ID** (n=12)	**ID** (n=11)	**no-ID** (n=9)	**ID** (n=10)	**no-ID** (n=10)	**ID** (n=10)	**no-ID** (n=10)
Age (months)	49 ± 12 [28-70]				49 ± 14 [22-72]			
NVIQ	73 ± 22 [34-113]				73 ± 23 [31-123]			

Table 1: Demographic and main clinical features of ASD and controls group*; abbreviations*: ASD, autism spectrum disorders; NVIQ, non-verbal intelligence quotient; ID, with intellectual disability; no-ID, without intellectual disability.

Language level is not equally spread between ASD and controls, because subjects with ASD have a lower language degree. In fact, within the ASD group 13 children have a language development at level 1, 13 at level 2, 3 at level 3, 11 at level 4 and 1 child at level 5. By contrast, within the control group 6 children are a level 1, 9 at level 2, 3 at level 3, 11 at level 4 and 11 at level 5.

Language levels are summarized in Table 2.

Variable	Subject group			
	ASD (n=41)		**Controls** (n=40)	
	Males (n=21)	**Females** (n=20)	**Males** (n=20)	**Females** (n=20)
Level 1	6	7	3	3
Level 2	5	8	3	6
Level 3	3	0	3	0
Level 4	7	4	6	5
Level 5	0	1	5	6

Table 2: Language levels of ASD and controls groups.

In the ASD group autism severity has been analyzed by ADOS-G scores (Language and Communication-scores, Reciprocal Social Interaction-scores and Total-scores). All subjects, except 2 females, have Total-scores above the clinical cut-off for ASD. In males mean Total-score is 13.1 ± 3.5 (range 7-18); in females is 14.1 ± 5.5 (range 6-22).

CBCL1½-5 scores were accounted to assess autism severity also. In the ASD group 7 children (4 males and 3 females) have not any CBCL1½-5 scores so in the statistical analysis of this clinical feature they were not taken into account because missing data.

Inter-rater reliability

Inter-rater reliability, assessed with a paired-two-sample t-test on volumes of 10 subjects MRI images, shows a good degree of concordance among raters. Particularly, inter-rater disagreement on CC volumes is not significant for significance level $p<0.05$ (CC-Posterior, $p=0.831$; CC-Mid-Posterior, $p=0.271$; CC-Central, $p=0.466$; CC-Mid-Anterior, $p=0.188$; CC-Anterior, $p=0.163$).

Total Brain volume

TBV is higher in ASD when compared to controls, when age and sex were considered as covariates ($p=0.006$). This difference is driven mainly by differences between males, because TBV was significantly higher in males ASD compared to control males ($p=0.017$), when age is considered as covariate; instead TBV does not show significant differences between ASD

females and control females (p=0.169). TBV results are summarized in Table 3; Figure 1 shows TBV differences between ASD and controls.

Males + Females				
	Mean ± std		**Anova (covariates: age, sex)**	
	ASD	**CTRL**	**F**	**p**
TBV	(1.08 ± 0.12) x 10^6	(1.01 ± 0.13) x 10^6	7.72	0.006 *
Males				
	Mean ± std		**Anova (covariate: age)**	
	ASD	**CTRL**	**F**	**p**
TBV	(1.14 ± 0.11) x 10^6	(1.05 ± 0.14) x 10^6	6.14	0.017 *
Females				
	Mean ± std		**Anova (covariate: age)**	
	ASD	**CTRL**	**F**	**p**
TBV	(1.02 ± 0.10) x 10^6	(0.98 ± 0.12) x 10^6	1.96	0.169

Table 3: TBV comparison between ASD and controls (* significance level p <0.05).

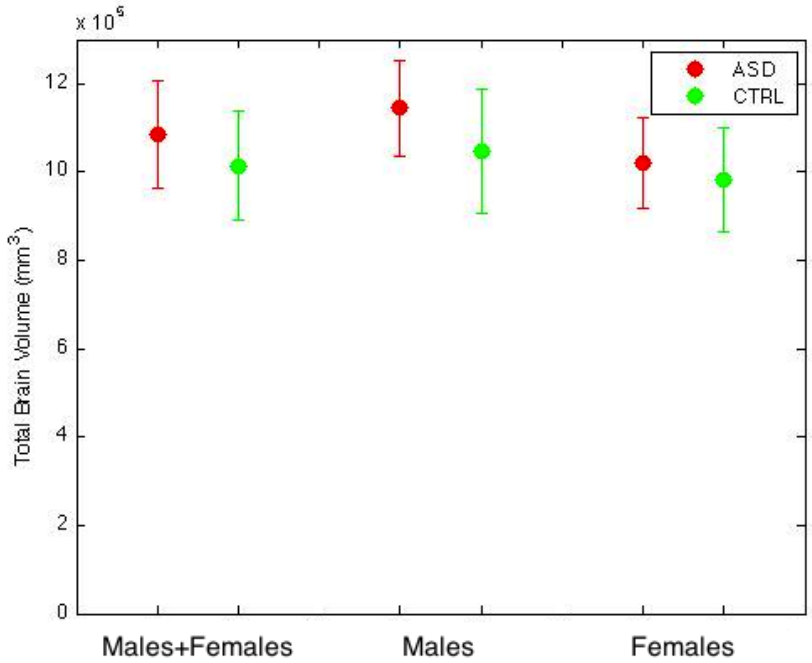

Figure 1: Differences in TBV between ASD and controls, both with and without gender distinction (males+females, males and females); *abbreviations*: ASD, autism spectrum disorder; CTRL, controls.

Corpus callosum total volume

CC total volume (CC-tot) is greater in the ASD group compared to controls, and if no covariate was used (two-sample t-test) CC-tot is significantly greater in ASD subjects (2875 ±

585 mm³) compared to controls (2544 ± 577 mm³; p=0.012) and this difference is mainly driven by males (2971 ± 532 mm³ in ASD; 2526 ± 597 mm³ in controls; p=0,015) rather than by females (2774 ± 635 mm³ in ASD; 2562 ± 570 mm³ in controls; p=0.272).

CC-tot does not significantly differ when using either TBV (p=0,159) or TBV + age (p=0,100) as covariates between ASD group and controls group.

Table 4 summarizes CC-tot analysis results.

Males + Females				**covariate: TBV**		**covariates: TBV + Age**		**Two-sample t-test**	
	Mean ± std								
	ASD		**CTRL**	**F**	**p**	**F**	**p**	**t**	**p**
CC-tot	2875 ± 585	>	2544 ± 577	2,021	0,159	2,776	0,100	2,56	0,012*
Males									
	Mean ± std								
	ASD		**CTRL**	**F**	**p**	**F**	**p**	**t**	**p**
CC-tot	2971 ± 532	>	2526 ± 597	0,32	0,575	1,545	0,222	2,52	0,015*
Females									
	Mean ± std								
	ASD		**CTRL**	**F**	**p**	**F**	**p**	**t**	**p**
CC-tot	2774 ± 635	>	2562 ± 570	0,402	0,530	0,946	0,337	1,11	0,272

Table 4: CC-tot analysis results (* significance level p<0.05).

No differences in ASD CC-tot were found, when compared to controls and using covariates, either among males (p=0.575 when TBV is used; p=0.222 when TBV + age is used), or females (p=0.530 when TBV is used; p=0.337 when TBV + age is used). If no covariate was used (two-sample t-test) differences found in CC-tot is driven mainly by males ASD subjects who show greater CC-tot than controls (p=0.015); instead females show no significant difference (p=0.272).

See Table 4 for CC-tot results. Figure 2 shows differences within groups.

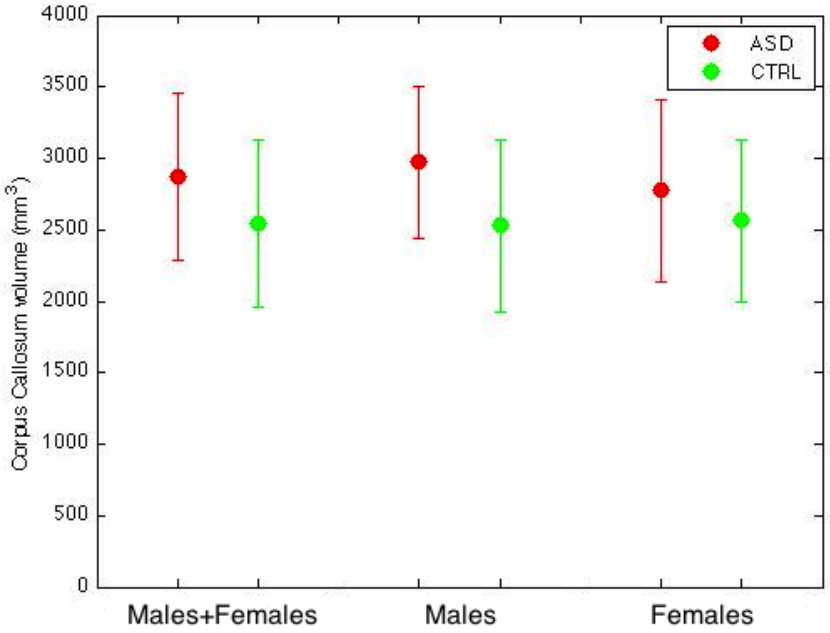

Figure 2: Differences in CC-tot volume between ASD and controls, both with and without gender distinction (males+females, males and females); *abbreviations*: ASD, autism spectrum disorder; CTRL, controls.

Sub-regions volumes

Sub-regions volumes tend to be larger in ASD when compared to controls, but when covariates were considered (TBV, CC-tot, TBV + age, or CC-tot + age), sub-regions volumes do not differ significantly within groups, except that for females in Mid-Ant sub-region,

where ASD have a smaller volume than controls (p = 0.004, using CC-tot as covariate; p = 0.001, using CC-tot + age as covariate). See table 4 for sub-regions volumes analysis results. Figure 3 shows CC sub-regions segmentation performed by Freesurfer.

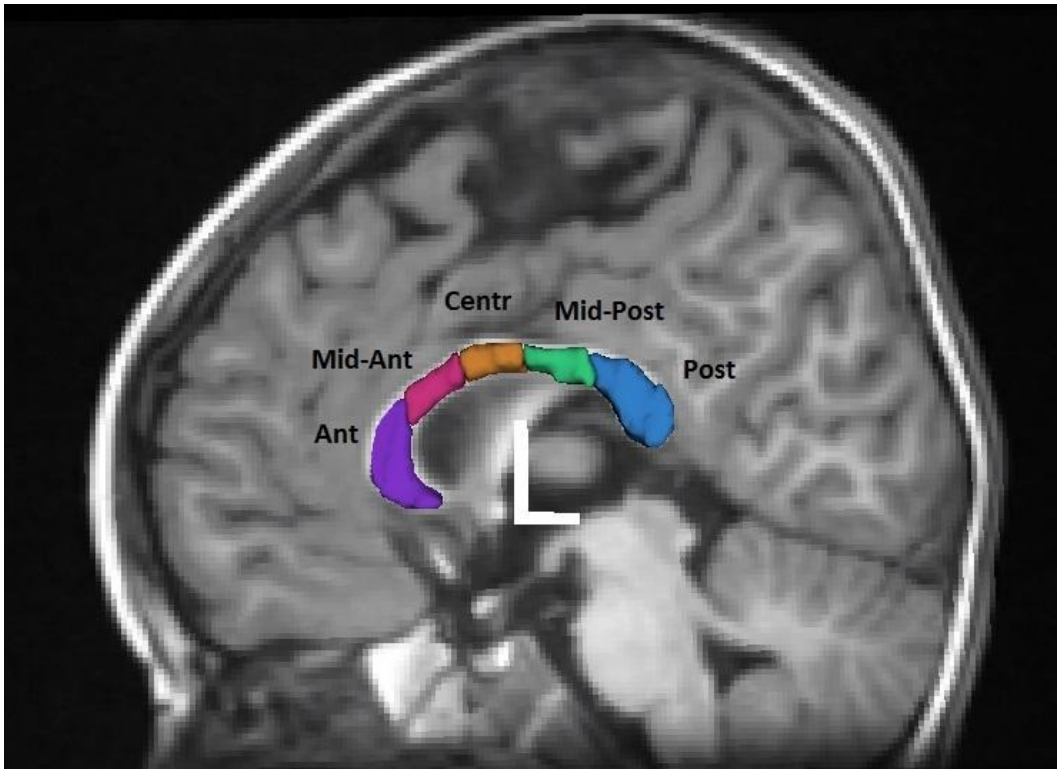

Figure 3: CC sub-regions as segmented by Freesurfer; *abbreviations*: Ant, anterior; Mid-Ant, mid-anterior; Centr, central; Mid-Post, mid-posterior; Post, posterior.

Males + Females			**covariate: TBV**		**covariate: CC-Tot**		**Covariates: TBV+Age**		**covariates: CC-Tot+Age**	
Sub-region	**Mean±std**									
	ASD	**CTRL**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**

CC_Post	710 ± 117	>	638 ± 143	1,782	0,186	0,401	0,528	2,187	0,143	0,383	0,538
CC_Mid_post	373 ± 108	>	321 ± 102	1,16	0,285	0,021	0,885	1,617	0,207	0,038	0,846
CC_Cen	433 ± 108	>	372 ± 116	1,936	0,168	0,05	0,824	2,846	0,096	0,137	0,712
CC_Mid_Ant	546 ± 170	>	495 ± 199	0,089	0,766	3,284	0,074	0,228	0,634	3,292	0,073
CC_Ant	813 ± 195	>	719 ± 151	2,675	0,106	0,701	0,405	3,046	0,085	0,6	0,441
Males											
Sub-region	**Mean±std**										
	ASD		**CTRL**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
CC_Post	713 ± 95	>	648 ± 152	0,062	0,805	0,321	0,574	0,059	0,81	0,337	0,565
CC_Mid_post	408 ± 88	>	308 ± 99	4,999	0,031	4,607	0,038	4,919	0,033	4,484	0,041
CC_Cen	462 ± 98	>	359 ± 120	3,592	0,066	2,533	0,12	3,745	0,061	2,712	0,108
CC_Mid_Ant	593 ± 157	>	454 ± 179	2,565	0,118	0,654	0,424	2,877	0,098	0,841	0,365
CC_Ant	796 ± 189	>	757 ± 133	0,087	0,77	5,831	0,021	0,089	0,767	6,82	0,013
Females											
Sub-region	**Mean±std**										
	ASD		**CTRL**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
CC_Post	706 ± 139	>	628 ± 135	2,019	0,164	2,413	0,129	3,058	0,089	2,795	0,103
CC_Mid_post	338 ± 118	>	333 ± 106	0,244	0,624	2,972	0,093	0,053	0,819	2,656	0,112
CC_Cen	403 ± 112	>	385 ± 113	0	0,992	1,988	0,167	0,095	0,76	1,77	0,192
CC_Mid_Ant	497 ± 172	<	535 ± 214	1,19	0,282	0,368	0,004*	0,998	0,324	13,89	0,001*
CC_Ant	831 ± 204	>	680 ± 162	5,32	0,027	5,553	0,024	7,441	0,01	6,348	0,016

Table 4: CC sub-regions volumes (mm^3) analysis results (* significance level $p<0.01$, after Bonferroni correction); *abbreviations*: Ant, anterior; Mid_Ant, mid-anterior; Cen, central; Mid_Post, mid-posterior; Post, posterior.

Demographic correlations: sex and age

Sex

In the analysis of gender differences, both among ASD and controls, no significances were found. Particularly no significant sex differences were observed, among ASD subjects, both in CC-tot, for significance level $p<0.05$ ($p=0.258$), or CC-subregions for $p<0.01$ (see Table 5 for detailed sex analysis results). No significant difference were found among controls also in CC-tot ($p=0.458$) and its sub-regions.

However, although not significant, ASD males subjects show a tendency for a larger CC-tot compared to females; among controls the trend is reversed. In ASD, males have almost all sub-regions larger, compared to females, except the Anterior sub-region, that is smaller in males than females ($796 \pm 189 < 831 \pm 204$). Controls show more heterogeneity among sub-regions, and the Anterior sub-regions is larger in males than in females ($757 \pm 133 > 680 \pm 162$).

Figure 4 shows sex differences in CC-tot among ASD and controls.

ASD					
	Mean ± std			**ANOVA (covariates: TBV, age)**	
	Males		**Females**	**F**	**p**
CC_tot	2971 ± 532	>	2774 ± 635	1,32	0,2581
CC_Posterior	713 ± 95	>	706 ± 139	4,29	0,0453
CC_Mid_Posterior	408 ± 88	>	338 ± 118	0,00	0,9772
CC_Central	462 ± 98	>	403 ± 112	0,00	0,9922
CC_Mid_Anterior	593 ± 157	>	497 ± 172	0,02	0,8842
CC_Anterior	796 ± 189	<	831 ± 204	4,16	0,0485
Controls					
	Mean ± std			**ANOVA (covariates: TBV, age)**	
	Males		**Females**	**F**	**p**
CC_tot	2526 ± 597	<	2562 ± 570	0,56	0,4585
CC_Posterior	648 ± 152	>	628 ± 135	0,16	0,6910
CC_Mid_Posterior	308 ± 99	<	333 ± 106	2,03	0,1627
CC_Central	359 ± 120	<	385 ± 113	1,32	0,2581
CC_Mid_Anterior	454 ± 179	<	535 ± 214	2,55	0,1191
CC_Anterior	757 ± 133	>	680 ± 162	2,56	0,1181

Table 5: sex differences of callosal volumes (mm^3) in ASD group and in control group (significance level $p<0.05$ for CC-tot; $p<0.01$ for sub-regions).

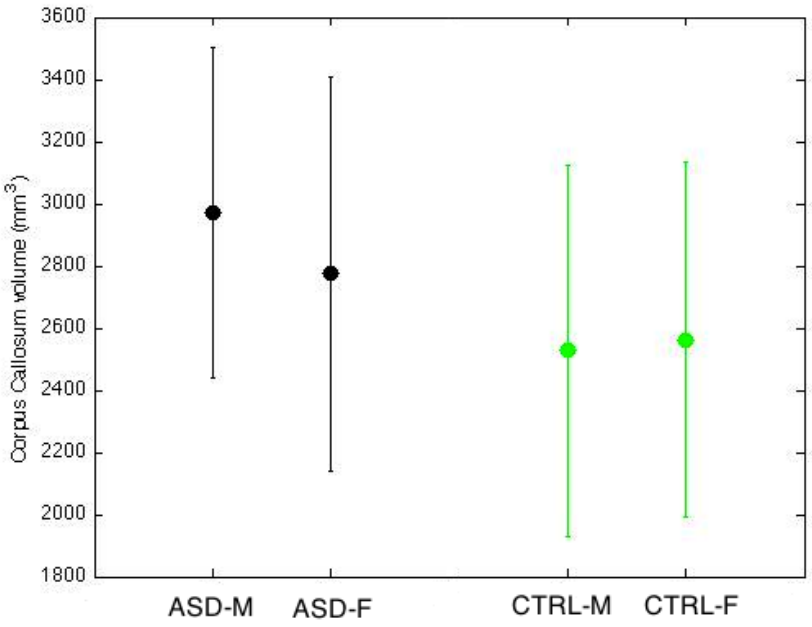

Figure 4: sex differences in CC-tot among ASD group and control group; *abbreviations*: ASD-M, ASD males; CTRL-M, controls males; ASD-F, ASD females; CTRL-F, controls females.

Age

CC-tot positively correlated with age in both ASD and controls. The correlation is significant only in the control group (p = 0.009, r = 0.406), instead it is not significant in ASD (p = 0.050, r = 0.308). In age correlation, the comparison between males and females reveals gender differences in both groups: in ASD group, the correlation is driven mostly by females (p = 0.005, r = 0.600), while males do not show significant CC volume correlations with age (p = 0.485, r = -0.161). On the contrary, in control group opposite results are found: the correlation is driven mostly by males (p = 0.040, r = 0.463), while females do not show significant CC volume correlations with age (p = 0.118, r = 0.361).

Table 5 summarizes age correlations results. Figure 5 shows age distribution in ASD and controls.

ASD		
	p-value	**Pearson's index**
Males + Females	0,050	0,308
Males	0,485	-0,161
Females	0,005*	0,600

Controls		
	p-value	**Pearson's index**
Males + Females	0,009*	0,406
Males	0,040*	0,463
Females	0,118	0,361

Table 5: CC-tot correlations with age (* significance level p<0.05).

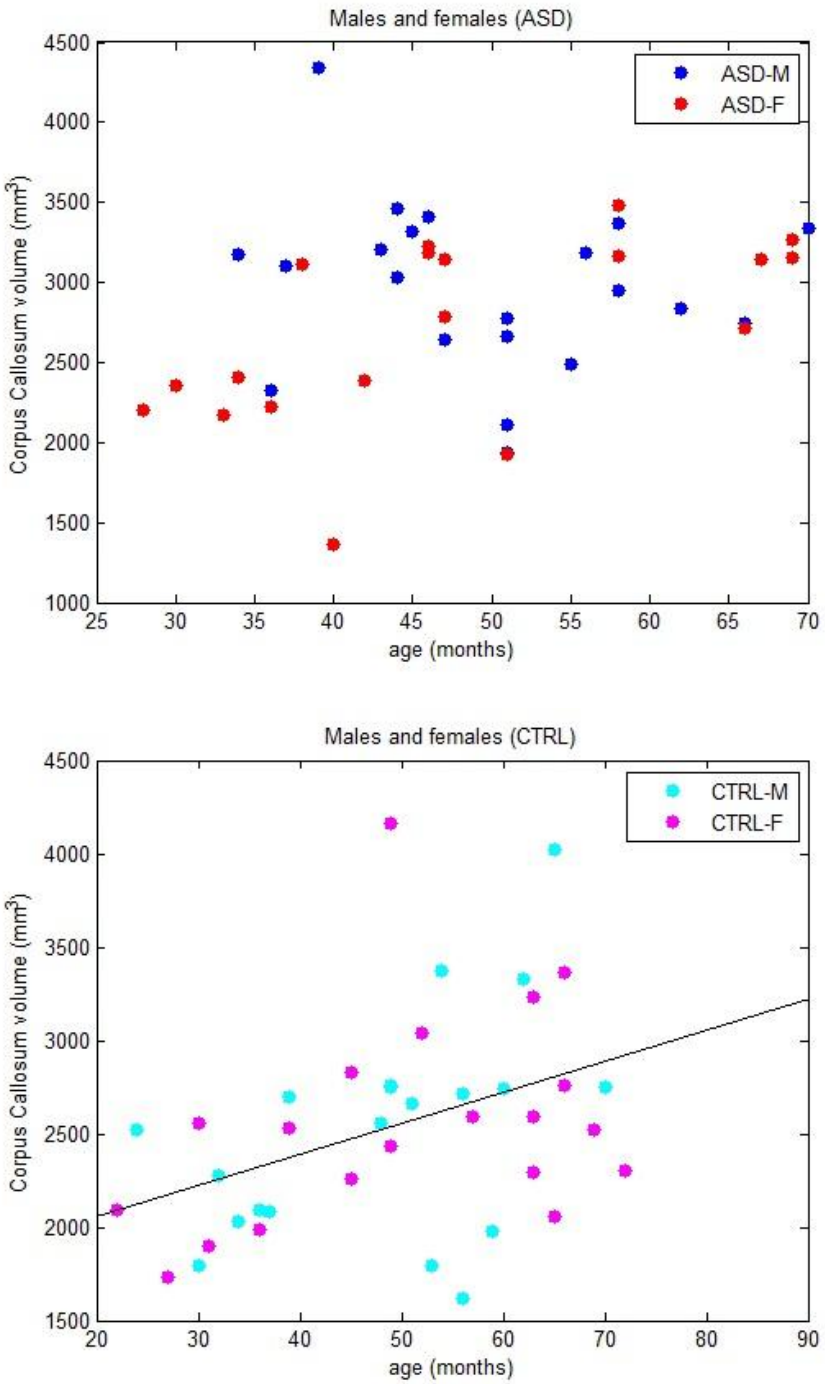

Figure 5: CC-tot correlation with age in the ASD group and in the control group; sex are represented by different colours; a line is plotted only for significant correlations ($p<0.05$); *abbreviations*: ASD-M, ASD males; CTRL-M, controls males; ASD-F, ASD females; CTRL-F, controls females.

To better explore age correlation we analyzed CC-tot relations with age in two groups of ages: children younger than 49 months of age (named Group1: age ≤ 49 months, N=42) and older than 49 months of ages (named Group 2: age > 49 months, N=39). Both in Group 1 and in Group 2 no differences were found in the analysis of CC-tot when ASD are compared to controls and TBV, age and sex were used as covariates (Group 1, p=0.211; Group 2 p=0.400). A significant difference emerges between younger males: CC-tot is significantly larger in the ASD group compared to controls (p=0.008), when TBV and age were used as covariates. In older males the difference loses significance (p=0.508). In the female set no differences were found in Group 1 (p=0.503) and Group 2 (p=0.075).

Figure 6 shows CC volume differences between ASD and controls in Group 1 and Group 2 respectively. Table 6 and Table 7 summarize respectively Group 1 and Group 2 features and CC volume results.

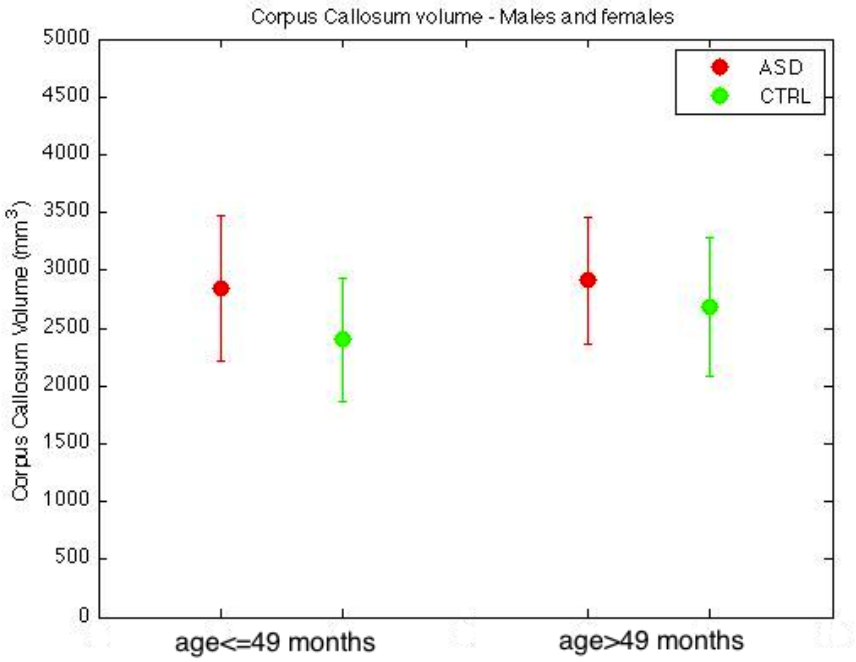

Figure 6: CC volume differences between ASD and controls in Group 1 (age ≤ 49 months) and Group 2 (age > 49 months) respectively

Group 1 (age ≤ 49 months, N=42)				
Males + Females				
	Mean ± std		**Univariate analysis**	
	ASD (N=22)	**CTRL (N=20)**	**(covariates: TBV, age, sex)**	
			F	**p**
CC total	2843.8 ± 629.8	2401.3 ± 532.7	1.62	0.211
Males				
	Mean ± std		**Univariate analysis**	
	ASD (N=10)	**CTRL (N=10)**	**(covariates: TBV, age)**	
			F	**p**
CC total	3199.5 ± 533.1	2354.6 ± 344.3	9.03	0.008 *
Females				
	Mean ± std		**Univariate analysis**	
	ASD (N=12)	**CTRL (N=10)**	**(covariates: TBV, age)**	
			F	**p**
CC total	2547.4 ± 560.2	2448 ± 689.7	0.47	0.503

Table 6: Group1 (age ≤ 49 months) features and CC total volume analysis results (* significance level p<0.05).

Group 2 (age > 49 months, N=39)				
Males + Females				
	Mean ± std		**Univariate analysis**	
	ASD (N=19)	**CTRL(N=20)**	**(covariates: TBV, age, sex)**	
			F	**p**
CC total	2911.1 ± 543.9	2686.9 ± 596.5	0.72	0.400
Males				
	Mean ± std		**Univariate analysis**	
	ASD (N=11)	**CTRL (N=10)**	**(covariates: TBV, age)**	
			F	**p**
CC total	2763.6 ± 458.4	2698.3 ± 754.7	0.46	0.508
Females				
	Mean ± std		**Univariate analysis**	
	ASD (N=8)	**CTRL (N=10)**	**(covariates: TBV, age)**	
			F	**p**
CC total	3113.8 ± 615.9	2675.5 ± 425.7	3.68	0.075

Table 7: Group2 (age > 49 months) features and CC total volume analysis results (* significance level p<0.05).

Clinical correlations: NVIQ and Language

Non verbal IQ (NVIQ)

CC-tot does not correlate with NVIQ either in ASD (p=0.685, r=0.065) or control group (p=0.617, r=-0.081), after Bonferroni correction (significance level p<0.05). Although not significantly, CC-tot of control subjects seems to have a negative correlation trend with NVIQ (r=-0.081), instead CC-tot of subjects with ASD, shows a positive, not significant, correlation with NIVQ (r=0.065); in this group the positive trend is mainly due to females (p=0,255; r=0,267), instead males with ASD show a negative correlation trend (p=0,360; r=-0,210), as controls do.

In Table 8 NVIQ analysis results are shown. Figure 7 shows NIVQ and CC-tot distribution in ASD and controls.

ASD		
	p-value	**Pearson's index**
Males + Females	0,685	0,065
Males	0,360	-0,210
Females	0,255	0,267

Controls		
	p-value	**Pearson's index**
Males + Females	0,617	-0,081
Males	0,885	-0,034
Females	0,587	-0,129

Table 8: NVIQ correlation analysis (significance level p<0.05).

Language

CC-tot does not correlate significantly with expressive language level achieved in either the ASD group (p=0.883, r=-0.024) or the control group (p=0.331, r=0.158). Although not significantly, ASD group tends to correlate negatively with language level, both in males and females, conversely than controls.

Table 9 shows language correlation analysis. Figure 8 shows speech and CC-tot distribution in ASD and controls.

ASD		
	p-value	**Pearson's index**
Males + Females	0,883	-0,024
Males	0,279	-0,248
Females	0,553	-0,141

Controls		
	p-value	**Pearson's index**
Males + Females	0,331	0,158
Males	0,472	0,171
Females	0,532	0,148

Table 9: Language correlation analysis (significance level p<0.05).

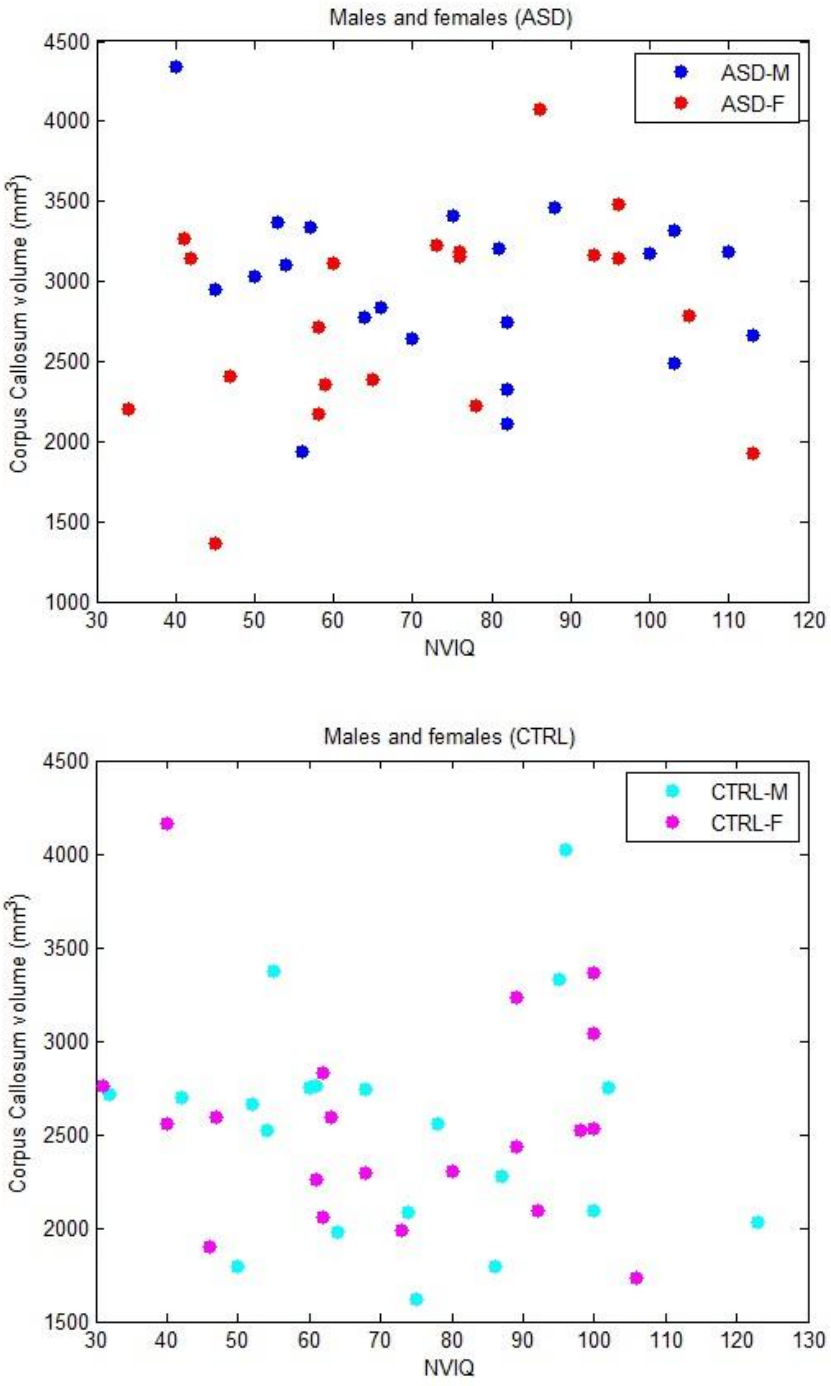

Figure 7: CC-tot correlation with NVIQ in the ASD group and in the control group; sex are represented by different colours; no line is plotted because no significant correlations were found ($p<0.05$); *abbreviations*: ASD-M, ASD males; CTRL-M, controls males; ASD-F, ASD females; CTRL-F, controls females.

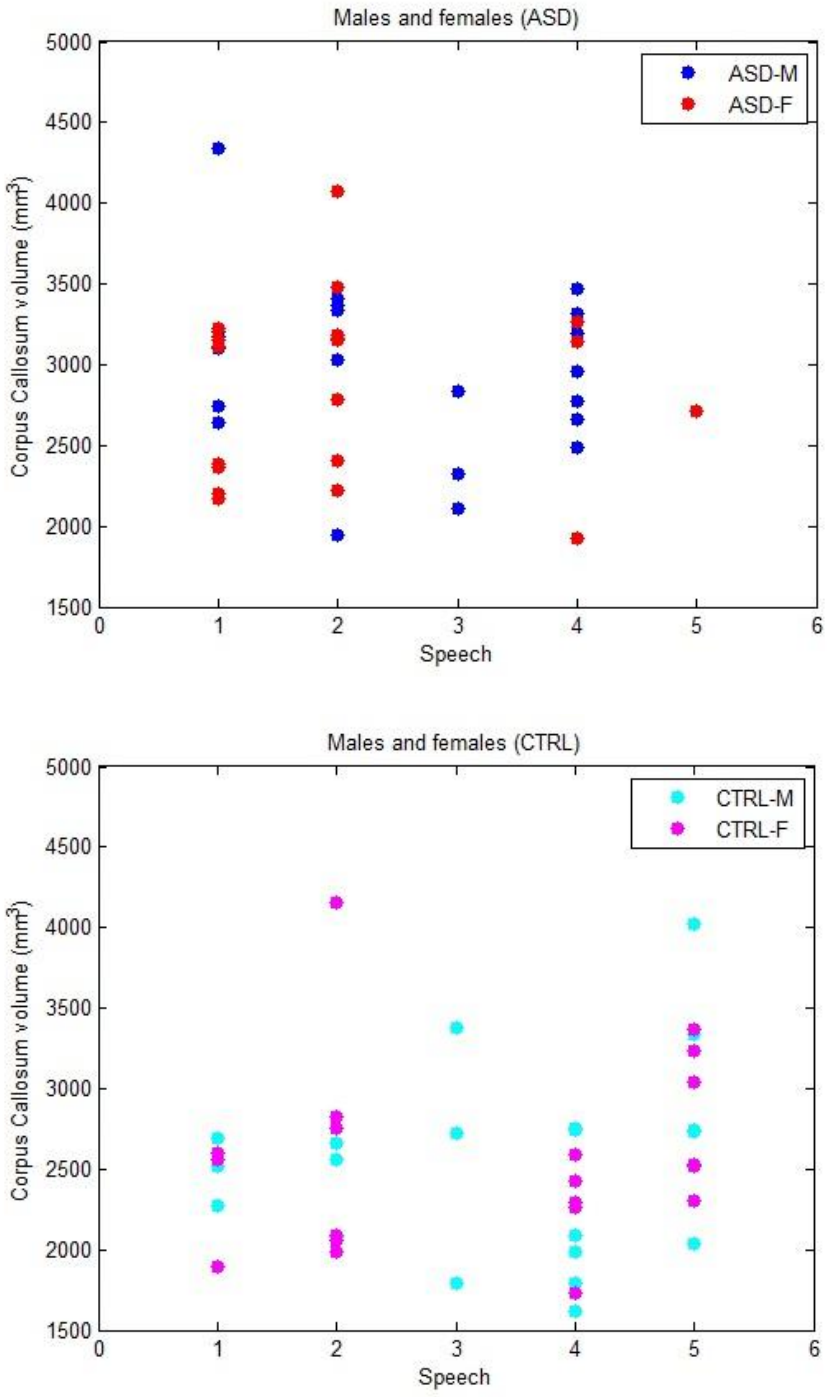

Figure 8: CC-tot correlation with speech (expressive language level) in the ASD group and in the control group; sex are represented by different colours; no line is plotted because no significant correlations were found ($p<0.05$); *abbreviations*: ASD-M, ASD males; CTRL-M, controls males; ASD-F, ASD females; CTRL-F, controls females; level 1, Preverbal Communication; level 2, First Words; level 3, Word Combinations; level 4, Sentences; level 5, Complex Language.

Clinical correlations: autism severity

ADOS-G scores

CC-tot significantly correlates ($p<0.05$), in a negative direction, with ADOS-G Total scores ($p=0.018$, $r= -0.372$). All ADOS-G scores show a negative trend correlation with CC-tot, both in males and females. A negative significant correlation ($p<0.025$) was found for Language and Communication scores ($p=0.017$, $r= -0.376$), after Bonferroni correction, in the ASD group (males+females). Reciprocal social interaction scores shows a negative correlation trend but it does not result significant after Bonferroni correction ($p=0.034$, $r= -0.336$).

Table 10 shows correlations between ADOS scores and CC-tot in the ASD group. Figures 9a, 9b, 9c represent ADOS-G scores correlation with CC-tot.

ADOS-G and CC-tot		
	p-value	**Pearson's index**
Males + Females		
ADOS-G - Language and Communication	0,017 *	-0,376
ADOS-G - Reciprocal Social Interaction	0,034	-0,330
ADOS-G Total	0,018 *	-0,372
Males		
ADOS-G - Language and Communication	0,279	-0,248
ADOS-G - Reciprocal Social Interaction	0,295	-0,240
ADOS-G Total	0,233	-0,272
Females		
ADOS-G - Language and Communication	0,048	-0,459
ADOS-G - Reciprocal Social Interaction	0,114	-0,374
ADOS-G Total	0,076	-0,416

Table 10: ADOS-G scores correlations with CC-tot (* significance level $p<0.05$ for Total scores, $p<0.025$ for Language and Communication scores and Reciprocal Social Interaction scores).

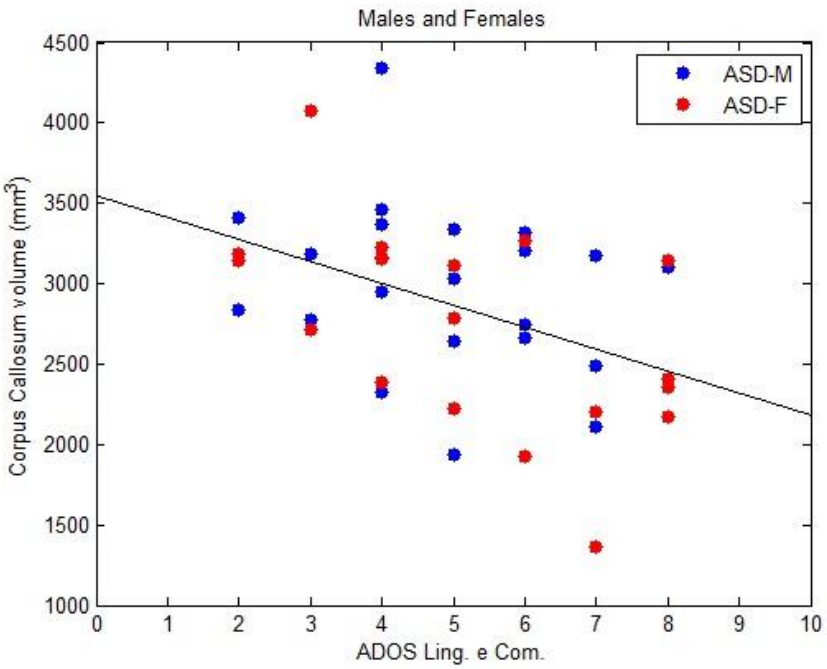

Figure 9a: CC-tot correlation with ADOS-G Language and Communication scores in the ASD group; sex are represented by different colours; a line is plotted for significant correlations ($p<0.025$); *abbreviations*: ASD-M, ASD males; ASD-F, ASD females.

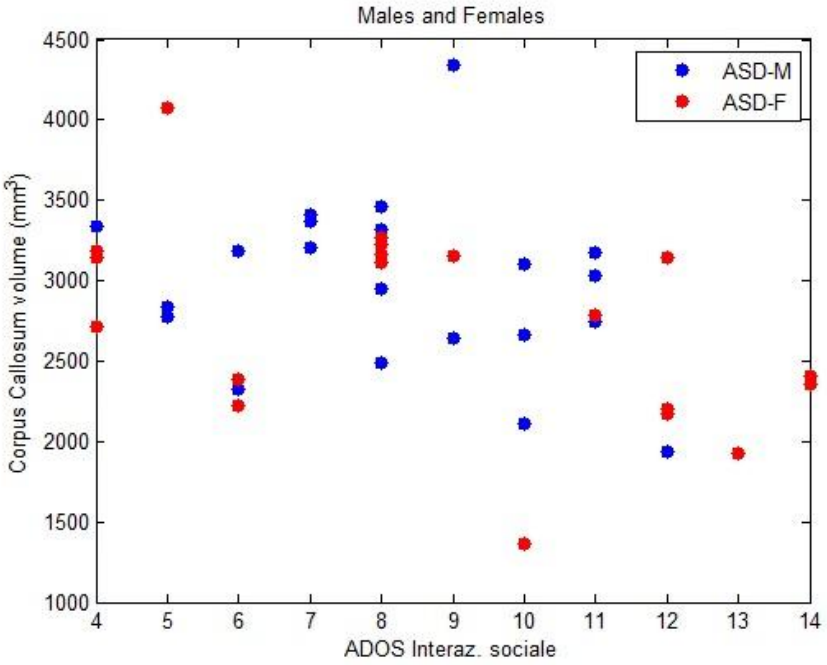

Figure 9b: CC-tot correlation with ADOS-G Reciprocal Social Interaction scores in the ASD group; sex are represented by different colours; no line is plotted because no significant correlation was found ($p<0.025$); *abbreviations*: ASD-M, ASD males; ASD-F, ASD females.

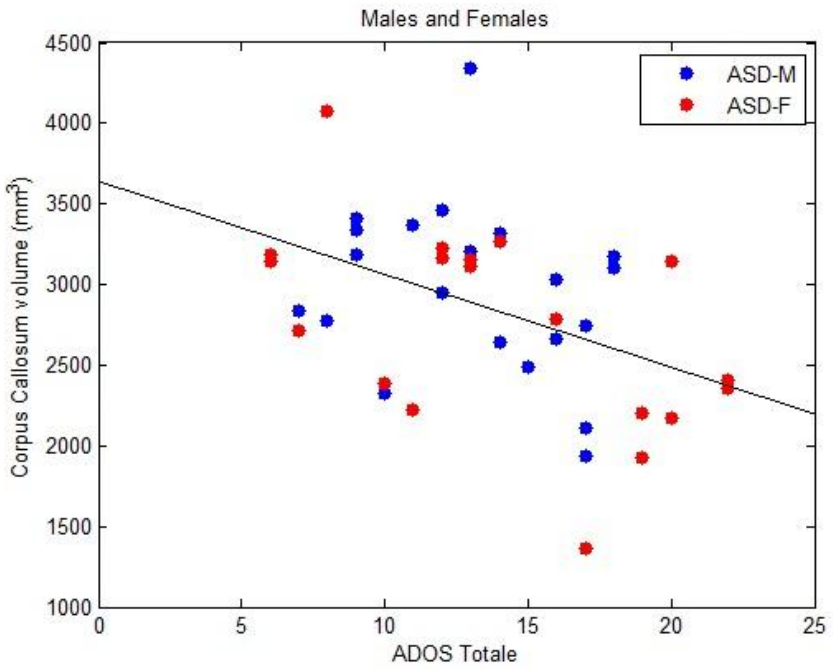

Figure 9c: CC-tot correlation with ADOS-G Total scores in the ASD group; sex are represented by different colours; a line is plotted for significant correlation ($p<0.05$); *abbreviations*: ASD-M, ASD males; ASD-F, ASD females.

We search for a potential ADOS-G scores and TBV correlation, but no significant relationship was found.

Table 11 show ADOS-G and TBV correlation analysis.

ADOS-G and TBV		
	p-value	**Pearson's index**
Males + Females		
ADOS-G - Language and Communication	0,3729	-0,1447
ADOS-G - Reciprocal Social Interaction	0,2421	-0,1893
ADOS-G Total	0,2620	-0,1816
Males		
ADOS-G - Language and Communication	0,5116	0,1517
ADOS-G - Reciprocal Social Interaction	0,8278	-0,0505
ADOS-G Total	0,8638	0,0398
Females		
ADOS-G - Language and Communication	0,1035	-0,3851
ADOS-G - Reciprocal Social Interaction	0,3237	-0,2394
ADOS-G Total	0,2100	-0,3013

Table 11: correlation analysis between ADOS-G scores and TBV (* significance level $p<0.05$ for Total scores, $p<0.025$ for Language and Communication scores and Reciprocal Social Interaction scores).

CBCL 1½-5 scores

CC-tot does not correlate with CBCL1½-5 scores, in none of the analyzed scales: Internalizing Problems (p=0.275), Externalizing Problems (p=0.613) and Total Problems (p=0.714), Withdrawn (p=0.284), Attention Problems (p=0.706), and DSM-oriented Pervasive Development Problems (p=0.242).

However, among other, a tendency for negative, not significant, correlations for the DSM-oriented scale Pervasive Development Problems (p=0.242, r = -0.210) and Withdrawn scale were found (p=0.282, r= -0.192).

Table 11 summarizes CBCL1½-5 scores correlations with CC-tot results. Figure 10 shows data about DSM-oriented Pervasive Development Problems and Withdrawn scales.

CBCL 1½-5		
	p-value	**Pearson's index**
Males + Females		
Int	0,275	-0,196
Ext	0,613	0,091
Tot	0,714	-0,066
Withd	0,284	-0,192
Atten	0,706	0,068
DSM-PDP	0,242	-0,210
Males		
Int	0,993	-0,002
Ext	0,859	0,047
Tot	0,831	0,056
Withd	0,952	-0,016
Atten	0,401	0,218
DSM-PDP	0,490	0,180
Females		
Int	0,218	-0,326
Ext	0,668	0,116
Tot	0,649	-0,123
Withd	0,259	-0,300
Atten	0,851	-0,051
DSM-PDP	0,101	-0,425

Table 11: CC-tot correlations with CBCL1½-5 scores analysis (significance level p<0.05); *abbreviations*: Int, Internalizing problems scale; Ext, Externalizing problems scale; Tot, Total problems scale; Withd, Withdrawn scale; Atten, Attention problems scale; DSM-PDP, DSM-oriented Pervasive developmental problems scale.

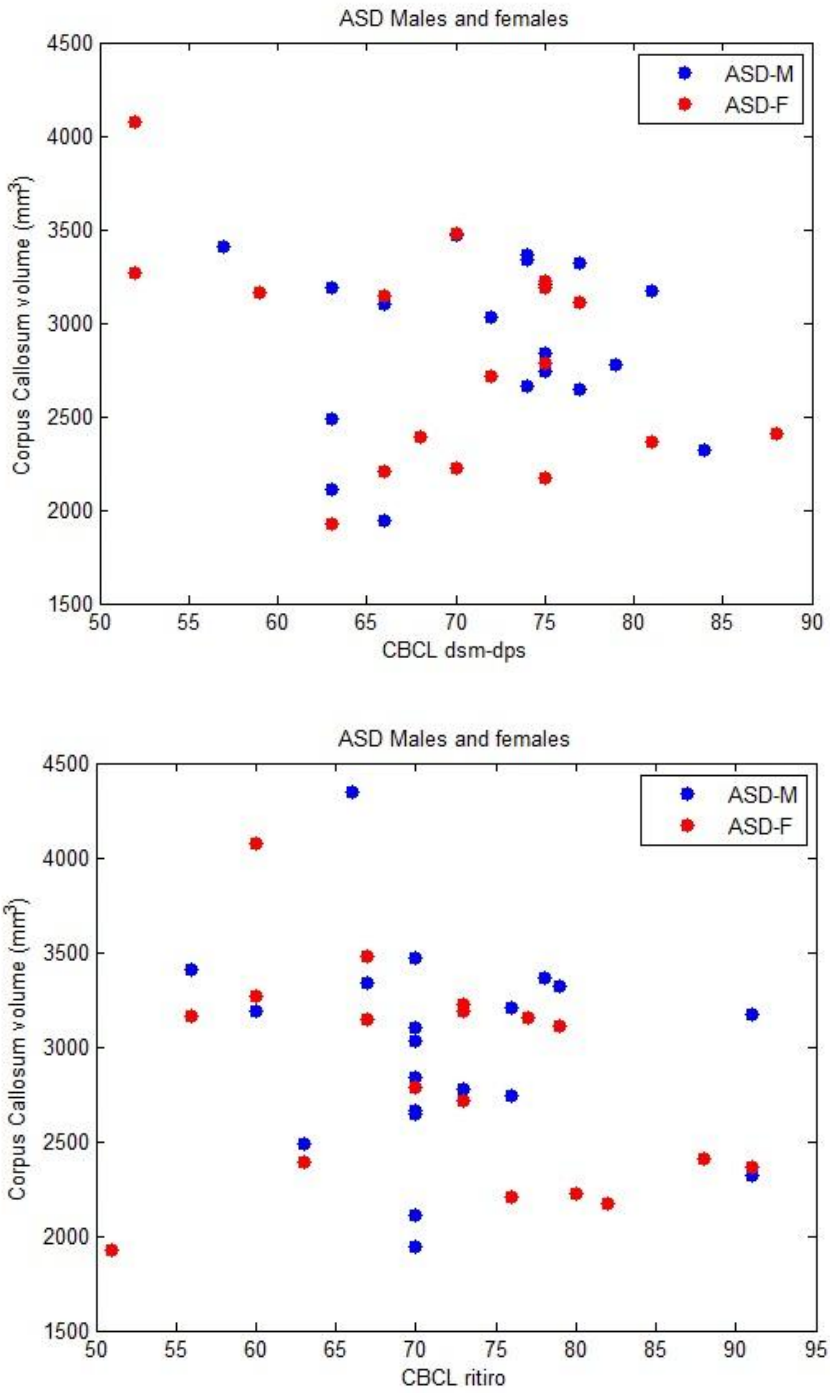

Figure 10: CC-tot correlation with CBCL1½-5 scores in the ASD group; sex are represented by different colours; a line is plotted for significant correlation ($p<0.05$); *abbreviations*: ASD-M, ASD males; ASD-F, ASD females; CBCL dsm-dps, DSM-oriented Pervasive developmental problems scale; CBCL ritiro, Withdrawn scale.

Discussion

Autism Spectrum Disorder (ASD) are an heterogeneous spectrum of neurodevelopmental disorders characterized by early-onset abnormalities in social communication and interaction, and atypically restricted and repetitive behaviors and interests. The impaired social interaction and communication, although permanent, is expressed by symptoms that can vary over time during development. Symptoms often are present in the early developmental period, but may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life.

Despite its high and increasing prevalence (recently estimated in about 1 child every 68; Blenner, 2014), it is not yet clear what causes autism, and neurobiological investigations about ASD etiology have helped to create an impressive number of studies in literature.

Genetics has a key role in the etiology of autism, and twins studies have suggested that autism has high heritability, that occurs in the context of environmental pre- and/or post-natal risk factors. This gene-environment interplay, in epigenetic mechanisms, causes neurobiological anomalies in brain development, resulting in the heterogenic spectrum of autistic symptoms.

Many studies have identified several atypicalities in brain of subjects with ASD and possible neuroanatomical, cellular, and molecular underpinnings etiological factors of autism have been identified.

One of the most replicated data on neuroanatomical studies of ASD, is the abnormal overgrowth of brain volume (TBV) in early development (Courchesne et al., 2001; 2004; 2011; Schumann et al., 2010; Nordahl et al., 2011; Shen et al., 2013), with over 90% of autistic toddlers (2-3 years of age) exhibited abnormally larger total brain, compared to neurotypical peers. Before 2 years of age, brains of subjects with ASD show an abnormal expansion trajectory, as result of increased rate of brain growth from early infancy through preschool period, followed by an abnormally slow cerebral volume increase during late childhood, puberty and adolescence. These and other evidences from electrophysiology, functional neuroimaging, molecular genetics and information processing studies, have given rise to the idea that autism is characterized by atypical neural connectivity, rather than by anomalies in different brain regions.

Although yet consistency about connectivity is low, data support the hypothesis that neural networks in autism are atypical, leading to the "disconnection syndrome theory" (Frith et al, 2004; Melillo et al., 2009) and the "under-connectivity theory" of autism (Just et al., 2012). Recent fMRI data reveal hyper-connectivity in subcortical networks and hypo-connectivity in cortico-cortical and interhemispheric functional networks, in a large sample of males with ASD (Di Martino et al., 2014).

In the disconnection hypothesis framework, a growing body of literature studies corpus callosum (CC), as the largest white matter structure in human brain and the main connection and information interhemispheric transfer structure. A further boost to the study of CC in autism has been given by the detection of autistic symptoms in individuals with agenesis of the CC (Booth et al., 2011; Paul et al., 2014), supporting the hypothesis that congenital disruption of the CC constitutes a risk factor for developing ASD. Thus, to date, we can find

192 papers about CC and autism on the main engine of scientific research, and many of these studies are focused on callosal size (volume or area).
Most of these studies identified reductions of the CC size in subjects with ASD (see review by Bellani et al., 2013). However findings are not so consistent and some studies reveal no CC differences between ASD and controls (Waiter et al. 2004; Bonilha et al. 2008; Ke et al. 2008; Ecker et al. 2010; Toal et al. 2010; Cheng et al. 2011; Mengotti et al. 2011; Hong et al. 2011; Calderoni et al. 2012; Lefebvre et al., 2014). Interestingly no study published, in our knowledge, reports CC size increased in ASD. Moreover some studies report a reduction of the entire volume of the CC (Hardan et al., 2009; McAlonan et al., 2009; Duan et al., 2010; Anderson et al., 2011), others report a reduction of one or more parts of the CC, mainly in the anterior region (Alexander et al., 2007; Keary et al., 2009; Thomas et al., 2011), others mainly in the posterior region (Waiter et al., 2005) or simultaneously in the anterior and posterior regions (Vidal et al., 2006).
Volumetric reduction of CC size has been found both in adults and children, but studies performed on children involved mainly school-age subjects and adolescents, and only few studies are carried out on preschoolers.
Data about size of CC in preschoolers with ASD are few, inconsistent and not comparable, due to differences on sample selection (age, sex, clinical features of participants) and size parameters used (volume vs area). Among this publications, some studies report a reduction in CC size in ASD compared to controls (Duan et al., 2010; Boger-Mediddo et al., 2006; Prigge et al., 2013), others found no differences (Riva et al., 2011; Calderoni et al., 2012; Xiao et al., 2014).
The main goal of our study is to compare the CC volume of a well-selected group of 41 preschoolers with ASD and 40 age-, sex- and NVIQ-matched controls subjects. We analyzed CC subregions volume in both groups and callosal size relations to demographic and clinical variables of ASD and control group (gender, age, non-verbal IQ, and language) have been examined. Lastly, in the ASD group we assessed callosal volume relationship with autism severity.

Corpus callosum and its hypothetical growth trajectory

In preschoolers with ASD of our sample, CC is larger than their peers without autism. CC total volume (CC-tot) in fact is significantly greater in ASD group when compared to controls, and this difference is mainly driven by males rather than by females. But when TBV only, or TBV and age together, are considered, CC-tot does not significantly differ between ASD group and control group, because in ASD children TBV is significantly larger than controls during early development, as already widely described in literature (Courchesne et al., 2001; 2004; 2011; Schumann et al., 2010; Nordahl et al., 2011; Shen et al., 2013), especially in males (Nordahl et al., 2011). Our results are consistent with other data reported in literature (Xiao et al, 2014), resulting from a comparable sample groups (males and females preschoolers with ASD, with and without intellectual deficits) and size measurement data, using volume measures instead area. In our opinion the volume measure describes better CC dimensions than area measure, considering callosal tridimensional features, and better represent real size of the structure. Interestingly, previous studies that used area as measure parameter, found that CC is smaller in ASD subjects compared to controls (Boger-Mediddo et

al., 2006; Prigge et al., 2013), instead studies using volume as measurement value found no differences. This incongruity could be explained by anomalies in the shape of CC. Duan (2010) reported a significant reduction in the callosal length in ASD subjects, but the difference in the width was not significant, suggesting that the decrease found in CC size, especially in studies using mid-sagittal area, could be due to the decrease in the anterior-posterior length, rather than to the tri-dimensional reduction.

Results of these studies about CC area are not comparable, given that ASD samples are very different: in fact Boger-Mediddo analyzed CC area of 45 preschoolers ASD (38-54 months of age, 89% males), instead Prigge examined a wide range age sample (3-36 years old, only males). Anyway both reported that CC is smaller in ASD subjects when TBV is considered and not in absolute manner and, interestingly, callosal area inversely correlates with autism severity.

Other studies, using volume measurement, reported no differences in CC or other white matter structure of ASD subjects compared to controls, but as previously said, also these data are not comparable, given that sample selections are different: Calderoni (2012) involved only females and Riva (2011) recruited older children (age range 3-10 years) only with intellectual impairment. Another study about CC volume in ASD is that by Duan (2010), where a reduction in CC-tot was described, especially in its anterior portion. This reduction becomes more significant when considering TBV. Again this study result is not comparable to our data because sample age is very different to our, given that they analyzed MRI data from a 3-30 years old aged sample. Maybe the CC-tot reduction reported by Duan is mainly due to older subjects, consistently whit other literature data describing smaller callosal size in older ASD subjects.

In fact, it is interesting to note that, whereas older ASD subjects (school-aged, adolescents and young adults) have widely reported smaller CC than controls (Bellani et al., 2013), data on preschoolers are less consistent, and those studies using volume as measure parameter report no differences in callosal size of preschoolers ASD compared to controls (Riva et al., 2011; Calderoni et al., 2012; Xiao et al., 2014). Taken together, these data lead to the hypothesis that CC could have an abnormal growth trajectory in ASD compared to controls, characterized by a greater development in early ages, followed by a slower rate of growth in older ages, resulting in smaller CC in adolescents and adults patients. Larger reductions in CC size were found in advanced age in a meta-analysis involving subjects whose mean age was approximately 14 years (Frazier-Hardan, 2009). This hypothesis is not surprising, because this hypothetical and atypical CC growth trajectory seems to reflect abnormal TBV growth trajectory in ASD, that, as widely reported, is characterized by a rapid overgrowth with larger brain volumes in early stage of development, whereas subsequently in older children cerebral volume growth is slower, so in adults brain could be normal sized or smaller than controls.

The abnormal growth pattern of CC in ASD children is confirmed by the different correlation between CC-tot and age found in our study, along all the age-range used (54 months, from 18 to 72 months of age). CC size positively correlates with age both in ASD group and in control group, but this relationship is statistically significant only in preschoolers without ASD and it is not significant in ASD subjects. This results could mean that in preschoolers ASD the CC is

initially larger but tends to grow slowly than control, resulting smaller in older children and adults, as reported in literature. This hypothesis is supported by our results about CC-tot in a subgroup of younger children (≤ 49 months of age), particularly males, who have a significantly larger CC than their peers without ASD, when considering TBV also, whereas in older preschoolers (> 49 months of age) this difference disappear.

In a developmental perspective comparison of CC mid-sagittal area across 30-years age range, Prigge (2013) found that similar age-related changes were found in autism and controls (3-36 years old), although a slower isthmus growth was found. Though it was not a longitudinal study, it supported the brain maturation abnormalities hypothesis in autism. The first longitudinal study of the CC in autism was that by Frazier and Hardan group (2012), that investigated the volumetric changes of the CC during 2 years, in 7-13 years old subjects with ASD. Authors reported that volume of the CC increases with age in a similar measure between ASD and controls. This finding contrasted with the meta-analysis by the same group (2009), that reported a worsening of the reduction in the volume of the CC over time, except in the rostral region. However these studies involved older children, adolescents and young adults, thus they are poorly helpful to explain early developmental anomalies in callosal growth.

The hypothesis of atypical development of CC is also supported by other data about callosal white matter integrity in preschoolers. Some DTI-based studies already described abnormalities in the integrity of the CC in ASD population; in adolescents and adults findings are quite consistent, reporting a reduction of the FA in the CC in ASD (Alexander et al., 2007; Keller et al., 2007; Kumar et al., 2010; Travers et al., 2012; Aoki et al., 2013), although with some exceptions that found no differences (Cheng et al., 2010; Thomas et al., 2011; Hanaie et al., 2014). Other studies reported microstructural white matter anomalies in CC of ASD children also, particularly an increased fractional anisotropy (FA) value was found in preschoolers with ASD (Ben Bashat et al., 2007; Weinstein et al., 2011; Xiao et al., 2014), leading Authors to speculate an early and accelerated abnormal maturation of white matter in ASD young children. FA assess the degree of anisotropic diffusion in tissue and it is very sensitive to microstructural anomalies (Alexander et al., 2007), such as axonal density, size, myelination and organization of fibers within a white matter voxel. Whereas FA is increased in preschoolers ASD, it is reported to be reduced in older ASD subjects, as described in a quite recent review of literature (Travers et al., 2012). Some Authors recently reported an increased apparent diffusion coefficient (ADC), an indirect measure of white matter integrity, in CC of preschoolers with ASD (Razek et al., 2014). Its increasing is positively correlated to autism severity. In older children ADC decreases in ASD but not in controls (Mengotti et al., 2011). Authors underlined that age correlates negatively with callosal ADC only in the ASD group and not in neurotypical children, supporting the role of the altered trajectory of white matter growth in ASD during childhood. An altered myelination of the CC has been found in preschoolers with ASD, particularly in region II and III of Hofer-Frahm segmentation (Gozzi et al., 2012).

To explain the atypical overgrowth of brain and CC in ASD, some Authors speculate that this early brain overgrowth should result in a greater-than-normal amount of pruning of long-

distance connectivity, thus a greater brain overgrowth results in a greater connections pruning (Lewis et al., 2012). ASD show an inverse relation between callosal fiber length and CC size, supporting the hypothesized impact of fiber length in the over-pruning during development, resulting in a smaller CC.

Taken together, these finding about macro-structural (i.e., volumetric) and micro-structural features of CC in ASD children support not only the theory of atypicalities in connectivity and in white matter tracts, particularly in CC, but lead also to the hypothesis that these anomalies are age-related in developmental stages. Specific growth trajectory of CC in ASD is still unknown but it may be abnormal, as all brain growth is, and these findings support this hypothesis. Therefore it is important to consider the role of abnormal developmental trajectories in studying neuroanatomical features of ASD brains and maybe longitudinal studies are needed to better explore atypical changes during development in young children with ASD. Longitudinal studies could also explain if callosal and brain growths have a linear proportion or they have a different and indipendent growth trajectory one to each other.

Higher variability in CC size was reported in ASD compared to controls (Alexander et al., 2007jan; Prigge et al., 2013). Also in our sample, ASD children, especially females, show higher variability in CC volume than controls.

Sex differences

In preschoolers with ASD, CC-tot is greater in males than females, but this difference does not reaches statistic significance. Particularly no significant sex difference, among ASD subjects, emerges both in CC-tot or CC sub-regions, when TBV and age were considered. No significant sex-related difference is found, in CC-tot or sub-regions, among control group also. Although not significant, however ASD males children show a tendency for a larger CC-tot compared to females; among controls the trend is reversed. In ASD, males have almost all sub-regions larger, compared to females, except the Anterior sub-region, that is smaller in males than females. Instead controls show more heterogeneity among sub-regions, and the Anterior sub-regions is larger in males than in females.
Recent studies continue to report in the prevalence of ASD a male bias and to suggest sex differences in phenotypic presentation (such as fewer restricted and repetitive behaviors, greater social communication impairment, lower cognitive ability, weaker adaptive skills in females than males; see as example the recent study about behavioral and cognitive sex-related differences in ASD, by Frazier et al., 2014). Sex-related genetic differences (females are protected from the effects of heritable and de-novo ASD risk variants) and sex-related different in genetic-hormonal modulation were also reported (sex chromosomal genes and/or sex hormones, especially testosterone, may modulate the effects of genetic variation on the presentation of the phenotype; see as example Werling et al., 2013).
The investigation of sex-related differences in brain structure is relevant to understanding the pathophysiology of ASD, but to date very limited neuroimaging data are available to evaluate sex-related brain features, and, to date, no study, to our knowledge, analyses CC sex-differencs in preschoolers with ASD. Moreover studies performed on adults report inconsistent findings. In a high-functioning adults sample, quite recently Lai (2013) found

that the CC (splenium) is greater in females with autism than typical females, insted males with autism have CC equal to typical males. More recently, unpublished data about CC size in the ABIDE large sample (Lefebvre et al., 2014), reported that callosal volume in ASD adults was significantly smaller among females compared with males; however this difference is explained by the significant difference in TBV between females and males (as adding TBV as a covariate made the sex effect not statistically significant). In another high-functioning adults sample, with respect to gender, only TBV was significantly increased in males compared with females, resulting in a significantly decreased CC/TBV ratio in males (independently from gender and fully attributed to brain size). This finding is in accordance with the hypothesis that brain size, per se, is the relevant factor and contradicts the sexual dimorphism hypothesis of the human CC (Tepest et al., 2010).

Also in healthy subjects findings about CC sexual dimorphism are inconsistent. Several studies in fact reported the presence of a sexual dimorphism in the human CC, however it is not yet clear whether CC morphological differences described in literature are related to differences in brain size (Bishop and Wahlsten, 1997; Tepest et al., 2010; Luders et al., 2014), typically greater in males than in females, or are themselves linked to sex dimorphism (Sullivan et al, 2001). Disagreement also exists with respect to the direction of the sexual differences, with some studies reporting larger CC in men (Luders et al., 2014) and other studies reporting larger callosal regions in women (Tepest et al., 2010). Several studies also failed to detect any significant sexual difference in size of CC (Allen et al.,1991).

In this scenery, our findings are important but poorly comparable. However they support the hypothesis that males and females with ASD may have different not only clinical but also neuroanatomical features, such as CC volume and volume distribution among its sub-regions, though few substantial. Globally we can assume that females ASD have callosal features more similar to controls than males ASD. CC-tot in ASD females in fact does not significantly differ than control females callosal volume, instead volume differences are bigger among males, given that males with ASD have CC-tot significantly larger than control peers (if any TBV is not considered). In ASD, males and females show different CC growth pattern also: while in males CC shows an early overgrowth followed by a slower growth, in females callosal growth is similar to those of controls and homogenous.

Obviously this hypothesis has to be better explored with further studies, considering brain size and age as potential interfering factors. Although few studies analyzed developmental sex-related features in brains of subjects with ASD, some sex differences in the neuroanatomy of these children were reported, especially in the early brain overgrowth (Sparks et al., 2002; Bloss and Courchesne, 2007; Schumann et al., 2010; Nordahl et al., 2011) and interemispheric functional connectivity (Schulte et al., 2010). Our data supports the hypothesis that that some aspects of the neuroanatomy of autism are sex-dependent and males and females may have different structural neurophenotypes, but how these neuroanatomical differences relate to clinical presentation of ASD in boys and girls remains to be understood.

Sub-regions analysis

Another difference between males and females CC, regards sub-regions volume.
Quite all sub-regions tend to be larger in preschoolers with ASD when compared to controls, but when global volumes, such as TBV or CC-tot, and age were considered as covariates, sub-regions volumes do not differ significantly between groups.
This finding is in contrast with several other studies analyzing sub-regions volumes. Whereas some of them, in fact report in ASD subjects a reduction of the entire CC and all, or almost all, its sub-regions (Hardan et al. 2009; McAlonan et al. 2009; Anderson et al. 2011; Alexander et al. 2007), most of studies report internal differences in callosal segmentation size, mainly a reduction of the anterior region (Keary et al. 2009; Frazier-Hardan, 2009; Duan et al. 2010;), in the posterior region (Waiter et al. 2005; Freitag et al., 2009; Prigge et al., 2013) or simultaneously in the anterior and posterior regions (Vidal et al. 2006; Chung et al, 2004). Among these, studies performed with preschoolers subejcts are those by Duan (2010) and Prigge (2013), reporting in children with ASD a reduction in each sub-regions of the CC or only in the isthmus, respectively.
In our sample, the only sub-regions maintaining significance, when CC-tot and CC-tot + age are used as covariates, is the Mid-Anterior sub-region, that in females with ASD is significantly smaller when compared to females without ASD; this finding survives Bonferroni correction (significance value $p<0.01$). In our study, segmentation of CC was automatically performed with the software used for image processing (Freesurfer). This software subdivide CC in 5 regions on the basis of equally distant segments along its main axis, so no functional subdivision is done, but only a dimensional one (see Appendix for observation about methodological techniques). However we can assume, simply on a perceptive basis, that Mid-Anterior portion corresponds approximately to rostral body and part of the anterior mid-body of CC, according to Witelson subdivision. Many hypothetical scheme for CC segmentation were proposed in time (as example Witelson, 1989; Hofer-Frahm, 2006; Chao et al., 2009; Fabri-Palonara, 2013), often with inconsistent results. The most used in studies about CC is the historical and geometrically-based Witelson scheme, but more recently Hofer-Frahm, starting from Witelson scheme, revisited CC topography on the basis of DTI-based tractography and cortical connectivity information. Mid-Anterior sub-region, as identified by Freesurfer software, approximately overlap rostral body and the first part of anterior mid-body of Witelson subdivision, that corresponds to region II of Hofer-Frahm scheme, which contains fibers projecting to premotor and supplementary motor cortical areas (figure 11 shows a comparison between Freesurfer segmentation, A; Witelson subdivision, B; and Hofer-Frahm scheme, C). Both premotor and supplementary motor cortical areas are mainly involved in movements control. The premotor cortex is an area of motor cortex lying within the frontal lobe just anteriorly to the primary motor cortex and occupies part of Brodmann's area 6 (Barr - Kiernan, 1995). It is involved in the direct control of movement, especially in fine motor tasks, playing a role in planning (Purves et al., 2001), and in the spatial and sensory guidance of movement (such as selection of movements based on external events) and speech motor planning; thanks to the "mirror neurons system", situated in the ventral premotor cortex, this area is involved in social cognition also, such as understanding others' actions and their intentions behind them, and it underlies mechanisms of

observational learning and imitation (Cattaneo et al., 2009). The supplementary motor area is located in the medial part of Brodmann's area 6. Its neurons project directly to the spinal cord and may play a role in the direct control of movement, such as the control of postural stability during stance or walking, coordinating temporal sequences of actions, bimanual coordination, and the initiation of internally generated movement (as opposed to stimulus driven movement) and it is involved in relation and adaptation to the surroundings. Anatomically and functionally related, premotor cortex, particularly the "mirror neuron system" (Iacoboni et al., 2007), and supplementary motor cortex (Saugstad, 2008) are both involved in autism symptoms (Puzzo et al 2010; Peeva et al., 2013). Meta-analysis on CC size performed by Frazier and Hardan (2009) underlined that the portion showing the largest reduction in the CC of ASD is the rostral body, and the normalization of the rostral body, observed in adolescents, is associated with a decrease in externalizing and repetitive behavior, improvement in social interaction, and fine motor coordination (Frazier et al., 2012). This callosal region has less white matter density and higher ADC value in ASD subjects, and these findings are associated with a severer disoerder (Hong et al., 2011).These findings appear inconsistent and insignificant, but taken together they lead to the hypothesis that CC sub-regions may vary independently one to each other, and changes may be age and sex-related. Mid-Anterior (or rostral body) region seems to have a key role in ASD, thanks to its fibers originating from cortical areas involved in social cognition and motor coordination, and its development may have a peculiar growth trajectory compared to other sub-regions in subjects with autism. Why, in our sample, only females show this anomaly in sub-regions volume, should be further explored. It can be explained by a severer disoerder in females (in our sample females with ASD have higher ADOS-G scores than males), but also females may have intrinsic differences in callosal structure than males.

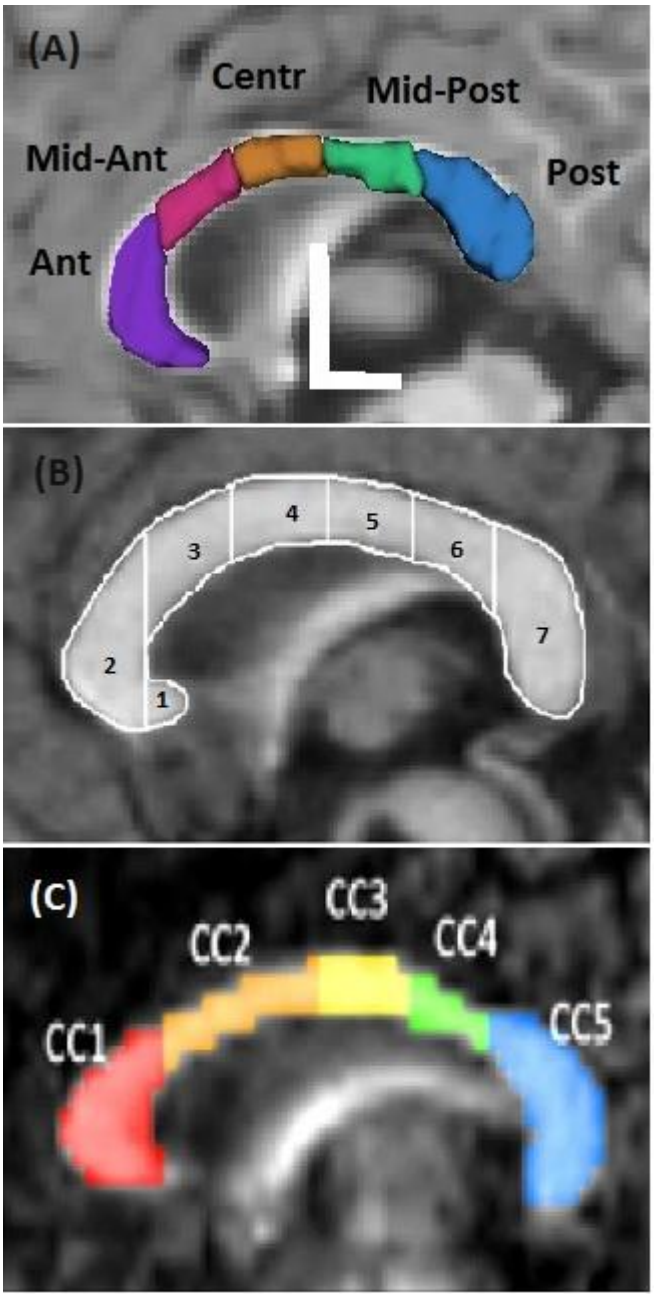

Figure 11: comparison between Freesurfer segmentation, A; Witelson subdivision (Witelson, 1989), B; and Hofer-Frahm scheme (Hofer-Frahm, 2006), C; *abbreviations*: Ant, anterior; Mid-Ant, mid-anterior; Centr, central; Mid-Post, mid-posterior; Post, posterior; 1, rostrum; 2, genu; 3, rostral body; 4, anterior mid-body; 5, posterior mid-body; 6, isthmus; 7, splenium; CC1, CC2, CC3, CC4, CC5, regions I, II, III, IV, V respectively).

Clinical correlations

ASD are frequently associated with cognitive impairment and a reduced CC size has been related, in subjects with autism, to lower IQ (Freitag et al., 2009; Verhoeven et al., 2009; Prigge et al., 2013) and poor performance in neuropsychological tests (Keary et al., 2009). Alexander (2007jan) described a specific subgroup of autistic patients with smaller CC volume, reduced WM integrity (lower FA values and higher MD values), poorer intellectual abilities and slower processing speed. Social and communication deficits have been reported in patient with other type of callosal disruption, as in subjects with AgCC (Lau et al., 2013; Paul et al., 2014), a congenital syndrome frequently associated to intellectual deficit, and among this patients those with a diagnosis of ASD show a lower IQ also (Booth et al., 2011). Callosal sizes were related to intelligence in neurotypical children also, and the observed negative correlations between callosal thickness and IQ in the pediatric population contrast with the positive correlations typically reported in adult samples, suggesting again that relationships between callosal morphology and clinical and demographic features are highly dynamic during brain maturation (Luders et al., 2011). Studies about CC size and intelligence relationship performed with children samples are few, and to our knowledge, the only one involving preschoolers with autism is that by Prigge (2013), who reported, in his wide age-range sample (3-36 years), an increased callosal area associated with an higher intelligence in the ASD group.
In our well selected and matched sample, no significant correlation was found between CC volume and non verbal IQ, either in ASD group or in control group. Although not significantly, callosal size of control subjects seems to have a negative correlation trend with non verbal intelligence, instead children with autism have a not significant correlation in the opposite direction, thus those with larger CC reached better performance in non verbal tasks and, conversely, children with smaller CC have lower IQ. In our ASD group the positive correlation trend is mainly due to females, instead males show a negative correlation trend, as controls do, suggesting again that sex-related differences in ASD regard both clinical and structural features. This findings are consistent to the few data of literature reported in this specific population, but due to the heterogeneity of sample selection and clinical assessment data, a real comparison can not be done. Regarding sample selection should be underlined again the importance of further studies on children population, with small age-range selection, in consideration of the dynamic nature of neuroanatomical features during development, as reported in neurotypical children also. Moreover given that our sample includes children in the early stage of preschool age (age-range is from 18 months of age), we choose to select only non verbal abilities assessment, to avoid bias potentially derived from the presence or the absence of language skills, instead other studies used also verbal type assessment tests, and this variability creates further inaccuracy in a findings comparison.

Language correlation to callosal size was separately analyzed and, as observed for non verbal IQ, no statistically significant relationship was found between expressive verbal skills and CC volume. However, although weak, an opposite correlation was found between groups: ASD subjects with higher language level have smaller CC, conversely than controls. In literature, some fMRI-based studies are performed to explore anatomical correlation to language skills, and a weaker interemispheric synchronization (ie., functional connectivity) in language

cortical areas was found in subjects with ASD (Just et al., 2004; Dinstein et al., 2011) as an evidence of under-connectivity. Studies based on DTI-analysis found no correlation between ADC of CC and language in ASD (Razek et al., 2014). Instead no study aim to assess the correlation between verbal abilities and callosal size. However CC represent the larger interemispheric structure in human brain and could reflect functional connectivity (Booth et al., 2011; Just et al., 2012). Recent unpublished data (Di Martino) found a negative correlation between functional interemispheric connectivity and expressive language level in preschoolers with ASD. These results are consistent with the weak negative correlation found in our study about language and CC volume. This finding could be explained by the hypothesis that a less inter-connected and more lateralized language function tends to be more efficient, as occurs in neurotypical population, whose verbal function are lateralized in the dominant left hemisphere.

It is interesting to note that the two different features of cognitive skills, such as non verbal IQ and language abilities, are inversely related with CC: in the ASD group in fact lower IQ is associated with smaller CC, instead a poorer verbal expression is associated with a larger CC. In the control group we observe the opposites correlations, both in non verbal and verbal skills. Maybe this apparent incongruity could be explained by the different nature of these cognitive functioning areas, subtended by different neuroanatomical network. These findings, although statistically insignificant, support again the hypothesis that the atypical cognitive profile, both verbal and non verbal, of children with ASD have some kind of abnormal correlation with underlying brain structures when compared to subjects without autism.

CC is larger, though not significantly, in preschoolers with ASD, but callosal volume inversely correlates with autism severity, measured by ADOS-G Total scores (the analysis of correlation performed using CBCL1½-5 reveals no significances, though DSM-oriented Pervasive Development Problems scores show a negative correlation trend with CC volume). Instead no correlation was found between ADOS-G scores and TBV. These findings together may mean that CC, that tends to be larger in ASD, is smaller in children with a severer disorder, indipendently by brain size. Maybe this apparent incongruity could be explained by the presence of a subgroup of patients with a severe form of autism, within the ASD, who have a smaller CC. This finding is consistent with other studies reporting a smaller CC in severer ASD (Hardan et al., 2009), in the preschooler population also (Boger-Megiddo et al., 2006; Prigge et al., 2013). These results suggest the hypothesis that different clinical phenotypes within the autism spectrum could be subtended by different neuroanatomical substrates, probably reflecting not in CC size only but in callosal functionality also. In this sense, children with autism with a larger CC could likely have a CC with a better functioning, although macroscopically it is farther from the mean size of children without ASD. Conversely, those autistic children with a smaller CC, although with normal callosal size, may have more microstructural anomalies or less compensatory mechanisms, resulting in a severer symptomatology. Higher FA values in the CC of ASD young children were found (Ben Bashat et al., 2007; Weinstein et al., 2011; Xiao et al., 2014), but no correlations with autism severity were searched; ADC is increased in CC of preschoolers with ASD and correlates with autism severity (Razek et al., 2014). Maybe further studies performed about functional integrity of the CC in relation to its volume and to detailed clinical and demographic features

of ASD preschoolers sample, could better outline phenotypic variability of autism in early age. Another possible explanation of the relation between a severer autism and a smaller CC could be that children presenting more autistic symptoms may have an earlier reduction of CC size, that in children with a mild phenotype begins afterwards.

The apparent incongruity of our results could be caused by the presence of a greater heterogeneity in clinical and anatomopathological anomalies of the ASD. In fact, a grater intrinsic variability in the structure of CC has been described in autism compared to neurotypical population (Alexander et al., 2007; Prigge et al., 2013), thus this heterogeneity may reflect in the variability of clinical manifestations and development trajectories of autistic spectrum.

It is interesting to note that among ADOS-G subscale, particularly ADOS-G Language and Communication scores correlate, in a negative and statistically significant manner, with CC volume, thus a smaller CC is associated with lower communication skills. Instead, the not significant correlation found in the language analysis, goes in the opposite direction: a smaller CC is associated with better language skills. This appearent incongruity could be caused by the different nature of these clinical measures: language represents the expressive verbal skills of subjects, ADOS-G scores reflect the social nature of communication abilities. Thus, given that correlation with CC volume is stronger and negative for the ADOS-G Language and Communication scores, it could be assumed that mostly the social communication is related to grater CC anomalies, such as a reduction of callosal volume.

In sum, CC is abnormal in preschoolers with ASD compared to controls, and this study complicates the research about CC in ASD: it is not simply a question about reduction or not of callosal size, but study of CC in autism implicates many clinical and demographic variables that should be considered to better explore how and when changes in CC may occur and how these changes reflect clinical phenotype. In this study CC of preschoolers with ASD reflects the atypical growth trajectory of the brain and tends to be larger in early ages. However, in ASD, a smaller CC is associated with a greater impairment, particularly with a lower IQ and mostly with a severer autism. Children with ASD may have micro-structural anomalies that maybe are somehow counterbalanced by a bigger CC (more or larger or more myelinated fiber?) or, vice versa, smaller CC may also have greater micro-structural anomalies resulting in a worse functioning CC. The complex variability, resulted by gene-environment inter-play, can be observed in the micro- and macro-structural anomalies of brains of ASD, related to gender and age also, and it is reflected in heterogeneity of clinical phenotypes of this wide spectrum of disorders.

Conclusions

Autism is an heterogeneous spectrum of neurodevelopmental disorders characterized by early-onset anomalies in social communication and unusually restricted, repetitive behavior and interests. It is subtended by atypical brain and neural development and genetics has a key role in the etiology of autism, in association with early environmental epigenetic factors. It is characterized by an atypical neural connectivity as widely reported in recent literature. CC represent the larger interemispheric structure in human brain and callosal size could reflect functional brain connectivity. CC size is highly heritable in human brains and significant heritability was found for several CC sub-regions (Woldehawariat et al., 2014). Genetic factors play an important role in CC size among individuals and distinct genetic factors seem to be involved in far callosal portions, such as caudal and rostral regions, consistent with the divergent functional specialization of these brain areas.

CC size reflects brain connectivity and brain volume. Both CC and brain volume show an atypical growth trajectory during development in ASD children compared to their neurotypical peers and these atypicalities might be responsible for some of the behavioral impairments in patients. The early brain and callosal overgrowth is followed, during childhood, by a decrease in this structures volumes, probably subtended by micro- and macro-structural neuropathological changes (e.g. reduction in number and/ or size of axons, impaired myelination, excessive synaptic pruning) that are genetically related.

CC shows greater variability in the ASD and maybe this heterogeneity could explain the heterogeneity of clinical phenotypes, such as autistic symptoms, language skills and intelligence, and it can be influenced by demographic features of individuals, such as age and sex.

This study is based on a well selected and well matched sample of preschoolers with ASD and it is the first study, to our knowledge, to analyze CC volume in this population and to correlate it with all these variables. Among main limitations of this study is to underline the poor possibility of comparison with other literature data, given that comparable studies are few, and some inconsistency emerged could be related to sample features, to methodological differences but also to the possible existence of subgroup of ASD subjects with different clinical and neuroanatomical phenotype. Future dedicated studies, performed on large sample with restricted range of clinical and demographic data, should aim to address these issues more specifically. Another limitation could be the use of a semi-automated software used to get callosal volume and sub-regions segmentation (see Appendix for further discussion).

Appendix

The subdivision of the CC in 5 sub-regions is performed with the semi-automated software for brain images analysis Freesurfer, that divides CC into 5 segments along its eigenaxis. In a perceptive analysis of callosal images segmented by the software, some errors have been noticed in the subdivisions drawings. These inaccuracies have been confirmed by volume analysis, and callosal volume of each subjects may have an intrinsic variability of about 5%.
Freesurfer is a widely used software for CC segmentation analysis, in the pediatric age and in subjects with autism also (Johnson et al., 2012; Salat et al., 2005; Vatta et al., 2011; Francis et al., 2011; Lefebvre et al., 2014), and it is, in our knowledge, the only one that automatically parcellates CC subregions. Segmentations performed by Freesurfer were judged to be of excellent quality and relatively accurate for brain parenchyma (i.e. GM + WM) volumetry, although it was noted that they tend to over-estimated WM volume when compared to manual segmentations (Klauschen et al., 2009). Some Authors described their confidence in this methodology, and unpublished data showing a high correlation (about r=0.95) between Freesurfer segmentation of the CC and manual measurements were reported (Johnson et al., 2012). However Authors concluded writing that "errors in registration or parcellation occurring in the automated processing of the scans" are possible.
Freesurfer is still being improved and these inaccuracy have been described to the Authors of the software. However possible errors and corresponding volume differences, if present, involve both ASD group and controls group, likewise. Thus, although aware of possible inaccuracy in our analysis, we can assume that this potential errors can not significantly affect our work.

References

Aboitiz F, Scheibel AB, Fisher RS, Zaidel E. Fiber composition of the human corpus callosum. Brain Res. 1992 Dec 11;598(1-2):143-53.

Achenbach, T.M., & Rescorla, L.A. Manual for the ASEBA Preschool forms and Profiles (2000).

Achiron R. and a. Achiron A. Development of the human fetal corpus callosum: a high-resolution, cross-sectional sonographic study- Ultrasound Obstet Gynecol 2001; 18: 343–347

Alexander AL, Lee JE, Lazar M, Boudos R, DuBray MB, Oakes TR, Miller JN, Lu J, Jeong EK, McMahon WM, Bigler ED, Lainhart JE. Diffusion tensor imaging of the corpus callosum in Autism. Neuroimage. 2007 Jan 1;34(1):61-73.

Alexander Andrew L., Jee Eun Lee, Mariana Lazar, and Aaron S. Field- Diffusion Tensor Imaging of the Brain- Neurotherapeutics. Jul 2007; 4(3): 316–329. doi: 10.1016/j.nurt.2007.05.011

Allen Laura S., Mark F. Richey, Yee M. Chai, and Roger A. Gorski- Sex Differences in the Corpus Callosum of the Living Human Being- The Journal of Neuroscience, April 1991, 1 f(4): 933-942

American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders - 5, 2013

American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders - IV- Text Revision, 2000

Anderson JS, Druzgal TJ, Froehlich A, DuBray MB, Lange N, Alexander AL, Abildskov T, Nielsen JA, Cariello AN, Cooperrider JR, Bigler ED, Lainhart JE. Decreased interhemispheric functional connectivity in autism. Cereb Cortex. 2011 May;21(5):1134-46.

Aoki Y, Abe O, Nippashi Y, Yamasue H.- Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies. MolAutism (2013) 4(1):25.

Arnone, D., Mcintosh,A.M., Chandra,P., and Ebmeier,K.P. (2008). Meta-analysis of magnetic resonance imaging studies of the corpus callosum in bipolar disorder. Acta Psychiatr.Scand. 118, 357–362.

Arnone, D., Mcintosh,A.M., Chandra,P., and Ebmeier,K.P. (2008). Meta-analysis of magnetic resonance imaging studies of the corpus callosum in schizophrenia. Schizo-phr.Res. 101, 124–132.

Aylward E.H., N.J. Minshew, K. Field, B.F. Sparks, N. Singh, Effects of age on brain volume and head circumference in autism, Neurology 59 (2002) 175–183.

Bailey A, Phillips W, Rutter M. Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. J Child Psychol Psychiatry . 1996 Jan;37(1):89-126.

Bailey A., P. Luthert, P. Bolton, A. Le Couteur, M. Rutter, B. Harding, Autism and megalencephaly, Lancet 341 (1993) 1225–1226.

Baird G, Cass H, Slonims V., Diagnosis of autism., BMJ. 2003 Aug 30;327(7413):488-93.

Baribeau Danielle A. and Evdokia Anagnostou - A comparison of neuroimaging findings in childhood onset schizophrenia and autism spectrum disorder: a review of the literature - Front Psychiatry. 2013 Dec 20;4:175. eCollection 2013.

Barkovich AJ. MR of the normal neonatal brain: assessment of deep structures. AJNR Am J Neuroradiol. 1998 Sep;19(8):1397-403.

Baron-Cohen S et al., Psychological markers in the detection of autism in infancy in a large population, Br J Psychiatry , 1996, 168: 158-163.

Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R. Why are autism spectrum conditions more prevalent in males? PLoS Biol 2011; 9: e1001081.

Barr M.L., Kiernan J.A., Anatomia del sistema nervoso umano, sesta edizione - McGraw-Hill, 1995

Bartak L., Rutter M., Differences between mentally retarded and normally intelligent autistic children. Journal of autism and child schizofrenia, 1976; 6, 109-120.

Beachera F.D., L. Minatia, S. Baron-Cohend, M.V. Lombardod, M.-C. Laid, M.A. Graya, N.A. Harrisona, and H.D. Critchley - Autism Attenuates Sex Differences in Brain Structure: A Combined Voxel-Based Morphometry and Diffusion Tensor Imaging Study- AJNR 2012 33: 83-89

Beaulé V, Tremblay S, Théoret H. Interhemispheric control of unilateral movement. Neural Plast. 2012;2012:627816. doi: 10.1155/2012/627816. Epub 2012 Dec 6.

Bellani M, Calderoni S, Muratori F, Brambilla P. Brain anatomy of autism spectrum disorders II. Focus on amygdala. EpidemiolPsychiatrSci (2013) 22(4):309–12. doi: 10.1017/ S2045796013000346

Bellani M., S. Calderoni, F. Muratori and P. Brambilla. Brain anatomy of autism spectrum disorders I. Focus on corpus callosum. Epidemiology and Psychiatric Sciences, 22, pp 217-221 doi:10.1017/S2045796013000139 (2013)

Ben Bashat D, Kronfeld-Duenias V, Zachor DA, Ekstein PM, Hendler T, Tar- rasch R,et al. Accelerated maturation of white matter in young children with autism: a high b value DWI study. Neuroimage (2007) 37(1):40–7. doi:10.1016/j.neuroimage.2007.04.060

Berlucchi Giovanni - Frontal callosal disconnection syndromes - Cortex. 2012 Jan;48(1):36-45. doi: 10.1016/j.cortex.2011.04.008. Epub 2011 May 1. Review.

Billstedt E, Gillberg IC, Gillberg C. Autism in adults: symptom patterns and early childhood predictors. Use of the DISCO in a community sample followed from childhood. J Child Psychol Psychiatry. 2007 Nov;48(11):1102-10.

Bishop KM, Wahlsten D. Sex differences in the human corpus callosum: myth or reality? -Neurosci Biobehav Rev. 1997 Sep;21(5):581-601.

Blenner S, Augustyn M. -Is the prevalence of autism increasing in the United States?- BMJ. 2014 May 9;348:g3088. doi: 10.1136/bmj.g3088.

Bloss CS, Courchesne E. MRI neuroanatomy in young girls with autism: a preliminary study. J Am Acad Child Adolesc Psychiatry. 2007 Apr;46(4):515-23.

Blumenthal JD, Baker EH, Lee NR, Wade B, Clasen LS, Lenroot RK, Giedd JN - A pediatric magnetic resonance imaging study- Neuroimage Clin (2013)

Boersma M1, Kemner C, de Reus MA, Collin G, Snijders TM, Hofman D, Buitelaar JK, Stam CJ, van den Heuvel MP. Disrupted functional brain networks in autistic toddlers. Brain Connect. 2013;3(1):41-9. doi: 10.1089/brain.2012.0127.

Bonilha L, Cendes F, Rorden C, Eckert M, Dalgalarrondo P, Li LM, Steiner CE (2008). Gray and white matter imbalance–typical structural abnormality underlying classic autism? Brain and Development 30, 396–401

Boger-Megiddo I, Shaw DW, Friedman SD, Sparks BF, Artru AA, Giedd JN, Dawson G, Dager SR. Corpus callosum morphometrics in young children with autism spectrum disorder. J Autism Dev Disord. 2006 Aug;36(6):733-9.

Booth R., G. L. Wallace, F. Happé - Connectivity and the corpus callosum in autism spectrum conditions: Insights from comparison of autism and callosal agenesis- Progress in Brain Research, Vol. 189 ISSN: 0079-6123- 2011

Brambilla Paolo, Antonio Hardan, Stefania Ucelli di Nemi, Jorge Perez , Jair C. Soares, Francesco Barale Brain anatomy and development in autism: review of structural MRI studies, Brain Research Bulletin 61 (2003) 557–569

Bryson S. E. , Brief report: epidemiology of autism, Journal of autism and developmental disorders, 1996, 26, pp. 165-168.

Bryson SA, Corrigan SK, McDonald TP, Holmes C. Characteristics of children with autism spectrum disorders who received services through community mental health centers. Autism. 2008 Jan;12(1):65-82.

Calderoni S, Retico A, Biagi L, Tancredi R, Muratori F, Tosetti M. Female children with autism spectrum disorder: an insight from mass-univariate and pattern classification analyses. Neuroimage. 2012 Jan 16;59(2):1013-22. doi: 10.1016/j.neuroimage.2011.08.070.

Camaioni L, Perucchini P, Muratori F, Parrini B, Cesari A., The communicative use of pointing in autism: developmental profile and factors related to change, EurPsychiatry, 2003 Feb; 18(1):6-12.

Cao, Q., Sun, L., Gong,G., Lv,Y., Cao, X., Shuai, L.,Zhu,C., Zang,Y., and Wang,Y. (2010). The macrostructural and microstructural abnormalities of corpus callosum in children with attention deficit/hyperactivity disorder: a combined morphometric and diffusion tensor MRI study. BrainRes. 1310, 172–180

Carpenter Laura - DSM5 Autism spectrum disorder, Guidelines & criteria exemplars, Feb 2013.

Cattaneo L, Rizzolatti G. The mirror neuron system. Arch Neurol. 2009 May;66(5):557-60. doi: 10.1001/archneurol.2009.41.

Chao YP, Cho KH, Yeh CH, Chou KH, Chen JH, Lin CP. Probabilistic topography of human corpus callosum using cytoarchitectural parcellation and high angular resolution diffusion imaging tractography. Human Brain Mapping. 2009;30(10):3172–3187

Charman T, Swettenham J, Baron-Cohen S, Cox A, Baird G, Drew A, Infants with autism: an investigation of empathy, pretend play, joint attention, and imitation. Dev Psychol,1997.33:781-9.

Chasen S, Birnholz J, Gurewitsch E, Skupski D, Chervenak F. Antenatal growth of the corpus callosum. Am J Obstet Gynecol 1997; 176: S66

Cheng Y, Chou KH, Chen IY, Fan YT, Decety J, Lin CP. Atypical development of white matter microstructure in adolescents with autism spectrum disorders. NeuroImage. 2010; 50(3):873– 882.10.1016/ j.neuroimage.2010.01.011

Cheng Y, Chou KH, Fan YT, Lin CP (2011). ANS: aberrant neurodevelopment of the social cognition network in adolescents with autism spectrum disorders. PLoS ONE 6, e18905

Chung MK, Dalton KM, Alexander AL, Davidson RJ.- Less white matter concentration in autism: 2D voxel-based morphometry. Neuroimage. 2004 Sep;23(1):242-51.

Courchesne E, Campbell K, Solso S. Brain growth across the life span in autism: age-specific changes in anatomical pathology. BrainRes (2011) 1380:138–45. oi:10.1016/j.brainres.2010.09.101

Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, et al. Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. Neurology 2001;57:245–254.

Courchesne E, Mouton PR, Calhoun ME, et al. Neuron number and size in prefrontal cortex of children with autism. JAMA 2011; 306: 2001–10.

Courchesne E, Redcay E, Kennedy DP. - The autistic brain: birth through adulthood. CurrOpinNeurol (2004) 17(4):489–96.doi:10.1097/01.wco. 0000137542.14610.b4

Courchesne E.- Brain development in autism: early overgrowth followed by premature arrest of growth.- Ment Retard Dev Disabil Res Rev. 2004;10(2):106-11.

Courchesne, E., Pierce, K., Schumann, C. M., Redcay, E., Buckwalter, J. A., Kennedy, D. P., et al. (2007). Mapping early brain development in autism. Neuron, 56, 399–413.

Crespi B.- Developmental heterochrony and the evolution of autistic perception, cognition and behavior. BMC Med. 2013 May 2;11:119. doi: 10.1186/1741-7015-11-119.

Cyprien, F., Courtet,P., Malafosse,A., Maller,J., Meslin,C., Bonafe,A., LeBars,E., DeChampfleur, N.M., Ritchie, K.,and Artero,S.(2011). Suicidal behavior is associated with reduced corpus callosum area. Biol. Psychiatry 70, 320–326.

Di Martino A, C-G Yan, Q Li, E Denio, FX Castellanos, K Alaerts, JS Anderson, M Assaf, SY Bookheimer, et al., The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism- Molecular Psychiatry (2014) 19, 659–667

Dickstein DP, Pescosolido MF, Reidy BL, et al. Developmental meta-analysis of the functional neural correlates of autism spectrum disorders. J AmAcadChildAdolescPsychiatry (2013) 52(3):279–89.e16.doi:10.1016/j.jaac.2012.12.012

Dinstein I, Pierce K, Eyler L, Solso S, Malach R, Behrmann M et al. Disrupted neural synchronization in toddlers with autism. Neuron 2011; 70: 1218–1225.

Dodero L, Damiano M, Galbusera A, Bifone A, Tsaftsaris SA, Scattoni ML, Gozzi A. Neuroimaging evidence of major morpho-anatomical and functional abnormalities in the BTBR T+TF/J mouse model of autism.- PLoS One. 2013 Oct 16;8(10):e76655.

Doherty D., Tu, S., Schilmoeller, K. & Schilmoeller, G. Health-related issues in individuals with agenesis of the corpus callosum. Child Care Health Dev. 32, 333–342 (2006).

Doyle-Thomas KA, Duerden EG, Taylor MJ, Lerch JP, et al. - Effects of age and symptomatology on cortical thickness in autism spectrum disorders. ResAutismSpectrDisord (2013) 7(1):141–50.doi:10.1016/j. rasd.2012.08.004

Duan Ye, Qing He, Xianfeng Gu, Kevin Karsch, and Judith Miles- Detecting corpus callosum abnormalities in autism subtype using planar conformal mapping - International Journal for Numerical Methods in Biomedical Engineering Volume 26, Issue 2, pages 164–175, 2010

Duerden EG, Mak-Fan KM, Taylor MJ, Roberts SW. Regional differences in grey and white matter in children and adults with autism spectrum disorders: an activation likelihood estimate(ALE) meta-analysis. AutismRes (2012) 5(1):49–66.doi:10.1002/aur.235

Eaves LC, Ho HH. Young adult outcome of autism spectrum disorders. J Autism Dev Disord. 2008 Apr;38(4):739-47. Epub 2007 Sep 1.

Ecker C, Rocha-Rego V, Johnston P,Mourao-Miranda J, Marquand A, Daly EM, BrammerMJ,Murphy C, Murphy DG; MRC AIMS Consortium (2010). Investigating the predictive value ofwhole-brain structuralMR scans in autism: a pattern classification approach. NeuroImage 49, 44–56

Ecker C, Ginestet C, Feng Y, Johnston P, Lombardo MV, Lai MC, et al. Brain surface anatomy in adults with autism: the relationship between surface area, cortical thickness, and autistic symptoms. JAMAPsychiatry (2013) 70(1):59–70. doi:10.1001/jamapsychiatry.2013.265

Ecker C, Shahidiani A, Feng Y, Daly E, Murphy C, D'Almeida V, Deoni S, Williams SC, Gillan N, et al., The effect of age, diagnosis, and their interaction on vertex-based measures of cortical thickness and surface area in autism spectrum disorder. J Neural Transm. 2014 Apr 22.

Edmonson C, Ziats MN1, Rennert OM.- Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. Mol Autism. 2014 Jan 10;5(1):3. doi: 10.1186/2040-2392-5-3.

Egaas B, Courchesne E, Saitoh O. - Reduced size of corpus callosum in autism. - Arch Neurol. 1995 Aug;52(8):794-801.

Esbensen AJ, Seltzer MM, Lam KS, Bodfish JW. Age-related differences in restricted repetitive behaviors in autism spectrum disorders. J Autism Dev Disord. 2009 Jan;39(1):57-66. doi: 10.1007/s10803-008-0599-x. Epub 2008 Jun 20.

Fabri Mara and Gabriele Polonara -Functional Topography of Human Corpus Callosum: An fMRI Mapping Study- Neural Plast. 2013; 2013: 251308. published online Feb 14, 2013. doi: 10.1155/2013/251308

Floris D., Chura LR, Holt RJ, Suckling J, Bullmore ET, Baron-Cohen S, Spencer MD. - Psychological correlates of handedness and corpus callosum asymmetry in autism: the left hemisphere dysfunction theory revisited. - J Autism Dev Disord. 2013 Aug;43(8):1758-72.

Fombonne E et al., Is there an epidemic of autism? Pediatrics, 2001, 107: 411-2.

Fombonne E, Quirke S, Hagen A. Epidemiology of pervasive developmental disorders. In: Amaral DG, Dawson G, Geschwind DH, eds. Autism spectrum disorders. New York, NY: Oxford University Press, 2011: 90–111.

Fombonne E., The prevalence of Autism. JAMA. 2003 Jan 1;289(1):87-9

Francis AN, Bhojraj TS, Prasad KM, Kulkarni S, Montrose DM, Eack SM, Keshavan MS. Abnormalities of the corpus callosum in non-psychotic high-risk offspring of schizophrenia patients. Psychiatry Res. 2011 Jan 30;191(1):9-15. doi: 10.1016/j.pscychresns.2010.09.007. Epub 2010 Dec 9.

Frazier TW, Hardan AY. -A meta-analysis of the corpus callosum in autism. Biol Psychiatry. 2009 Nov 15;66(10):935-41. doi: 10.1016/j.biopsych.2009.07.022. Epub 2009 Sep 12.

Frazier TW, Keshavan MS, Minshew NJ, Hardan AY. A two-year longitudinal MRI study of the corpus callosum in autism. J Autism Dev Disord. 2012 Nov;42(11):2312-22. doi: 10.1007/s10803-012-1478-z.

Frazier TW, Georgiades S, Bishop SL, Hardan AY- Behavioral and cognitive characteristics of females and males with autism in the Simons Simplex Collection.- J Am Acad Child Adolesc Psychiatry. 2014 Mar;53(3):329-40.e1-3.

Freitag CM, Luders E, Hulst HE, Narr KL, Thompson PM, Toga AW, Krick C, Konrad C. Total brain volume and corpus callosum size in medication-naïve adolescents and young adults with autism spectrum disorder. Biol Psychiatry. 2009 Aug 15;66(4):316-9.

Frith, C. (2004). Is autism a disconnection disorder? Lancet Neurology, 3(10), 577.

Fritz G. Lehnhardt, Kai Vogeley- Corpus callosum size in adults with high-functioning autism and the relevance of gender- Psychiatry Research: Neuroimaging 183 (2010) 38–43

Fuster Joaquin M., The Prefrontal Cortex, Fourth Edition. Boston: Academic Press. 2008 ISBN 0-12-373644-7

Gillberg C, Ehlers S, Schaumann H, Jakobsson G, Dahlgren SO, Lindblom R, Bagenholm A, Tjuus T, Blidner E. Autism under age 3 years: a clinical study of 28 cases referred for autistic symptoms in infancy. J Child Psychol Psychiatry. 1990; 31:921-934.

Gillberg, C., Cederlund, M., Lamberg, K., Zeijlon, L., 2006. Brief report: ''the autism epidemic''. The registered prevalence of autism in a Swedish urban area. J. Autism Dev. Disord. 36, 429–435.

Gilmore JH1, Lin W, Prastawa MW, Looney CB, Vetsa YS, Knickmeyer RC, Evans DD, Smith JK, Hamer RM, Lieberman JA, Gerig G. Regional gray matter growth, sexual dimorphism, and cerebral asymmetry in the neonatal brain. J Neurosci. 2007 Feb 7;27(6):1255-60.

Gozzi M., Nielson D., Lenroot R., Ostuni J., et al., A magnetization tranfer Imaging study of corpus callosum myelination in young children with autism – Biol psychiatry, 2012, 72: 215-220

Gregory RL, ed. The Oxford Companion to the Mind. Oxford: Oxford University Press, 1987: 104–10

Greimel E, Nehrkorn B, Schulte-Ruther M, et al. Changes in grey matter development in autism spectrum disorder. BrainStructFunct (2013) 218(4):929–42.doi:10.1007/ s00429-012-0439-9

Hadjikhani N, Joseph RM, Snyder J, Tager-Flusber H. Anatomical differences in the mirror neuron system and social cognition network in autism. Cereb Cortex (2006) 16(9):1276–82.doi:10.1093/cercor/bhj069

Han, X., Jovicich, J., Salat, D., van der Kouwe, A., Quinn, B., Czanner, S., Busa, E., Fischl, B., et al., Reliability of MRI-derived measurements of human cerebral cortical thickness: the effects of field strength, scanner upgrade and manufacturer. Neuroimage 2006. 32, 180-194.

Hanaie R, Mohri I, Kagitani-Shimono K, Tachibana M, Matsuzaki J, Watanabe Y, Fujita N, Taniike M. Abnormal Corpus Callosum Connectivity, Socio-communicative Deficits, and Motor Deficits in Children with Autism Spectrum Disorder: A Diffusion Tensor Imaging Study. J Autism Dev Disord. 2014 Mar 25.

Hardan AY, Libove RA, Keshavan MS, Melhem NM, Minshew NJ. A preliminary longitudinal magnetic resonance imaging study of brain volume and cortical thickness in autism. BiolPsychiatry (2009) 66(4):320–6.doi:10.1016/j. biopsych.2009.04.024

Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ. An MRI study of increased cortical thickness in autism. AmJPsychiatry (2006) 163(7):1290–2.doi:10.1176/appi.ajp.163.7.1290

Hardan AY, Pabalan M, Gupta N, Bansal R, Melhem NM, Fedorov S, Keshavan MS, Minshew NJ -Corpus callosum volume in children with autism.- Psychiatry Res. 2009 Oct 30;174(1):57-61. doi: 10.1016/j.pscychresns.2009.03.005. Epub 2009 Sep 24.

Harsan LA, Poulet P, Guignard B, Steibel J, Parizel N, de Sousa PL, Ghandour MS. - Brain dysmyelination and recovery assessment by noninvasive in vivo diffusion tensor magnetic resonance imaging. Journal of Neuroscience Research. 2006; 83(3):392–402.10.1002/jnr.20742

Hazlett HC, Poe M, Gerig G, Smith RG, Provenzale J, Ross A, etal. Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. ArchGenPsychiatry (2005) 62(12):1366–76. doi:10.1001/archpsyc.62.12.1366

Hazlett HC, Poe MD, Gerig G, Styner M, Chappell C, Smith RG, et al. Early brain overgrowth in autism associated with an increase in cortical surface area before age 2years. ArchGenPsychiatry (2011) 68(5):467–76. doi:10.1001/archgenpsychiatry.2011.39

Herbert MR, Harris GJ, Adrien KT, Ziegler DA, Makris N, Kennedy DN, Lange NT, Chabris CF, Bakardjiev A, Hodgson J, Takeoka M, Tager-Flusberg H, Caviness VS J- Abnormal asymmetry in language association cortex in autism. Ann Neurol. 2002 Nov;52(5):588-96.

Hiller RM, Young RL, Weber N. Sex Differences in Autism Spectrum Disorder based on DSM-5 Criteria: Evidence from Clinician and Teacher Reporting. J Abnorm Child Psychol. 2014 Jun 3.

Hofer S., Frahm J., Topography of the human corpus callosum revisited - Comprehensive fiber tractography using diffusion tensor magnetic resonance imaging- NeuroImage 32 (2006) 989–994

Hong S, Ke X, Tang T, Hang Y, Chu K, Huang H, Ruan Z, Lu Z, Tao G, Liu Y (2011). Detecting abnormalities of corpus callosum connectivity in autism using magnetic resonance imaging and diffusion tensor tractography. Psychiatry Research: Neuroimaging 194, 333–339

Howlin P, Goode S, Hutton J, Rutter M. Adult outcome for children with autism. J Child Psychol Psychiatry. 2004 Feb;45(2):212-29.

Hua X, Thompson PM, Leow AD,et al. Brain growth rate abnormalities visualized in adolescents with autism. HumBrain Mapp (2013) 34(2):425–36.doi:10.1002/hbm.21441

Iacoboni M, Mazziotta JC.- Mirror neuron system: basic findings and clinical applications. Ann Neurol. 2007 Sep;62(3):213-8.

Innocenti GM, Price DJ. Exuberance in the development of cortical networks. Nat Rev Neurosci. 2005 Dec;6(12):955-65. Review.

Jasmin E, Couture M, McKinley P, Reid G, Fombonne E, Gisel E. Sensori-motor and Daily Living Skills of Preschool Children with Autism Spectrum Disorders. J Autism Dev Disord. 2008 Jul 16.

Jiao Y, Chen R, Ke X, Chu K, et al., Predictive models of autism spectrum disorder based on brain regional cortical thickness. Neuroimage (2010) 50(2):589–99.doi:10.1016/ j.neuroimage. 2009.12.047

Johnson SL, Greenstein D, Clasen L, Miller R, Lalonde F, et al., Absence of anatomic corpus callosal abnormalities in childhood-onset schizophrenia patients and healthy siblings. Psychiatry Res. 2013 Jan 30;211(1):11-6. doi: 10.1016/j.pscychresns.2012.09.013. Epub 2012 Nov 13.

Jones W, Carr K, Klin A., Absence of preferential looking to the eyes of approaching adults predicts level of social disability in 2-year-old toddlers with autism spectrum disorder., Arch Gen Psychiatry. 2008 Aug;65(8):946-54.

Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and anatomical cortical underconnectivity in autism: Evidence from an fMRI study of an executive function task and corpus callosum morphometry. Cerebral Cortex. 2007; 17:951–961.

Just MA, Cherkassky VL, Keller TA, Minshew NJ. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 2004; 127(Part 8): 1811–1821.

Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. Sentence comprehension in autism: Thinking in pictures with decreased functional connectivity. Brain. 2006; 129:2484–2493.

Ke X, Hong S, Tang T, Zou B, Li H, Hang Y, Zhou Z, Ruan Z, Lu Z, Tao G, Liu Y (2008). Voxel-based morphometry study on brain structure in children with high-functioning autism. Neuroreport 19, 921–925

Keary CJ, Minshew NJ, Bansal R, Goradia D, Fedorov S, Keshavan MS, Hardan AY Corpus callosum volume and neurocognition in autism. J Autism Dev Disord. 2009 Jun;39(6):834-41. doi: 10.1007/s10803-009-0689-4. Epub 2009 Jan 23.

Keehn B, Wagner JB, Tager-Flusberg H, Nelson CA. Functional connectivity in the first year of life in infants at-risk for autism: a preliminary near-infrared spectroscopy study. FrontHumNeurosci (2013) 7:444.doi:10.3389/fnhum. 2013.00444

Keller TA, Kana RK, Just MA- A developmental study of the structural integrity of white matter in autism. NeuroReport (2007) 18 (1):23–27

Klauschen F, Goldman A, Barra V, Meyer-Lindenberg A, Lundervold A. - Evaluation of automated brain MR image segmentation and volumetry methods.- Hum Brain Mapp. 2009 Apr;30(4):1310-27. doi: 10.1002/hbm.20599.

Kočovská E, Billstedt E, Ellefsen A, Kampmann H, Gillberg IC, Biskupstø R, Andorsdóttir G, Stóra T, Minnis H, Gillberg C. -Autism in the Faroe Islands: diagnostic stability from childhood to early adult life.- ScientificWorldJournal. 2013;2013:592371. Epub 2013 Feb 17.

Koldewyn K, Yendiki A, Weigelt S, Gweon H, Julian J, Richardson H, et al., Differences in the right inferior longitudinal fasciculus but no general disruption of white matter tracts in children with autism spectrum disorder. Proc Natl Acad Sci U S A. 2014 Feb 4;111(5):1981-6.

Kopp S, Gillberg C.The Autism Spectrum Screening Questionnaire (ASSQ)-Revised Extended Version (ASSQ-REV): an instrument for better capturing the autism phenotype in girls? … Res Dev Disabil. 2011 Nov-Dec;32(6):2875-88. doi: 10.1016/j.ridd.2011.05.017. Epub 2011 Jun 12.

Korkmaz B, Njiokiktjien C & Verschoor CA (2013) Children's social relatedness: an embodied brain process. A clinical view of typical development and disorders. Amsterdam, Suyi Publications, 888 pp

Krupa K., Bekiesinska-Figatowska M. - Congenital and Acquired Abnormalities of the Corpus Callosum: A Pictorial Essay- BioMed Research International Volume 2013, Article ID 265619

Kumar A, Sundaram SK, Sivaswamy L, Behen ME, Makki MI, Ager J, Janisse J, Chugani HT, Chugani DC.- Alterations in frontal lobe tracts and corpus callosum in young children with autism spectrum disorder. Cereb Cortex. 2010 Sep;20(9):2103-13. doi: 10.1093/cercor/bhp278. Epub 2009 Dec 17.

Laat KF, Tuladhar AM, van Norden AG, Norris DG, Zwiers MP, de Leeuw FE. Loss of white matter integrity is associated with gait disorders in cerebral small vessel disease. Brain. 2011 Jan;134(Pt 1):73-83. doi: 10.1093/brain/awq343. Epub 2010 Dec 14.

Lai MC, Lombardo MV, Pasco G, Ruigrok AN, Wheelwright SJ, Sadek SA, Chakrabarti B; MRC AIMS Consortium, Baron-Cohen S. A behavioral comparison of male and female adults with high functioning autism spectrum conditions. PLoS One. 2011;6(6):e20835

Lai MC, Lombardo MV, Ruigrok AN, Chakrabarti B, Wheelwright SJ, Auyeung B, Allison C; MRC AIMS Consortium, Baron-Cohen S. Cognition in males and females with autism: similarities and differences. PLoS One. 2012; 7(10):e47198. doi:10.1371/journal.pone.0047198.

Lai Meng-Chuan, Michael V Lombardo, Simon Baron-Cohen- Autism - Lancet 2014; 383: 896–910

Lai Meng-Chuan, Michael V. Lombardo, John Suckling, Amber N. V. Ruigrok, Simon Baron-Cohen et al., Biological sex affects the neurobiology of autism- Brain 2013: 136; 2799–2815

Lainhart JE, Bigler ED, Bocian M, et al., Head circumference and height in autism: a study by the Collaborative Program of Excellence in Autism., Am J Med Genet A. 2006 Nov 1;140(21):2257-74.

Lanza G., Anatomia Umana. Edi-Ermes, 1993

Lau Y. C. , L. B. N. Hinkley, P. Bukshpun , Z. A. Strominger, M. L. J. Wakahiro, S. Baron-Cohen , C. Allison , B. Auyeung , et al, - Autism Traits in Individuals with Agenesis of the Corpus Callosum - J Autism Dev Disord; 2013 DOI 10.1007/s10803-012-1653-2

Lefebvre Aline, Anita Beggiato, Thomas Bourgeron, Roberto Toro- Neuroanatomical diversity of corpus callosum and brain volume in the Autism Brain Imaging Data Exchange (Abide) project- doi: http://dx.doi.org/10.1101/002691- Posted February 15, 2014.

Lenroot Rhoshel K. and Pui KaYeung - Heterogeneity within autism spectrum disorders: what have we learned from neuroimaging studies? - Frontiers in Neuroscience, 30 October 2013 doi: 10.3389/fnhum.2013.00733

Lewis, J. D., & Elman, J. L. (2008). Growth-related neural reorganization and the autism phenotype: A test of the hypothesis that altered brain growth leads to altered connectivity. Developmental Science, 11, 135–155

Lewis, John D., Rebecca J. Theilmann, Vladimir Fonov, Pierre Bellec, Alan Lincoln, Alan C. Evans, and Jeanne Townsend- Callosal Fiber Length and Interhemispheric Connectivity in Adults with Autism: Brain Overgrowth and Underconnectivity - Human Brain Mapping 00:000–000 (2012)

Li W, Mai X, Liu C. -The default mode network and social understanding of others: what do brain connectivity studies tell us. -Front Hum Neurosci. 2014 Feb 24;8:74. eCollection 2014. Review.

Lord C, Risi S, DiLavore PS, Shulman C, Thurm A, Pickles A. Autism from 2 to 9 years of age. Arch Gen Psychiatry. 2006 Jun; 63(6):694-701.

Lord C, Schopler E , The role of age at assessment, developmental level, and test in the stability of intelligence scores in young autistic children. J Aut Dev Disord, 1989, 19: 483-499.

Lord C. Follow-up of two-year-olds referred for possible autism. J Child Psychol Psychiatry. 1995;36:1365-1382.

Lord Catherine, Susan Risi, Linda Lambrecht, et al., The Autism Diagnostic Observation Schedule–Generic: A Standard Measure of Social and Communication Deficits Associated with the Spectrum of Autism- Journal of Autism and Developmental Disorders, Vol. 30, No. 3, 2000

Loser JD, Alvord EC. Agenesis of the corpus callosum. Brain 1968; 91: 553–70

Luders E, Toga AW. Sex differences in brain anatomy.- Prog Brain Res. 2010;186:3-12. doi: 10.1016/B978-0-444-53630-3.00001-4.

Luders Eileen, Nicolas Cherbuin, Paul M. Thompson, Boris Gutman, Kaarin J. Anstey, Perminder Sachdev, and Arthur W. Toga - When more is less: associations between corpus callosum size and handedness lateralization- Neuroimage. 2010 Aug 1;52(1):43-9.

Luders Eileen, Paul M. Thompson, Katherine L. Narr, Alen Zamanyan, Yi-Yu Chou, Boris Gutman, Ivo D. Dinov, and Arthur W. Toga- The link between callosal thickness and intelligence in healthy children and adolescents- Neuroimage. 2011 February 1; 54(3): 1823–1830.

Luders E., ArthurW. Toga, Paul M. Thompson -Why size matters: Differences in brain volume account for apparent sex differences in callosal anatomy The sexual dimorphism of the corpus callosum - NeuroImage 84 (2014) 820–824

Mak-Fan KM, Taylor MJ, Roberts W, Lerch JP. Measures of cortical grey matter structure and development in children with autism spectrum disorder. J AutismDevDisord (2012) 42(3):419–27.doi:10.1007/s10803-011-1261-6

Mandy W, Chilvers R, Chowdhury U, Salter G, Seigal A, Skuse D.Sex differences in autism spectrum disorder: evidence from a large sample of children and adolescents. J Autism Dev Disord. 2012 Jul;42(7):1304-13. doi: 10.1007/s10803-011-1356-0.

Marans W. D. , Valutazione comunicativa. In: Cohen D. J, Volkmar F. R. (1997). Autismo e disturbi generalizzati dello sviluppo. Vol. I - Diagnosi e assessment. Tr. It. Vannini Editrice Brescia

McAlonan GM, Cheung C, Cheung V, Wong N, Suckling J, Chua SE.- Differential effects on white-matter systems in high-functioning autism and Asperger's syndrome. Psychol Med. 2009 Nov;39(11):1885-93. doi: 10.1017/S0033291709005728. Epub 2009 Apr 9.

McAlonan GM, Cheung V, Cheung C, Suckling J, Lam GY, Tai KS, et al. Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. Brain (2005) 128(Pt2):268–76. doi:10.1093/brain/awh332

McGovern CW, Sigman M. Continuity and change from early childhood to adolescence in autism. J Child Psychol Psychiatry. 2005;46:401-408.

Melillo R, Leisman G.- Autistic spectrum disorders as functional disconnection syndrome. -Rev Neurosci. 2009;20(2):111-31.

Mengotti P., S. D'Agostini, R. Terlevic, C. De Colle, P. Brambilla et al., Altered white matter integrity and development in children with autism: A combined voxel-based morphometry and diffusion imaging study- Brain Research Bulletin 84 (2011) 189–195

Menzler K, Belke M, Wehrmann E, Krakow K, Lengler U, et al., Men and women are different: diffusion tensor imaging reveals sexual dimorphism in the microstructure of the thalamus, corpus callosum and cingulum. Neuroimage. 2011 Feb 14;54(4):2557-62.

Miles, J.H., Hillman, R.E., 2000. Value of a clinical morphology examination in autism. Am. J. Med. Genet. 91, 245–253

Ming X, Brimacombe M, Wagner GC. Prevalence of motor impairment in autism spectrum disorders. Brain Dev. 2007 Oct;29(9):565-70. Epub 2007 Apr 30.

Moes P, Schilmoeller K, Schilmoeller G. Physical, motor, sensory and developmental features associated with agenesis of the corpus callosum. Child Care Health Dev. 2009 Sep;35(5):656-72. doi: 10.1111/j.1365-2214.2009.00942.x. Epub 2009 Mar 23.

Moore V, Goodson S. How well does early diagnosis of autism stand the test of time? follow-up study of children assessed for autism at age 2 and development of an early diagnostic service. Autism. 2003;7:47-63.

Morriss MC, Zimmerman RA, Bilaniuk LT. Changes in brain water diffusion during childhood. Neuroradiology. 1999;41(12):929–34.

Mukherjee P, Miller JH, Shimony JS, Conturo TE, Lee BC, Almli CR, McKinstry RC. Normal brain maturation during childhood: developmental trends characterized with diffusion-tensor MR imaging. Radiology. 2001 Nov;221(2):349-58.

Mundy, P., C. Annotation : The neural basis of social impairments in autism : the role of the dorsal medial-frontal cortex and anterior cingulate system, Journal of Child Psychology and Psychiatry, 2003, 44 (6), 793-809.

Muñoz-Yunta JA, Palau-Baduell M, Salvadó-Salvadó B, Valls-Santasusana A, Rosendo-Moreno N, Clofent-Torrentó M, Manchado F., Autism, epilepsy and genetics Rev Neurol. 2008;46 Suppl 1:S71-7.

Muratori F, Narzisi A, Tancredi R, Cosenza A, Calugi S, Saviozzi I, Santocchi E, Calderoni S.- The CBCL 1.5-5 and the identification of preschoolers with autism in Italy. -Epidemiol Psychiatr Sci. 2011 Dec;20(4):329-38.

Nickl-Jockschat T, Habel U, Michel TM, Manning J, Laird AR, Fox PT, et al. Brain structure anomalies in autism spectrum disorder : a meta-analysis of VBM studies using anatomic likelihood estimation. HumBrainMapp (2012) 33(6):1470–89.doi:10.1002/hbm.21299

O'Connor, N and Hermelin, B. Low intelligence and special abilities Journal of Child Psychology & Psychiatry & Allied Disciplines 29(4):391-396, 1988

Osborn A. G., Diagnostic Imaging - Brain. Amirsys, 2007

Ozalay O, Calli C, Kitis O, Cagdas Eker M, Donat Eker O, Ozan E, Coburn K, Saffet Gonul A. The relationship between the anterior corpus callosum size and prefrontal cortex volume in drug-free depressed patients. J Affect Disord. 2013 Apr 5;146(2):281-5. doi: 10.1016/j.jad.2012.06.040.

Ozonoff S, Goodlin-Jones BL, Solomon M. Evidence-based assessment of autism spectrum disorders in children and adolescents. J Clin Child Adolesc Psychol 2005; 34: 523–40.

Ozonoff S, Iosif AM, Baguio F, et al. A prospective study of the emergence of early behavioral signs of autism. J Am Acad Child Adolesc Psychiatry 2010; 49: 256–66

Park J.S., Yoon U., Kwak, K.C., Seo,S. W., Kim,S.I., Na,D.L., and Lee,J.M. (2011). The relationships between extent and microstructural properties of the midsagittal corpus callosum in human brain. Neuroimage 56, 174–184

Paul L, Brown WS, Adolphs R, Tyszka JM, Richards LJ, Mukherjee P, Sherr EH.- Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity.- Neuroscience 2007; 8: 287-299

Paul Lynn K.- Developmental malformation of the corpus callosum: a review of typical callosal development and examples of developmental disorders with callosal involvement- J Neurodevelop Disord (2011) 3:3–27 DOI 10.1007/s11689-010-9059-y

Paul Lynn K., Christina Corsello, Daniel P. Kennedy and Ralph Adolphs- Agenesis of the corpus callosum and autism: a comprehensive comparison- Brain 2014: 137; 1813–1829 doi:10.1093/brain/awu070

Peeva MG, Tourville JA, Agam Y, Holland B, Manoach DS, Guenther FH. White matter impairment in the speech network of individuals with autism spectrum disorder. Neuroimage Clin. 2013 Aug 28;3:234-41. doi: 10.1016/j.nicl.2013.08.011. eCollection 2013.

Peltier J.,M. Roussel, Y. Gerard et al., "Functional consequences of a section of the anterior part of the body of the corpus callosum: evidence from an interhemispheric transcallosal approach," Journal of Neurology, vol. 259, no. 9, pp. 1860–1867, 2012.

Peters JM1, Taquet M, Vega C, Jeste SS, Fernández IS, Tan J, Nelson CA 3rd, Sahin M, Warfield SK. Brain functional networks in syndromic and non-syndromic autism: a graph theoretical study of EEG connectivity. BMC Med. 2013 Feb 27;11:54. doi: 10.1186/1741-7015-11-54.

Pilu G, Sandri F, Perolo A, Pittalis G, Grisolia G, Cocchi G, Foschini MP, Salvioli GP, Bovicelli L. Sonography of fetal agenesis of corpus callosum: a survey of 35 cases. Ultrasound Obstet Gynecol 1993; 3: 318–29

Prigge Molly B. D., Nicholas Langed, Erin D. Biglerc, Tricia L. Merkley, E. Shannon Neeley, Tracy J. Abildskov, Alyson L. Froehlich, Janet E. Lainhart et al.- Corpus Callosum Area in Children and Adults with Autism- Res Autism Spectr Disord. 2013 ; 7(2): 221–234.

Prior M., Is there an increase in the prevalence of autism spectrum disorders? J Paediatr Child Health, 2003. 39: 81-82.

Puzzo I, Cooper NR, Vetter P, Russo R. EEG activation differences in the pre-motor cortex and supplementary motor area between normal individuals with high and low traits of autism. Brain Res. 2010 Jun 25;1342:104-10. doi: 10.1016/j.brainres.2010.04.060. Epub 2010 May 18.

Purves D, Augustine GJ, Fitzpatrick D, et al., editors.- Neuroscience. 2nd edition.- Sunderland (MA): Sinauer Associates; 2001.

Rademaker KJ, Lam JN, Van Haastert IC, Uiterwaal CS, Lieftink AF, Groenendaal F, Grobbee DE, de Vries LS. Larger corpus callosum size with better motor performance in prematurely born children. Semin Perinatol. 2004 Aug;28(4):279-87.

Radua J, Via E, Catani M, Mataix-Cols D.- Voxel-based meta-analysis of regional white-matter volume differences in autism spectrum disorder versus healthy controls. PsycholMed (2011) 41(7):1539–50.doi:10.1017/S0033291710002187

Raine, A., Lencz,T., Taylor,K., Hellige, J.B., Bihrle,S., Lacasse,L., Lee,M., Ishikawa,S., and Colletti, P.(2003).Corpus callosum abnormalities in psychopathic antisocial individuals. Arch.Gen.Psychiatry 60, 1134–1142

Raybaud C., The corpus callosum, the other great forebrain commissures, and the septum pellucidum: anatomy, development, and malformation.- Neuroradiology 2010; 52:447–477

Razek Ahmed Abdel, Jehan Mazroa, Hemmat Baz - Assessment of white matter integrity of autistic preschool children with diffusion weighted MR imaging - Brain & Development 36 (2014) 28–34

Raznahan A, Wallace GL, Antezana L, Greenstein D, Lenroot R, Thurm A, et al. -Compared to what? Early brain overgrowth in autism and the perils of population norms. Biol Psychiatry (2013) 74(8):563–75.doi:10.1016/j.biopsych.2013. 03.022

Redcay E, Courchesne E. Deviant functional magnetic resonance imaging patterns of brain activity to speech in 2-3-year-old children with autism spectrum disorder. BiolPsychiatry (2008) 64(7):589–98.doi:10.1016/j.biopsych. 2008.05.020

Reuter, M., Schmansky, N.J., Rosas, H.D., Fischl, B. 2012. Within-Subject Template Estimation for Unbiased Longitudinal Image Analysis. Neuroimage. 2012 Jul 16;61(4):1402-18.

Riva D, Bulgheroni S, Aquino D, Di Salle F, Savoiardo M, Erbetta A. Basal forebrain involvement in low-functioning autistic children: a voxel-based morphometry study. AJNR Am J Neuroradiol. 2011 Sep;32(8):1430-5. doi: 10.3174/ajnr.A2527.

Rogers SJ, Hepburn SL, Stackhouse T, Wehner E., Imitation performance in toddlers with autism and those with other developmental disorders. J Child Psychol Psychiatry., 2003. 44: 763-81

Rosas HD, Lee SY, Bender AC, Zaleta AK, Vangel M, Yu P, Fischl B, et al., Altered white matter microstructure in the corpus callosum in Huntington's disease: implications for cortical "disconnection". Neuroimage. 2010 Feb 15;49(4): 2995-3004.

Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2003 Oct;2(5):255-67.

Ruigrok Amber N.V., Gholamreza Salimi-Khorshidi, Meng-Chuan Lai,Simon Baron-Cohen, Michael V. Lombardo, Roger J. Tait, John Suckling- A meta-analysis of sex differences in human brain structure-Neuroscience and Biobehavioral Reviews 39 (2014) 34–50

Ruth C.M. Philip , Maria R. Dauvermann, Heather C. Whalley, Katie Baynham, Stephen M. Lawrie, Andrew C. Stanfield -A systematic review and meta-analysis of the fMRI investigation of autism spectrum disorders- Neuroscience and Biobehavioral Reviews 36 (2012) 901–942

Rutter M, Bailey A, Bolton P, Le Couteur A., Autism and known medical conditions: myth and substance. J Child Psychol Psychiatry. 1994 Feb;35(2):311-22. Review.

Salat DH, Tuch DS, Greve DN, van der Kouwe AJW, Hevelone ND, Zaleta AK, Rosen BR, Fischl B, Corkin S, Diana Rosas H, Dale AM. Age-related alterations in white matter microstructure measured by diffusion tensor imaging. Neurobiology of Aging. 2005;26:1215–1227

Sarnat HB. Development disorders of the nervous system. In: Bradley WG, Daroff RB, Fenichel GM, Marsden CD, eds. Neurology in Clinical Practice. Boston: Butterworth-Heinemann, 1991, Vol. II: 1258–9

Saugstad LF. What is a psychosis and where is it located? Eur Arch Psychiatry Clin Neurosci. 2008 Jun;258 Suppl 2:111-7. doi: 10.1007/s00406-008-2014-1.

Scamvougeras, A., Kigar,D.L., Jones, D., Weinberger,D.R., and Witelson, S.F.(2003). Size of the human corpus callosum is genetically determined: an MRI study in mono and dizygotic twins. Neurosci.Lett. 338, 91–94.

Scheel C, Rotarska-Jagiela A, Schilbach L, et al. Imaging derived cortical thickness reduction in high-functioning autism: key regions and temporal slope. Neuroimage (2011) 58(2):391–400. doi:10.1016/j.neuroimage.2011.06.040

Schulte Tilman and Eva M. Müller-Oehring - Contribution of Callosal Connections to the Interhemispheric Integration of Visuomotor and Cognitive Processes- Neuropsychol Rev. 2010 June ; 20(2): 174–190. doi:10.1007/s11065-010-9130-1.

Schumann C.M., C.S. Bloss, C. Carter Barnes, G.M. Wideman, R.A. Carper, N. Akshoomoff, K. Pierce, D. Hagler, N. Schork, C. Lord, and E. Courchesne - Longitudinal MRI Study of Cortical Development through Early Childhood in Autism- Neurosci. 2010 March 24; 30(12): 4419–4427.

Sears L.L., C. Vest, S. Mohamed, J. Bailey, B.J. Ranson, J. Piven, An MRI study of the basal ganglia in autism, Prog. Neuropsychopharmacol. Biol. Psychiatry 23 (1999) 613–624.

Seltzer MM, Krauss MW, Shattuck PT, Orsmond G, Swe A, Lord C. The symptoms of autism spectrum disorders in adolescence and adulthood- J Autism Dev Disord. 2003 Dec;33(6):565-81.

Shen MD, Nordahl CW, Young GS, Wootton-Gorges SL, Lee A, Liston SE, Harrington KR, Ozonoff S, Amaral DG.- Early brain enlargement and elevated extra-axial fluid in infants who develop autism spectrum disorder.-Brain. 2013 Sep;136(Pt 9):2825-35.

Shimamura, A. P.- The role of the prefrontal cortex in dynamic filtering. Psychobiology 2000 - 28: 207–218. ISSN 0889-6313

Shin YW, Kim DJ, Ha TH, Park HJ, Moon WJ, Chung EC, Lee JM, Kim IY, Kim SI, Kwon JS.- Sex differences in the human corpus callosum: diffusion tensor imaging study. Neuroreport. 2005 May 31;16(8):795-8.

Silver J, Lorenz SE, Washten D, Coughlim J. Axonal guidance during development of the great cerebral commissures: description and experimental studies in vivo on the role of preformed glial pathways. J Comp Neurol 1982; 210: 10–29

Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G., Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample., J Am Acad Child Adolesc Psychiatry. 2008 Aug;47(8):921-9.

Società Italiana di NeuroPsichiatria dell'Infanzia e Adolescenza, Linee guida per l'autismo. 2005.

Solomon M, Miller M, Taylor SL, Hinshaw SP, Carter CS. Autism symptoms and internalizing psychopathology in girls and boys with autism spectrum disorders. J Autism Dev Disord. 2012 Jan;42(1):48-59. doi: 10.1007/s10803-011-1215-z.

Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, Maravilla KR, Giedd JN, Munson J, Dawson G, Dager SR. Brain structural abnormalities in young children with autism spectrum disorder. Neurology. 2002 Jul 23;59(2):184-92.

Stanfield AC, McIntosh AM, et al., Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. - Eur Psychiatry 23:289-299 (2007)

Stone WL, Lee EB, Ashford L, Brissie J, Hepburn SL, Coonrod EE, Weiss BH. Can autism be diagnosed accurately in children under 3 years? J Child Psychol Psychiatry. 1999;40:219-226.

Stoner R, Chow ML, Boyle MP, Sunkin SM, Mouton PR, Roy S, Wynshaw-Boris A, Colamarino SA, Lein ES, Courchesne E.- Patches of disorganization in the neocortex of children with autism.- N Engl J Med. 2014 Mar 27;370(13):1209-19. doi: 10.1056/NEJMoa1307491.

Sukhodolsky DG, Scahill L, Gadow KD, et al., Parent-rated anxiety symptoms in children with pervasive developmental disorders: frequency and association with core autism symptoms and cognitive functioning. J Abnorm Child Psychol. 2008 Jan;36(1):117-28. Epub 2007 Aug 3.

Sullivan EV, Rosenbloom MJ, Desmond JE, Pfefferbaum A. Sex differences in corpus callosum size: relationship to age and intracranial size.- Neurobiol Aging. 2001 Jul-Aug;22(4):603-11.

Supekar K., L. Q. Uddin, A. Khouzam, J.Phillips, W. D. Gaillard, L.E. Kenworthy, B. E. Yerys, C. J. Vaidya, V. Menon - Brain hyper-connectivity in children with autism and its links to social deficits - Cell Rep. 2013 November 14; 5(3): 738–747. doi:10.1016/j.celrep.2013.10.001

Tager-Flusberg H, Rogers S, Cooper J, Landa R, Lord C, Paul R, et al., Defining spoken language benchmarks and selecting measures of expressive language development for young children with autism spectrum disorders. J Speech Lang Hear Res. 2009 Jun;52(3):643-52.

Takao H., Naoto Hayashi, and Kuni Ohtomo, Sex Dimorphism in the White Matter: Fractional Anisotropy and Brain Size- Journal of magnetic resonance imaging 00:00–00 (2013)

Tepest, R., Jacobi, E., Gawronski, A., Krug, B., Moller-Hartmann, W., Lehnhardt, F.G.,Vogeley, K., 2010. Corpus callosum size in adults with high-functioning autism and the relevance of gender. Psychiatry Res. 183, 38–43.

Testa-Silva G1, Loebel A, Giugliano M, de Kock CP, Mansvelder HD, Meredith RM.- Hyperconnectivity and slow synapses during early development of medial prefrontal cortex in a mouse model for mental retardation and autism.- Cereb Cortex. 2012 Jun;22(6):1333-42.

Thomas C, Humphreys K, Jung KJ, Minshew N, Behrmann M. The anatomy of the callosal and visual-association pathways in high-functioning autism: a DTI tractography study. Cortex. 2011 Jul-Aug;47(7):863-73. doi: 10.1016/j.cortex.2010.07.006. Epub 2010 Aug 3.

Toal F, Daly EM, Page L, Deeley Q, Hallahan B, Bloemen O, Cutter WJ, BrammerMJ, Curran S, Robertson D,Murphy C, Murphy KC, Murphy DG (2010). Clinical and anatomical heterogeneity in autistic spectrum disorder: a structural MRI study. Psychological Medicine 40, 1171–1181

Tomasch J.- Size, distribution, and number of fibres in the human corpus callosum. The Anat. Rec., 119–135 (1954).

Travers BG, N Adluru, C Ennis, DPM Tromp, D Destiche, S Doran, ED Bigler, N Lange, JE Lainhart, and AL Alexander Diffusion Tensor Imaging in Autism Spectrum Disorder: A Review. Autism Res. 2012 October ; 5(5): 289–313. doi:10.1002/aur.1243.

Uddin LQ, Supekar K, Menon V. Reconceptualizing functional brain connectivity in autism from a developmental perspective. FrontHumNeurosci (2013) 7:458.doi:10.3389/fnhum.2013.00458

Vatta F, Di Salle F. Brain morphometry in autism spectrum disorders: a unified approach for structure-specific statistical analysis of neuroimaging data - biomed 2011. Biomed Sci Instrum. 2011;47:135-41.

Verhoeven JS1, De Cock P, Lagae L, Sunaert S. Neuroimaging of autism. Neuroradiology. 2010 Jan;52(1):3-14. doi: 10.1007/s00234-009-0583-y. Epub 2009 Dec 24.

Vidal CN, Nicolson R, DeVito TJ, Hayashi KM, Geaga JA, Drost DJ, Williamson PC, Rajakumar N, Sui Y, Dutton RA, Toga AW, Thompson PM. Mapping corpus callosum deficits in autism: an index of aberrant cortical connectivity. Biol Psychiatry. 2006 Aug 1;60(3):218-25.

Vissers ME, Cohen MX, Geurts HM: Brain connectivity and high functioning autism: a promising path of research that needs refined models, methodological convergence, and stronger behavioral links. Neurosci Biobehav Rev 2012, 36:604–625.

Volkmar F., Cohen D.J., Autismo e i disturbi generalizzati dello sviluppo. Vol.1: diagnosi e assessment. 1997

Volkmar FR, Lord C, Bailey A, Schultz RT, Klin A. Autism and pervasive developmental disorders. J Child Psychol Psychiatry. 2004 Jan;45(1):135-70.

Wade BS, Stockman M, McLaughlin MJ, Raznahan A, Lalonde F, Giedd JN. - Improved corpus callosum area measurements by analysis of adjoining parasagittal slices. Psychiatry Res. 2013 Mar 30;211(3):221-5. doi: 10.1016/j.pscychresns.2012.05.004. Epub 2012 Nov 11.

Waiter GD, Williams JH, Murray AD, Gilchrist A, Perrett DI, Whiten A (2004). A voxel-based investigation of brain structure in male adolescents with autistic spectrum disorder. NeuroImage 22, 619–625

Waiter GD, Williams JH, Murray AD, Gilchrist A, Perrett DI, Whiten A. Structural white matter deficits in high-functioning individuals with autistic spectrum disorder: a voxel-based investigation. Neuroimage. 2005 Jan 15;24(2):455-61.

Wallace, G. L., Dankner, N., Kenworthy, L., Giedd, J. N., & Martin, A. (2010). Age-related temporal and parietal cortical thinning in autism spectrum disorders. Brain, 133, 3745–3754

Wazana A, The autism epidemic: fact or artifact?,J Am Acad Child Adolesc Psychiatry, 46(6):721-30, 2007

Weinstein M, Ben-Sira L, Levy Y, Zachor DA, Ben Itzhak E, Artzi M, Ben Bashat D. Abnormal white matter integrity in young children with autism. Human Brain Mapping. 2011; 32(4):534– 543.10.1002/hbm.21042; 10.1002/hbm.21042

Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. Curr Opin Neurol. 2013 Apr;26(2):146-53. doi: 10.1097/WCO.0b013e32835ee548.

Westerhausen R1, Kompus K, Dramsdahl M, Falkenberg LE, Grüner R, Hjelmervik H, Specht K, Plessen K, Hugdahl K. - A critical re-examination of sexual dimorphism in the corpus callosum microstructure. - Neuroimage. 2011 Jun 1;56(3):874-80.

Wing L, Gould J. Severe impairments of social interaction and associated abnormalities in children: epidemiology and classification. J Aut Dev Disord 1979;9:11-29.

Wing L, Potter D., The epidemiology of autistic spectrum disorders: is the prevalence rising? Ment Retard Dev Disabil Res Rev. 2002;8(3):151-61.

Wing L., Aspects of Autism: Biological Research , London: Gaskell, 1988

Wing L., Language, social and cognitive impairments in autism and severe mental retardation. Journal of autism and developmental disorders, 1981; 11, 31-44.

Wing L., Manifestations of Social Problems in High Functioning Autistic People, in Schopler, E. & Mesibov, G. (eds.), High Functioning Individuals with Autism, 1992, New York, Plenum Press

Witelson SF. Hand and sex differences in the isthmus and genu of the human corpus callosum. A postmortem morphological study. Brain. 1989 Jun;112 (Pt 3):799-835.

Woldehawariat Girma, Pedro E. Martinez, Peter Hauser, David M. Hoover, Wayne W. C. Drevets, and Francis J. McMahon- Corpus Callosum Size Is Highly Heritable in Humans, and May Reflect Distinct Genetic Influences on Ventral and Rostral Regions PLoS One. 2014; 9(6): e99980.

Woolfenden S, Sarkozy V, Ridley G, Coory M, Williams K. - A systematic review of two outcomes in autism spectrum disorder: epilepsy and mortality.- Dev Med Child Neurol 2012; 54: 306–12.

Xiao Zhou, Ting Qiu, Xiaoyan Ke, Xiang Xiao, et al. - Autism Spectrum Disorder as Early Neurodevelopmental Disorder: Evidence from the Brain Imaging Abnormalities in 2–3 Years Old Toddlers- J Autism Dev Disord (2014) DOI 10.1007/s10803-014-2033-x

Zielinski BA, Prigge MB, Nielsen JA, Froehlich AL, Abildskov TJ, Anderson JS, Fletcher PT, Zygmunt KM, Travers BG, Lange N, et al., - Longitudinal changes in cortical thickness in autism and typical development. Brain. 2014 Jun;137(Pt 6):1799-812.

Zikopoulos Basilis and Helen Barbas- Altered neural connectivity in excitatory and inhibitory cortical circuits in autism - Front Hum Neurosci. 2013 Sep 27;7:609. doi: 10.3389/fnhum.2013.00609. eCollection 2013.

Zilbovicius M., B. Garreau, Y. Samson, P. Remy, C. Barthelemy, A. Syrota, G. Lelord, Delayed maturation of the frontal cortex in childhood autism, Am. J. Psychiatry 152 (1995) 248–252.

Zwaigenbaum L, Bryson S, Lord C, et al. Clinical assessment and management of toddlers with suspected autism spectrum disorder: insights from studies of high-risk infants. Pediatrics 2009; 123: 1383–91