Brain Development in Autism Spectrum Disorder

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PhD

November 2014

- WITH THANKS -

TO ALL THE FAMILIES WHO KINDLY TOOK PART IN THIS RESEARCH

ACKNOWLEDGMENTS

First and foremost I would like to extend my thanks to all the wonderful families who invited my research into their lives. It has been a privilege to work with such supportive parents and captivating young men, whom I have seen mature over the years of the study. They have given me many laughs and shown me some real struggles, but most significantly they have taught me some important life skills and have demonstrated to me the value of this research. I would also like to thank the Medical Research Council for funding this study.

I would like to express my sincere gratitude to the inspirational academics with whom I have had the pleasure of working with over the years. My first supervisor, Professor Steve Williams, who has always remained good humoured and calm in the face of the many difficulties of this study. Professor Declan Murphy, who has been an invaluable source of support and has always found time to respond to my questions. Professor Sean Deoni, my *de facto* professor, who has provided me with scholarly and technical guidance from across the pond. I would also like to thank Sean for his generosity and guidance on the numerous trips I made to work with his team at Brown University.

I would especially like to thank my second supervisor Dr Christine Ecker. Christine has been a pillar of support for me throughout my PhD and has gone above and beyond in her role as my second supervisor. I have developed at a remarkable pace, both academically and personally, under her tutelage.

Finally, words cannot express the love and gratitude I feel for my family and friends who have supported me through writing this thesis. My selfless mother Arghavan Fathieh and father Homayoun Shahidiani, thank you for always telling me to go after what I want - whatever it may be - as long as I did it well. To you I owe everything I am today. My kind little sister, and best friend Ghazal, you will always inspire me with everything you do. And to my new family, Hamed, thank you for being by my side. I hope that in life I repay you all with the love you have shown me.

برای مادربزرگ عزیزم

List of Abbreviations

5-HTP	5-Hydroxytryptophan
AD	Axial Diffusivity
ADC	Apparent Diffusion Coefficient
ADHD	Attention-Deficit Hyperactivity Disorder
ADI-R	Autism Diagnostic Interview-Revised
ADOS	Autism Diagnostic Observation Schedule
ASD	Autism Spectrum Disorder
BA	Brodman's Area
CC	Corpus Callosum
CDH10	Cadherin 10
CDH9	Cadherin 9
CNS	Central Nervous System
CNTNAP2	Contactin Associated Protein-Line 2
CNV	Copy Number Variation
CPMG	Carr-Purcell- Meiboom-Gill
CSF	Cerebrospinal Fluid
CST	Corticospinal Tract
CT	Cortical Thickness
DSM-5	Diagnostic & Statistical Manual Of Mental Disorders (5th Edition)
DSM-IV	Diagnostic & Statistical Manual Of Mental Disorders (4th Edition)
DSM-III	Diagnostic & Statistical Manual Of Mental Disorders (3rd Edition)
DTI	Diffusion Tensor Imaging
EEG	Electroencephalography
FA	Fractional Anisotropy
fMRI	Functional Magnetic Resonance Imaging
FOV	Field Of View
FSIQ	Full-Scale Intelligence Quotient
FTI	Diffusion Tensor Imaging
GABA	Gamma-Aminobutiric Acid
GAD	Glutamate Decarboxylase
GLM	General Linear Model
GP	General Practitioner
GM	Grey Matter
GWAS	Genome-Wide Association Study
ICD-10	International Classification Of Diseases (10th Revision)
IFOF	Inferior Fronto-Occipital Fasciculus

ILF	Inferior Longitudinal Fasciculus
IQ	Intelligence Quotient
mcDESPOT	Multicomponent Driven Equilibrium Single Pulse Observation Of T1 & T2
MCR	Multi-Component Relaxometry
MD	Mean Diffusivity
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MS	Multiple Sclerosis
MTR	Magnetization Transfer Ratio
MWF	Myelin Water Fraction
NICE	National Institute For Health & Care Excellence
NLGN3	Neuroligin 3
NLGN4X	Neuroligin 4 X-Linked
NMR	Nuclear Magnetic Resonance
OCD	Obsessive-Compulsive Disorder
PD	Proton Density
PET	Positron Emission Tomography
PLIC	Posterior Limbs Of The Internal Capsule
PSD	Post-Synaptic Density
qMT	Quantitative Magnetization Transfer
RD	Radial Diffusivity
RF	Radio Frequency
ROI	Region Of Interest
RUH	Radial Unit Hypothesis
SA	Surface Area
SEMA5A	Semophorin 5
SLF	Superior Longitudinal Fasciculus
TBSS	Tract-Based Spatial Statistics
TD	Typically Developing
TE	Echo Time
ToM	Theory Of Mind
TR	Repetition Time
UF	Uncinate Fasciculus
WASI	Wechsler Abbreviated Scale Of Intelligence
WM	White Matter

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ABSTRACT

Autism spectrum disorder is a lifelong neurodevelopmental condition accompanied by differences in brain anatomy and connectivity. Whilst the ASD brain has been widely studied under the lens of neuroimaging, results are both spatially and temporally heterogeneous. The most ubiquitous findings relate to global differences in the trajectory of early brain growth. Thus, there is a compelling need to characterize the neurodevelopmental trajectory of brain maturation in ASD beyond these early years and beneath the global level.

Therefore, the present work conducts an investigation into brain development in ASD, utilizing a variety of magnetic resonance metrics in a broad sample of children and adolescents with ASD and typically developing controls. We examine age-related differences in structural connectivity - measured by diffusion tensor imaging and myelin mapping techniques - alongside vertex-based measures of cortical anatomy, including cortical thickness, surface area and gyrification. In addition, we dissect these differences within a developmental framework by investigating linear, quadratic, and cubic age effects on each neuroanatomical component in order to identify the most appropriate model for examining between-group differences in the presence of significant age effects and age 'by' group interactions. Finally, we extend our cross-sectional investigations by carrying out a longitudinal study of myelination in ASD, showing for the first time that the ASD is accompanied by altered myelin development.

Our overarching finding is that ASD is characterised by age-related, region-specific brain differences. Importantly, these differences encompass the trajectories of both grey- and white-matter development, which we have dissected further into contributions from cortical-thickness, surface-area and gyrification, as well as white matter microstructure and myelination, respectively. Therefore, measures of grey- and white-matter morphology and connectivity should not be interpreted independently, but jointly as they jointly elicit the atypical patterns of brain development and connectivity typically observed in ASD.

INTRODUCTION

CHAPTER. 1

AUTISM SPECTRUM DISORDER

1.1.1 AUTISM THROUGH HISTORY

The term ‘autism' originates from the Greek word ‘autos’ meaning ‘self’, combined with the word ‘ismos’ meaning ‘state of being’. Taken together this roughly translates to a state of being absorbed in one’s self or an isolated self.

Our understanding of the word autism has developed throughout the past century. In the early nineteen hundreds Eugen Bleuler, who also coined the word ‘schizophrenia’, used autism to describe a subset of symptoms occurring in schizophrenia [2]. Decades later, Leo Kanner, in his influential paper ‘Autistic Disturbances of Affective Contact’ documented the characteristics of 11 children who he diagnosed with so called ‘infantile autism’. His work, along with that of Hans Asperger, forms the basis of our classical understanding of autism [3, 4]. At that time, children with autism fell into a discrete diagnostic category. Autism was seen as a rare and severe psychiatric condition characterized by distinct behavioural abnormalities including severe language delay, impaired cognitive skills, and a profound lack of emotional closeness with others [3]. In the 1950s and 1960s, Bruno

Bettelheim and his colleagues interpreted autistic behaviour in classic Freudian terms [5], and children with autism were portrayed as living in 'a glass bubble'. The condition was thought not to be of a biological origin but the outcome of an unaffectionate maternal relationship. This interpretation of 'refrigerator mothers' as they were called remained a focus through the 1950s and 1960s [3, 5].

Through the 1960s, however, our understanding of autism began to shift from being considered a psychological disorder towards being one of neurological origin. Infantile autism was first described as a neurological disorder with a strong genetic component in 1964 [6], with a prevalence estimated to affect 4 in every 10,000 children [7]. Moreover, in the late 1970s, Folstein and Rutter reported a significantly high concordance rate of autism in monozygotic twins (36%) compared to dizygotic twins (10%)[8], thus providing further support for the notion of autism being a highly heritable condition with genetic origins. Findings from this and other twin studies discredited those who advocated cold parenting theories of autism.

A few years later, the first working definition of autism was established and included in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III) [9]. The original characterization was strongly influenced by Michael Rutter's perspective of autism, characterized by an atypical social and communicative development, a propensity for sameness, and the onset of first symptoms prior to 30 months of age [10]. Subsequent modifications to the concept of autism in the DSM-IV and the 10th revision of the International Classification of Diseases (ICD-10) [11] then referred to autism as a 'pervasive developmental disorder', placing emphasis on the early onset of the typical 'triad' of symptoms such as (1) impaired social interaction; (2) impaired communication; and (3) restricted, repetitive, and stereotyped behaviours, interests, and activities. This behavioural definition further changed in 2013 with the publication of the 5th revision of the DSM (DSM-5) [12], which now uses the diagnosis of autism as an 'umbrella term' for wider autism spectrum disorders (ASD) (see below for current perspectives on diagnosing ASD).

1.1.2. Understanding Autism As A Spectrum Condition

In the late 1970s, Lorna Wing was the first to question the value of regarding autism as a discrete diagnostic condition [13]. Based on a prevalence study of autistic individuals with learning disabilities, Wing argued that autism fell on a wider 'spectrum,' and was not a distinct (i.e. binary) diagnostic category. According to Wing, individuals with autism fall on a wider autism spectrum that includes the syndromes originally described by Kanner and Asperger, but is broader and more dimensional [14].

This re-definition of ASD by Lorna Wing also affected the prevalence of the condition, which is now estimated to affect 1-2% of the general population [15-23]. Much of this increase in prevalence is due to the conceptual shift from a categorical to a spectrum view of autism, and a coincident widening of the diagnostic criteria. For example, the notion of an autism 'spectrum' means that the diagnosis includes individuals with milder symptom severities, and also recognises that ASD affects both people with and without language and/or learning difficulties, who nonetheless share the core diagnostic features of ASD.

The spectrum view of autism is further supported by the notion of "autistic traits" within the general population. Autistic traits assessed in the general population show a continuous distribution of severities through the normal range to the clinical extreme [24-27]. Furthermore, there is evidence to suggest that autistic symptoms and traits run in families and that those related to individuals with ASD are more likely to show a higher degree of autistic traits than those individuals without autistic kin. These individuals that show mild to moderate degrees of autistic symptoms or traits, but do not meet diagnostic cut-offs, exhibit what is know referred to as the "broader autism phenotype" (BAP) [28, 29]. Although people with the BAP show autistic symptoms that are subclinical, they do share the same familial influences and risk factors as their clinically diagnosed relatives [27]. Thus, understanding the aetiology of such inter-individual variation in autistic symptoms and traits between kin, will help to deepen our understanding of the possible genetic and environmental risk

factors underlying ASD, which may be elucidated by examining both patterns of resilience against – and a tendency for the condition.

Ultimately, the notion of a broader autism phenotype exemplifies the increasing sense of appreciation for the wide clinical and phenotypic diversity of ASD [30, 31], which has lead to a significant conceptual change in diagnosing and potentially treating ASD in the future (i.e. personalized/stratified medicine).

1.1.3. Current Definition And Epidemiology of ASD

The present definition of ASD refers to a group of behaviourally defined life-long neurodevelopmental conditions. The diagnostic hallmarks of ASD are visible in both syndromic and non-syndromic cases. The term syndromic autism refers to autism with a single defined cause (e.g. resulting from a known genetic mutation), such as fragile-X syndrome or tuberous sclerosis [32]. However, none of these aetiologies is specific to ASD because each of them includes a variable proportion of individuals with and without the condition [33]. More commonly, the ASD phenotype is non-syndromic, resulting from an unknown aetiology, which is thought to be the result of a complex synergy of genetic and non-genetic events [34].

It is essential to recognise that the ASD phenotype is diverse, including both individuals with normal, and opposing extremes of IQ. Between 40-55% of individuals with ASD have a learning disability (LD) and would be classed as 'low-functioning', compared to their 'high-functioning', non-LD counterparts [35-37]. Importantly, however, across all individuals on the ASD spectrum, the condition is hallmarked by impairments in three core domains (the traditional 'triad' of symptoms), which have been used to describe autism since the early 1980s. These include:

1. Qualitative impairments in communication
2. Limited reciprocal social interaction

3. Behaviours and interests that are restricted in range, and repetitive in nature.

However, with the arrival of DSM-5 and the adoption of ASD as an umbrella term for individuals on the wider autism spectrum, the traditional 'triad' of symptoms has been reorganized into a 'dyad', which encompasses the same behavioural deficits, now collapsed into two domains of (1) impaired social communication and interaction (combined as one domain); and (2) restricted and repetitive behaviour. For a schematic representation of these changes see figure 1.1

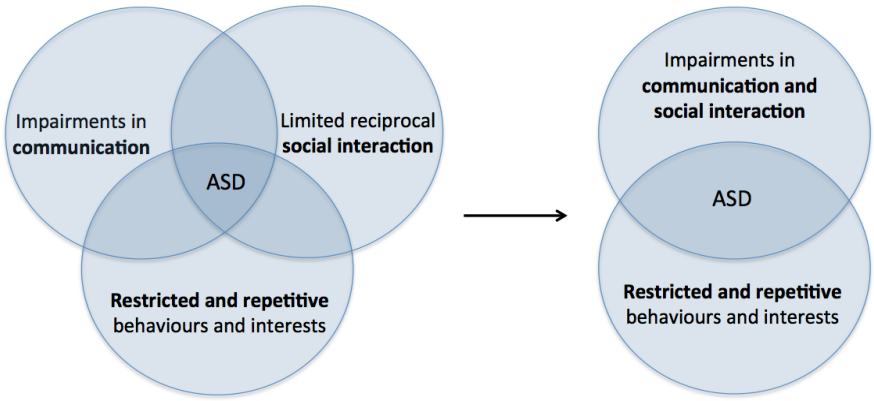

FIGURE 1.1

The reorganisation of the autistic 'triad' into a 'dyad'

These core impairments of ASD can occur in isolation or as a combination, and are often accompanied by other behavioural characteristics such as sensory hyper- and hypo-sensitivities (e.g. particular oversensitivity to specific sounds) restrictive eating habits, gastro-oesophageal reflux and constipation; sleep disturbances; unusual posture or gait, such as walking on tiptoes; and motor stereotypes (e.g. arm flapping) [38-47].

1.1.4. THE DIAGNOSIS OF ASD

Parents are often cognisant of their child's developmental problems within the first 18 months of life (for a descriptive outline of common indicators refer to Appendix 1.A). However, a reliable diagnosis of ASD can be made between the ages of 2 and 3 years - although it is typically made much later (e.g. at the age of 4-5 years). The referral and diagnostic process for ASD is extremely varied, and is heavily dependent on the geographical region, services and age [48, 49]. The overview provided here will mainly focus on the diagnosis of those individuals under 18 years of age, as the diagnosis of adults can be more variable.

In the UK, a child will initially be referred by their GP to a specialist mental health team where they will be formally assessed and diagnosed. A multidisciplinary team of expert clinicians normally carry out this assessment in line with NICE guidelines [1]. For an outline of what should be assessed in line with NICE guidelines, refer to Box 1. These guidelines are based around clinical ICD and DSM criteria for ASD. The preferred classification system used in UK institutions is currently the ICD-10 [11] - for ICD-10 diagnostic criteria for 'childhood autism', refer to Box 2. Although these diagnostic guidelines and criteria are extremely useful and provide a good streamline in the clinical setting, clinical diagnostic criteria for ASD have poor inter-rater reliability in complex cases (e.g. individuals on the wider autism spectrum, individuals with co-morbidities etc.), and also do not provide quantitative diagnostic cut-offs for disease severity, which are often required in the research setting. For research purposes gold-standard autism-specific diagnostic tools (as referred to in Box 1) are therefore employed to gather essential diagnostic information.

1.1.5. Research Reliable Diagnostic Tools

The gold standard tools used for diagnosis include the Autism Diagnostic Interview (ADI-R), and the Autism Diagnostic Observation Schedule (ADOS), in addition to various other checklists and instruments (e.g. the Childhood Autism Rating Scale (CARS)[50], and the Checklist for Autism in Toddlers (CHAT)[51]). The ADI is a semi-standardised interview of established reliability and validity [52]. The interview focuses primarily on the key diagnostic characteristics of ASD as specified in the ICD-10 [11] and DSM-5 [12], which include the core domains of abnormalities in reciprocal social interaction, abnormalities in communication, and restricted, repetitive, and stereotyped patterns of behaviour. There is also an emphasis on developmental milestones and potential delays. Individuals are objectively scored in each of these areas by an expert rater. The ADI interview is carried out with a parent or caregiver who has known the individual extremely well since early on in development. Notably, the ADI-R assesses autistic symptoms retrospectively, which quality for a diagnosis of 'childhood autism', and many items in the interview thus concentrate on the period between the ages of 4-5 years. Although the retrospective assessment of autistic symptoms yields valuable information about crucial developmental time-points, it can also be problematic – particularly in adults - as assessors rely on the availability of parents and their accurate recall and reporting of past events.

While the ADI probes past autistic symptoms and traits (i.e. at the age of 4-5 years), the ADOS assesses current symptoms. Thus, ADOS and ADI-R are typically used together in the gold-standard diagnosis of ASD. The ADOS [53] is a semi-structured assessment of communication, social interaction and behaviour. There are four different ADOS modules, which are each designed for use in individuals with varying degrees of verbal fluency and intellectual ability. The schedule follows a standardised interview and observational assessment consisting of a number of activities carried out with the individual. The ADOS offers opportunities to observe social communication skills, interests and repetitive behaviours, as well as the individual's sense of humour and

creativity within the assessment setting. As with the ADI, the ADOS includes a systematic scoring system and diagnostic thresholds.

These gold-standard assessments greatly enhance our ability to diagnose ASD, however they are both based on behavioural observations and/or clinical interviews, whether through parental report (in the case of the ADI) or an individuals self-portrayal (in the case of the ADOS), and measures are often subjective and could be affected by coping strategies acquired over the human life-span. The development of objective biomarkers that could aid the diagnosis of ASD would thus be of immense value.

Box 1

NICE guidelines for the diagnosis of ASD

Include in every autism diagnostic assessment:

- *Detailed questions about parent's or care giver's concerns and, if appropriate, the child's or young person's concerns*
- *Details of the child's or young person's experiences of home life, education and social care and developmental history, focusing on developmental and behavioural features consistent with ICD-10 or DSM-IV criteria (consider using an autism-specific tool to gather this information)*
- *Assessment (through interaction with and observation of the child or young person) of social and communication skills and behaviours, focusing on features consistent with ICD-10 or DSM-IV criteria (consider using an autism-specific tool to gather this information)*
- *A medical history, including prenatal, perinatal and family history, and past and current health conditions*
- *A physical examination*
- *Consideration of the differential diagnosis*
- *Systematic assessment for conditions that may coexist with autism*
- *Development of a profile of the child's or young person's strengths, skills, impairments and needs that can be used to create a needs-based management plan, taking into account family and educational context*
- *Communication of assessment findings to the parent or care giver and, if appropriate, the child or young person*

Taken from the National Institute for Health and Care Excellence [1]

Box 2

ICD-10 criteria for the diagnosis of AS

Childhood Autism - F84.0 (ICD-10)

A *Abnormal or impaired development is evident before the age of 3 years in at least one of the following areas:*

(1) receptive or expressive language as used in social communication;

(2) development of selective social attachments or of reciprocal social interaction;

(3) functional or symbolic play.

B *A total of at least six symptoms from (1), (2), and (3) must be present, with at least two from (1) and at least one from each of (2) and (3):*

(1) Qualitative abnormalities in reciprocal social interaction are manifest in at least two of the following areas:

(a) failure adequately to use eye-to-eye gaze, facial expression, body posture, and gesture to regulate social interaction;

(b) failure to develop (in a manner appropriate to mental age, and despite ample opportunities) peer relationships that involve a mutual sharing of interests, activities, and emotions;

(c) lack of socio-emotional reciprocity as shown by an impaired or deviant response to other people's emotions; or lack of modulation of behaviour according to social context; or a weak integration of social, emotional, and communicative behaviours;

(d) lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g a lack of showing, bringing, or pointing out to other people objects of interest to the individual).

(2) Qualitative abnormalities in communication are manifest in at least one of the following areas:

(a) a delay in, or total lack of, development of spoken language that is not accompanied by an attempt to compensate through the use of gesture or mime as an alternative mode of communication (often preceded by a lack of communicative babbling);

Continued overleaf…

(b) relative failure to initiate or sustain conversational interchange (at whatever level of language skills is present), in which there is reciprocal responsiveness to the communications of the other person;

(c) stereotyped and repetitive use of language or idiosyncratic use of words or phrases;

(d) lack of varied spontaneous make-believe or (when young) social imitative play

(3) Restricted, repetitive, and stereotyped patterns of behaviour, interests, and activities are manifest in at least one of the following areas:

(a) an encompassing preoccupation with one or more stereotyped and restricted patterns of interest that are abnormal in content or focus; or one or more interests that are abnormal in their intensity and circumscribed nature though not in their content or focus;

(b) apparently compulsive adherence to specific, non-functional routines or rituals;

(c) stereotyped and repetitive motor mannerisms that involve either hand or finger flapping or twisting, or complex whole body movements;

(d) preoccupations with part-objects or non-functional elements of play materials (such as their odour, the feel of their surface, or the noise or vibration that they generate).

C *The clinical picture is not attributable to the other varieties of pervasive developmental disorder: specific developmental disorder of receptive language (F80.2) with secondary socio-emotional problems; reactive attachment disorder (F94.1) or disinhibited attachment disorder (F94.2); mental retardation (F70-F72) with some associated emotional or behavioural disorder; schizophrenia (F20.-) of unusually early onset; and Rett's syndrome (F84.2).*

Taken from the International Classification of Diseases, 10th edition [5]

1.1.6. COMORBIDITY

ASD is a condition with a high degree of comorbidity. More than 70% of individuals with a diagnosis of ASD exhibit one or more co-existing medical, developmental, or psychiatric conditions in their lifetime [47, 54-56]. Some of the most common co-morbid conditions occurring in ASD are intellectual disability, Attention-deficit hyperactivity disorder (ADHD), anxiety disorders, motor abnormalities, sleep disturbances, gastrointestinal problems and various behavioural disorders. A more complete list of co-morbid conditions and their prevalence is given in Table 1.1. It is currently unknown whether the range of co-morbidities evident in ASD is the result of a common pathophysiology or disease mechanisms that may lead to overlapping symptomatic domains. This greatly complicates the diagnostic process and implies that research into ASD has to consider converging and diverging genetic determinants in order to correctly identify common as well as diverging genotypes and the associated phenotypes of co-occurring disorders, which will be an important future course of investigation.

TABLE 1.1

Comorbid Condition	Prevalence Alongside Autism Spectrum Disorder
Developmental	
Intellectual disability	40-55% [35-37]
Language deficits	Previously placed at 50-63% [48], language delay has now been removed from the DSM-5 as a defining feature of autism. Ergo pinpointing the prevalence of language deficits by current definition is challenging. An autism-specific language profile (independent of language disorders) does exist, but there is significant variability between individuals [57, 58].
Attention-deficit hyperactivity disorder	28-44% [47, 54, 55, 59]
Tic disorders	14-38% [47, 54, 55, 59] (including Tourette's)
Motor abnormalities	Approximately 79% [60-63] (including, motor delay, hypotonia, catatonia, deficits in coordination, preparation and planning, praxis, gait, and balance [60, 61])
Mental and Behavioural	
Anxiety	42-56% [47, 54, 55, 59] Most commonly social anxiety disorder [64] and generalised anxiety disorder [47, 54, 59].
Depression	Ranging from 12-70% from childhood (less common) to adulthood (more common) [47, 54, 55, 59].
Obsessive-compulsive disorder	7-24% [47, 54, 59]
Oppositional defiant disorder	16-28% [54, 55]
Psychotic disorders	12-17% [47, 59]
Personality disorders	Paranoid personality disorder (0-19%); schizoid personality disorder (21-26%); schizotypal personality disorder (2-13%); borderline personality disorder (0-9%); obsessive-compulsive personality disorder (19-32%); and avoidant personality disorder (13-25%) [47, 56]
Behavioural disorders	Aggressive behaviours (approximately 68% [65]); self injurious behaviours (approximately 50% [66]); Suicidal ideation (11-14% [67, 68])
General Medical	
Epilepsy	8-30% occurring more commonly in individuals with genetic syndromes and intellectual disability.
Gastrointestinal problems	9-70% [38] (including, chronic constipation; gastro-oesophageal reflux; inflammatory bowl disease; Crohn's disease; and colitis [38-40])
Immune dysregulation	Approximately 38% [69]
Genetic Syndromes [33]	Approximately 5% [70] (including, fragile X syndrome; Rett syndrome; and tuberous sclerosis)
Sleep disorders	50-80% [41, 42] Most commonly insomnia

1.1.7. GENDER BIAS

Research suggests that there may also be a gender-bias in the diagnosis of ASD and that females with ASD may have a different symptom profile. Of the 1-2% of individuals with ASD, 2-3 times more males are affected by the condition than females [16, 17, 19, 22]. Earlier epidemiological investigations had estimated autism to be 4–5 times more frequent in males than females [71]. Intelligence level has been shown to affect this sex ratio: males are considerably over-represented amongst high-functioning cases, whereas females and males are more equally represented amongst cases with severe intellectual disability [35, 71, 72].

Historically, females with autism have been under-recognised [73-75] and this may be due to numerous factors. High-functioning females are typically diagnosed much later than their male counterparts [76, 77], and also require more concurrent behavioural or cognitive deficits than males to be clinically diagnosed, despite equivalently high levels of autistic traits [78]. This diagnostic bias may be a result of skewed behavioural criteria for autism and gender stereotypes (ascertainment bias), or genuinely better adaptation or 'camouflage' in females [78-80]. In addition, studies indicate different symptom profiles for males and females, and these gender differences tend to reflect gender differences observed in the general population. Boys with ASD tend to exhibit more externalising behaviours than females do, such as aggression, hyperactivity, antisocial behaviour, and increased repetitive/restricted tendencies [35, 72, 81]. Females on the other hand show more internalizing symptoms than boys, such as anxiety, depression, and other emotional symptoms [81, 82]. In addition, a recent study found that women with anorexia nervosa possess a greater number of autistic traits than typical women, and concluded that autistic traits such as inflexibility of thinking and problems with social interactions may exacerbate factors that maintain the eating disorder [83].

These differences may also influence rates of diagnosis, as externalised behaviours are more noticeable and likely to cause concern in school, which would prompt referral.

Male predominance in terms of the prevalence of ASD is a consistent finding that might also carry aetiological indications. For example, a recent study of twins with ASD, suggested the existence of female (neuro)-protective effects [84]. In addition the multiple-threshold multifactorial liability model suggests that females require a comparatively greater aetiological load than males to manifest the same level of impairment [85, 86]. This model also predicts that the male relatives of female probands would have an increased genetic risk for ASD compared with the male relatives of male probands [85, 87]. Understanding the biology underlying ASD in females, as well as their resilience in terms of autistic symptoms, could thus aid our understanding of the behavioural autistic phenotype and the identification of potential protective factors.

1.1.8. The Wider Cognitive Phenotype Of ASD

Cognition and neurobiology are undoubtedly related, and it has been shown that the neurocognitive differences associated with ASD are indicative of the neural networks and substrates that underpin the condition [88-90]. As has been pointed out by Uta Frith in a seminal lecture 'The 38th Sir Frederick Bartlett Lecture', a small number of cognitive mechanisms in ASD might explain a large number of phenomena [88]. The cognitive mechanisms underlying ASD can be grouped into discrete domains, such as (1) socio-communicative function; (2) executive function; and (3) Local versus global hierarchical information processing. These domains of cognition are well characterized in ASD, and a number of neuropsychological tests might be used to assess these specific cognitive mechanisms (for examples, see; [91-94].

SOCIO-COMMUNICATIVE FUNCTION (SOCIAL COGNITION AND SOCIAL PERCEPTION)

Socio-communicative functioning includes gaze and eye contact [95], emotion perception [96], alexithymia (the inability to identify and describe one's own emotions)[88, 97], affective empathy and sympathy [98], facial recognition and processing [99], social attention, joint attention and orienting [100, 101], social motivation and social reward processing [102, 103], imitation [104], imagination and pretend play [105], theory of mind or mental perspective taking [106, 107], self-referential cognition and metacognitive awareness (introspective awareness of and access to our own mental states) [108, 109], and biological motion perception [110]. In ASD, this broadly translates to a lack of implicit mentalizing (i.e. difficulties in understanding and referring to mental states in both the self and others) [111, 112], and atypical social interaction and social communication.

Impaired theory of mind (first reported in autism in 1985 [93]) and difficulties with mentalizing are believed to constitute the core deficits in social and communication abnormalities associated with ASD. Studies have revealed that the developmental neuropsychological precursors for mentalizing (e.g., joint attention, eye contact, emotion perception, social orienting, and face processing) are atypical in ASD, even prior to the verbal expression of mentalizing [113, 114]. The early onset of these difficulties seems to be specific to ASD, although deficits in mentalizing are reported in other disorders later in life (for example, schizophrenia [115]). In higher functioning individuals where some degree of controlled or explicit mentalizing is achieved [116], the more subtle intuitive elements tend to remain impaired throughout life [107].

EXECUTIVE FUNCTION

Executive functioning includes cognitive flexibility, attention shifting, inhibitory control, planning and monitoring, generativity, and working memory [117]. In ASD executive dysfunction is thought to underpin both repetitive stereotyped behaviours and atypical social interaction and social communication [118], although it has also been suggested that impaired

performance in executive function tasks could, in fact, be underpinned by difficulties with mentalizing [119].

LOCAL VERSUS GLOBAL HIERARCHICAL INFORMATION PROCESSING

Local versus global information processing includes global versus local perceptual functioning, central coherence (processing bias for featural and local information) [120] and 'systemising' (the drive to construct and understand rule-based or factual systems) [58, 121]. In addition to atypical social interaction and social communication in ASD, deficits in these processing styles translate to idiosyncratic sensory-perceptual processing, and restricted interests and repetitive behaviours. In typically developing individuals, global functioning takes precedence. ASD individuals, however, demonstrate superior local-perceptual processing relative to global processing [120, 122, 123]. This difference in perceptual preference has also been used to explain the excellent attention to detail, enhanced sensory-perceptual processing and discrimination, and the strong preference to derive rule based systems typically associated with ASD [120]. It also explains the nature of 'special talents', and the exhaustive precision of 'savants', that are more frequently observed in the ASD population [124].

Taken together, these studies demonstrate that the neurocognitive profiles in individuals with ASD differ from typically developing individuals. Furthermore, these differences on the behavioural level have also significantly contributed to our current approach to diagnosing ASD at the behavioural level. In the following section, I will discuss the neurobiological underpinnings that may mediate these behavioural differences on the molecular and systems level.

1.2. THE NEUROBIOLOGY OF ASD

SECTION OUTLINE

The ASD brain has been studied at varying degrees of magnification and using various neuroscientific approaches that allow us to dissect the cellular, regional and systemic patterns of dysfunction associated with ASD. While differences at these varying levels of magnification play different roles in the development of brain anatomy, functioning and connectivity in ASD, there is currently no unifying biological hypothesis that could account for all autistic phenotypes. It is thus widely accepted that the biological determinants of autistic symptoms and traits are most likely multifactorial. In the following paragraphs I will briefly outline our current understanding of some of the main pathological pathways associated with ASD, bringing together findings from genetics, brain anatomy, and chemistry - categorised in terms of cellular and regional abnormality. Each of these areas will then be described in more depth in subsequent sections.

Cellular explanations have focussed on atypical synaptic structure and functioning, and an imbalance between excitation and inhibition (E/I) [125-127] - this might also explain the seizures that occur in a subset of patients with ASD. Robust arguments also exist for the contribution of serotonin [128]. ASD is highly comorbid with depression and a number of other conditions that have been linked to the deregulation of serotonergic systems [129-136]. Abnormal calcium signalling has similarly been posited [137] - a considerable proportion of ASD-related syndromes are associated with seizures or congenital cardiovascular abnormalities related to calcium signalling [138].

Regional explanations for ASD include differences in columnar organization [139, 140]. Post-mortem investigations have implicated reduced minicolumnar width in the cerebral cortex, with differences being most salient in areas involved in language processing and understanding [141-143]. Abnormalities of cerebellar architecture were among early hypotheses [144-147].

Additionally, neuroimaging studies suggest the involvement of frontal and anterior-temporal regions of the brain and their long-distance reciprocal and parietal connections [148]. These regions are thought to be involved in joint attention [149], which is often impaired in autism. Furthermore, these regional differences vary with age and there is ample evidence to suggest an atypical time-course of brain maturation in ASD [148, 150, 151].

Finally, for all the possibilities discussed above there are hosts of genes implicated. Thus, I will begin this section by discussing genetic influences on ASD before moving on to brain anatomy, connectivity and chemistry.

1.2.1. GENETICS OF ASD

It is well established that ASD is a highly heritable condition, and numerous lines of evidence support genetic factors as a principal cause of ASD. However, the genetic mechanisms are complex and include rare chromosomal anomalies, several individual genes of major effect, as well as numerous common variants with small effect. The relative risk of a child being diagnosed with ASD increases by 25 times over the population prevalence in families in which a sibling is affected [152]. The biological relatives of an affected person are also more likely than controls to show an attenuated phenotype of subtle cognitive or behavioural characteristics that are qualitatively similar to those observed in ASD, [153, 154] constituting a 'broader autism phenotype'. Twin studies, reveal that concordance rates for monozygotic (MZ) twins (70–90%) are many times higher than the corresponding rates seen in dizygotic (DZ) twins (0–10%) [155, 156].

However, it is important to note that concordance rates in MZ twin pairs are never reported at 100%, indicating the presence of epigenetic or environmental factors [157, 158]. It has recently been suggested that environmental factors are becoming increasingly more important and whilst genetic factors do play a fundamental role in ASD, they are of significantly lower magnitude than previously estimated. Hallmayer et al., recently

conducted a large-scale twin study and reported that concordance rates for MZ twins corroborated previous reports, but the concordance rates for DZ twin pairs were considerably higher than previously reported, with rates of 21% for male pairs and 27% for females [87]. They concluded that environmental factors common to twins actually explain around 55% of the liability to autism (ibid). These findings add additional dimensions to what is already a highly heterogeneous genetic landscape for ASD, with different types of genetic abnormalities positioned on almost all chromosomes with variable levels of penetrance [159, 160].

GENETIC SYNDROMES ASSOCIATED WITH ASD

There exists a growing body of literature demonstrating that mutations and/or structural differences in specific genes can considerably increase disease risk. The high heritability of the condition has been linked to several risk gene variants that significantly contribute to the genetic architecture of ASD [161]. Early insights into the genetics of ASD mostly stem from the observations that individuals with a handful of single-gene syndromes share the behavioural phenotype of ASD [162-167]. These disorders have often been referred to as 'autisms of known aetiology' (or syndromic autism). An example for an ASD-associated genetic syndrome is Fragile X syndrome, which results from expansion of the CGG trinucleotide repeat in the FMR1 gene, to 200 or more repeats. Other examples of single gene disorders include RETT syndrome (mutations in the X-linked MECP2 gene) [168], and Tuberous Sclerosis (TSC2 mutations) [169]. However, studies of single-gene disorders have major limitations with regards to isolating the ASD from the genetic disorder. Also, none of the molecules or syndromes currently linked to ASD have been known to selectively cause ASD. Rather, these genetic variants seem to result in a range of abnormal neuro-behavioural phenotypes, of which ASD is one [170, 171].

On the other hand, many lines of evidence suggest that idiopathic autism (i.e. in contrast to 'autisms of known aetiology') is not a single-gene disorder. Instead, it is mediated by a pattern of small and individually rare genetic variants, termed copy number variations (CNVs), which confer susceptibility. This is understandable, considering the varying degrees of symptom severity and diverse neurocognitive profiles of ASD individuals (and those with the broader autism phenotype). As previously mentioned, heterogeneity is a dominant characteristic in ASD, salient in both causative and phenotypic dimensions. CNVs, both *de novo* and inherited, are emerging as important causes of autism susceptibility, either as rare variants that robustly modulate risk or as potentially new syndromes linked to autism [172-174]. Rare variants have mainly been observed in genes encoding for synaptic cell adhesion molecules, which play a vital role in synaptogenesis and neuronal differentiation. For instance, numerous studies have pointed to CNVs in genes encoding for a family of proteins known as neurexins, neuroligins and SHANK3.

Neurexins and neuroligins are part of a family of cell adhesion molecules whose members are respectively localized, pre- and post-synaptically [175]. These proteins are thought to be involved in the formation and consolidation of synaptic contacts [176] and trans-synaptic signalling [177]. Depending on their particular subtype these proteins can induce the formation of both inhibitory and excitatory synapses [178]. Specifically, ASD has been related to CNVs in neuroligin genes such as neuroligin 3 (NLGN3) (involved primarily in excitatory synaptogenesis) and neuroligin 4 X-linked (NLGN4X) [175, 179]. Both excitatory-inhibitory imbalance [177, 180, 181] and abnormalities in synaptogenesis [174] have been linked to ASD, which is compatible with the discovery of these CNVs. Another member of the neurexin family, contactin associated protein-line 2 (CNTNAP2) has also been linked to ASD (as well as epilepsy, and language delay). This gene has been shown to modulate language function in ASD, and may also have a role in modulating long-range brain connectivity [182, 183]. During development, CNTNAP2 messenger RNA is elevated in the frontal and temporal lobes, and when adulthood is reached it is

expressed predominantly in the striatal circuits and the frontal cortices [161] - regions known to support cognitive specializations such as, speech and language. This cortical pattern of CNTNAP2 expression especially relates to frontal lobe connectivity, which has been described as atypical in ASD and ADHD [161, 182, 184].

Another CNV that may contribute to excitatory-inhibitory imbalance in ASD is SHANK3 [185, 186]. This is a gene encoding for a synaptic scaffolding protein that is largely located in the post-synaptic density (PSD) [187]. The PSD is a protein dense specialization attached to the postsynaptic membrane, where receptors, adhesion molecules and signalling molecules that are required for synaptogenesis and synaptic signalling, are assembled [188]. SHANK3 proteins are also involved in synapse formation and dendritic spine maturation through their interactions with neuroligins [189]. Mutations in SHANK3 strongly affects the development and morphology of dendritic spines and electrical activity [190, 191], and thus, could also contribute to an excitatory / inhibitory imbalance in ASD.

Cytogenetic studies have also commonly identified recurrent duplications of the maternally derived chromosome 15q11-q13 [192], and reciprocal 16p11.2 micro-deletions and duplications. As of yet, the 16p11.2 CNVs have not been found to confer an ASD phenotype, but this cannot be ruled out as clinical data is still limited. Although cytogenetic abnormalities have been identified on nearly every chromosome, they scarcely occur with a frequency suggestive of a specific autism gene location [162]. Interstitial duplication of 15q11-q13 is a highly penetrant contributor to autism susceptibility, and the observed phenotype correlates with the number of 15q copies. A maternal duplication of 15q which results in 3 copies for that region (trisomy), causes only subtle effects on the phenotype [193, 194], while children with four copies of 15q are characteristically more impaired and may show signs of hypotonia, seizures, microcephaly and severe developmental delay [195]. The paternally derived duplication on the other hand, has negligible, if any phenotypic effect, indicating genomic imprinting of this region [196].

GENOME-WIDE ASSOCIATION STUDIES IN ASD

Genome-wide association studies (GWAS), which examine the association of vast number of common polymorphisms with a particular trait, or between groups of individuals (for example, subjects with and without ASD), have identified a number of associations with ASD. For example, genes coding for neuronal cell adhesion, such as, Cadherin 10 (CDH10 and CDH9) [197] and Semophorin 5 (SEMA5A), which is thought to be involved in axonal guidance, [198] have been associated with ASD susceptibility. However, GWAS studies have discovered relatively few common genetic variants that can be reliably associated with ASD, possibly due to the large effect sizes that would be required to yield statistically significant results following multiple-comparison corrections, in addition to the breadth of autistic symptoms.

At present, the incidence of known genetic links to autism is posited at <5% for cytogenetically visible chromosomal abnormalities, 5-15% for CNVs and about 5% for single-gene disorders [192, 199-201]. There are sizeable limitations to our current understanding of genetic aetiology of autism. Foremost, penetrance is extremely variable, and both deletions and duplications may be inherited or occur de novo [202]. Additionally, unaffected family members can carry the same CNV as their ASD relative [203, 204]. These criticisms are compatible with the notion of a 'broader autism phenotype' that can be observed in the general population as well as with the phenotypic heterogeneity that we see in affected individuals. Thus, we might conclude that rather than 'causing' ASD, CNVs and other genetic variations may in fact simply increase the overall risk of ASD by affecting neural systems that mediate specific symptoms. The neural systems most associated with the condition will be discussed in the following section.

ENVIRONMENTAL TRIGGERS

It is important to include here that a number of potential environmental triggers for ASD are scientifically supported. Several authors support the hypothesis that environmental factors may interact with genetic factors to increase ASD risk [87, 205]. Arguments for an environmental contribution to ASD arise from a growing number of studies examining environmental factors, but also from the absence of a definitive genetic model. Because shared environments and early life experiences are common to twin pairs, the prenatal environment and early postnatal environment are of particular significance. It has been hypothesized that at least some of the environmental factors impacting susceptibility to ASD exert their effects during this crucial period in life [87]. Such non-genetic risk factors include parental age [206], maternal infections during pregnancy [207], multiple births [208], and low birth weight [209]. These factors, although very important, are beyond the scope of this thesis.

1.2.2. Insights From Typical Brain Development

The typically developing infant is born with imperfect processing and behavioural capacity as the neural circuitry is still incomplete in many regions of the brain [210]. Throughout life, the evolution of the functional ability of the brain rests on the formation and fine-tuning of its main neural circuitry. The process of brain development involves a sophisticated combination of constructive and subtractive processes that transform the brain on both microscopic and macroscopic levels.

Developmental changes in brain structure are well established by post mortem and neuroimaging studies (for an introduction to magnetic resonance imaging and the principles of neuroimaging refer to Chapter 2). In recent years the repertoire and technical advancement of neuroimaging techniques has also dramatically increased, thus improving our ability to assess brain development in vivo from infancy to old age. Understanding typical brain development is vital for establishing the relationship between changes in the brain and neurocognitive maturation, including the acquisition of higher cognitive functions. In addition, examining typical brain maturation is also the vital first step towards identifying abnormalities that occur in neurodevelopmental conditions such as ASD. For this purpose I will firstly outline what we know about typical development and then review the patterns of maturational abnormality typically observed in ASD.

From a microstructural perspective, brain development occurs in waves of cellular transformation, involving cell birth, differentiation, migration and elimination [211], which are also represented schematically in Figure 1.2. However, such changes are typically below the resolution of conventional neuroimaging techniques, which will be the main focus in the following section. Moreover, the gross-anatomical changes that are accessible with MRI are likely to be the result of multiple independent cellular processes, that involve various coordinated structural changes. Thus, in the interest of clarity, I will begin by discussing the development of MR detectable components such

as white matter (WM) and grey matter (GM) maturation, and refer to the underlying microstructural processes where relevant.

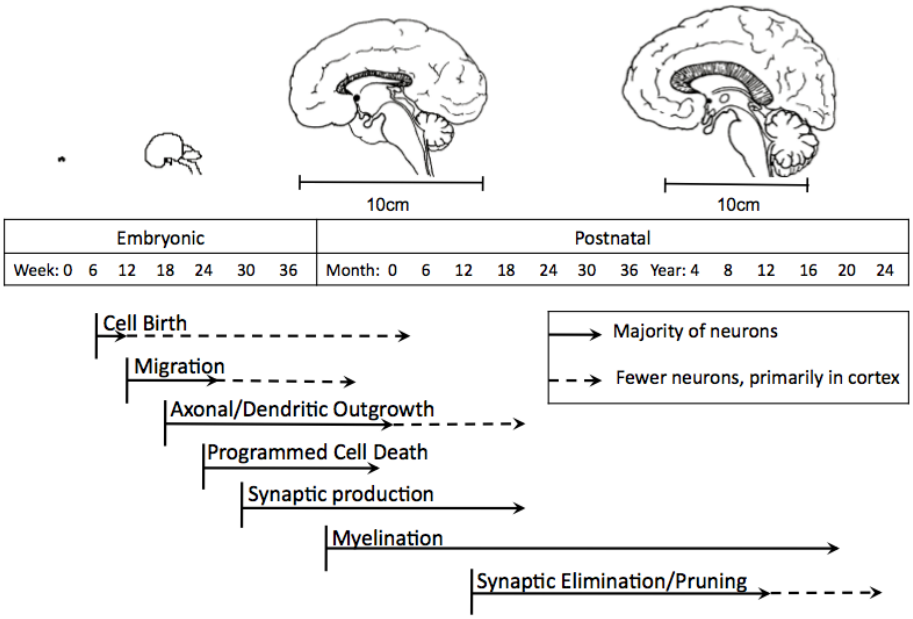

FIGURE 1.2

The stages of development. (Adapted from: [211])

Broadly, brain tissue may be classified into WM and GM. GM is distributed at the surface of cerebral hemispheres and cerebellum, making up the cerebral cortex and cerebellar cortex, respectively. It is also concentrated in the deep cortical and cerebellar nuclei and brainstem. WM is comprised of fibres that carry information between specific GM areas where that information is processed. Surrounding these fibres is a fatty layer called myelin, which acts as an insulating sheath and dramatically increases the speed at which information is transferred through saltatory or 'leaping' conduction. GM and WM have different origins and are known to develop differently.

Grey matter volume is known to increase well into adolescence, after which it plateaus and starts to decline [212-218] (shown in Figure 1.3). White matter, on the other hand, undergoes a linear increase in volume [219-221] and density [222] with age. The process of myelination continues throughout lifespan and even into middle age. Reductions in WM volume are only observed when

the balance between myelin production and the degenerative processes of aging tip in favour of white matter loss [223]. Thus, rather than simply ending or declining in development, it seems that developmental processes underlying brain maturation may in fact continue until they are counteracted by processes related to aging.

It has long been accepted that the trajectory of growth varies across brain regions [224], as well as over time [225]. For example, according to Shaw et al. 2008, there is considerable regional variation in complexity of the normal developmental trajectory of cortical thickness across the cerebral cortex, and the complexity of the growth trajectory (linear vs. quadratic vs. cubic) seems to correspond to the complexity of the laminar architecture of a particular brain region [224]. In addition, studies have shown that peak GM maturation differs between different lobes of the brain. For instance, the frontal and parietal lobes reach their maximum size in early adolescence, while temporal lobe grey matter plateaus later on in adolescence [212]. Maturation also differs significantly within the lobes, as can be seen in Figure 1.3; e.g. the prefrontal cortex and the posterior part of the superior temporal gyrus are particularly late maturing [217].

In contrast, it appears that dorsal parietal cortical regions that underpin sensory and perceptual functions may develop earlier in childhood [213]. This falls in line with the idea that phylogenetically older structures, such as those supporting vision and hearing, develop earliest in life, whilst areas with the most protracted course of development tend to be those that are phylogenetically most recent and responsible for higher order cognitive functions, such as the frontal and prefrontal cortices [217] and the posterior temporal cortex [214].

Multiple studies into normative brain maturation through childhood and adolescence have concluded that grey matter loss constitutes an essential step in sculpting the brain [213, 214, 218, 226-228]. Adolescence is also thought to be the critical time of cortical pruning [229]. The spatial and temporal pattern of change in GM and WM volume is consistent with known cellular maturational changes that occur through childhood and adolescence (such as synapse

elimination and dendritic pruning) as well as cognitive and behavioural changes [230]. The accelerated maturation of frontal lobe grey matter in adolescence, for example (before it plateaus), parallels the increased efficiency of executive functions that are not wholly developed before adolescence [231]. Further, it has been suggested that the phase of cortical thinning in adolescence (i.e. once peak maturation has been reached) may be associated with the refinement of neural circuits via experience-dependent synaptic pruning or elimination of synapses [232], possibly supporting maturing cognitive capabilities [233, 234]. Finally, it is important to note that the observed decrease in GM may also partly be explained by the on-going increase in WM [214]. More specifically, events that occur at the boundary between WM and GM, such as the proliferation of myelin into the peripheral cortical neurophil, could affect the grey- white-matter contrast [230].

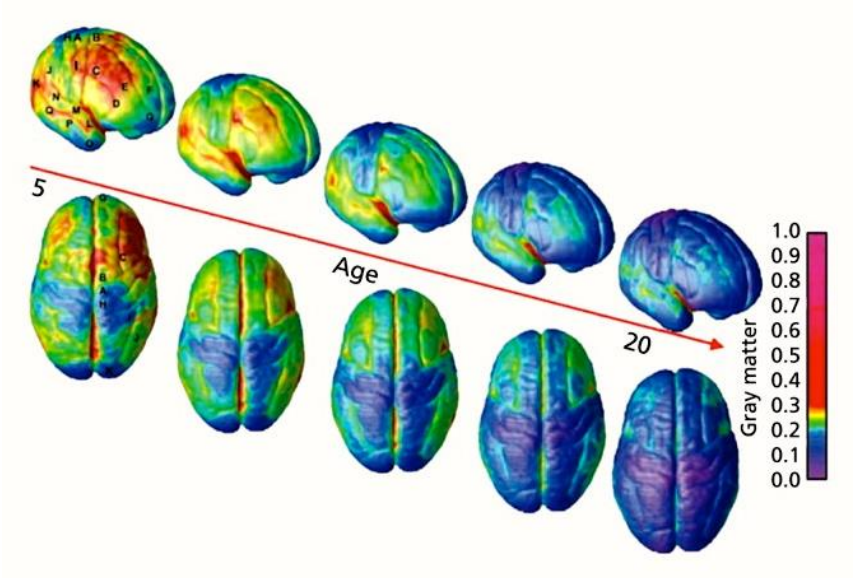

FIGURE 1.3

GM maturation over the cortical surface between the ages of 5 and 20 years. The sidebar shows a colour representation in units of GM. Taken from Gogtay, N., et al. Proc Natl Acad Sci USA, 2004;101(21): p. 8174-9 [217].

White-matter Development assessed via Diffusion Tensor Imaging

Although neuroimaging studies have provided many invaluable insights into the development and architecture of grey matter, conventional neuroimaging approaches (e.g. segmentation-based studies such as voxel-based morphometry) are less suited to the investigation of white-matter development. Alternative approaches that assess the complex WM structure by assessing myelin directly (e.g. potentially diffusion tensor imaging) might therefore be better suited to investigate white-matter development in the brain in vivo. In WM, the myelin sheath and the structure of axons impose boundaries to the course of water diffusion, so that diffusion largely occurs along the axis of the fibre bundles [235]. Diffusion tensor imaging (DTI) is a non-invasive MR tool, that takes advantage of this property, allowing us to visualize, at a gross level, the diffusion of water along these axons [236]. It can therefore be used to visualize axonal pathways, providing unique information about tissue microstructure. DTI yields an informative diffusion measure, termed fractional anisotropy (FA), a ratio that refers to the degree of water diffusion in one direction (along axons), relative to the secondary and tertiary orthogonal directions (the principles of DTI and diffusion measures are covered in depth in Chapter 2). FA has extensively been used to measure of WM integrity, for example higher FA values are conventionally interpreted to reflect increased myelination or enhanced development of individual tracts (or regions). Importantly, DTI measures have also been found to be reproducible across the lifespan, thus providing reliable biomarkers of typical development and ageing [237].

Measures of FA have been shown to increase through childhood and adolescence in prefrontal white matter regions, the corpus callosum, internal capsule, basal ganglia and thalamic pathways, and also the visual pathways [238-240]. These areas are important for cognitive functions such as attention and memory, as well as motor skills [238]. There is also evidence to suggest that this typical developmental trajectory of WM maturation may be altered in individuals with neurodevelopmental conditions, such as ASD, which affect the aforementioned cognitive and (in some cases) motor functions [241].

Eluvathingal et al (2007) found three differing patterns of FA change across 6 pathways in typically developing individuals, which possibly reflect different developmental stages of white matter maturation [242]. The first pattern is characterized by an increase in FA with a concomitant decrease in other measures of diffusivity. This spatial pattern includes the frontoparietal segment of bilateral arcuate; left inferior-longitudinal fasciculus; left inferior-fronto-occipital fasciculus; and bilateral uncinate [242]. These regions also subserve higher cognitive functions such as attention, language and visuo-spatial processing [243-246], which develop through childhood and adolescence [247]. In contrast, the second pattern was characterised by significant decreases in mean, transverse and axial diffusivity with age - without a significant change in FA. This pattern was specific to brain regions that support basic speech, motor control, and auditory and visual skills that develop prior to the elaboration of higher cognitive functions [247], namely, the fronto-temporal segment of left arcuate (speech), bilateral temporo-parietal segments of the arcuate (auditory spatial/audio-visual processing), and the left cortico-spinal tract (voluntary/fine motor movements) [243-246]. These areas likely undergo substantial maturation before the age of 6 years. Finally, a third pattern identified included regions/tracts with no significant age-related change, and only included the primary somatosensory pathway [242]. It has thus been suggested that the somatosensory pathway matures in early neonatal development and then does not change significantly during postnatal development [248].

Lebel et al., (2008) found that the developmental growth curves for measures of FA across tracts were best characterized by an exponential curve, as can be seen in Figure 1.4. Echoing the aforementioned studies, their results suggest a hierarchical pattern of maturation, whereby areas with fronto-temporal connections mature more slowly than others. The splenium and genu of the corpus callosum also develop relatively rapidly (i.e. increased slope of the growth curve), while the cingulum and uncinate fasciculus exhibit more gradual maturation. The fornix, a rudimentary tract involved in memory and emotion, showed no age-related change in the investigated age-group (5-30 years) [249]. In a similar study of over 800 subjects investigated over the lifespan

(11-90 years), Kochunov et al., (2012) showed that the 'age of peak FA' for the major WM tracts were in the 3rd and 4th decades of life (23.1–39.4 years), with the single exception of the cortico-spinal tract, which is known to reach maturity in early childhood [250]. The authors concluded that associative cerebral WM tracts responsible for higher order, multimodal cognitive functions, reach their peak FA values later in life than earlier maturing motor and sensory tracts [251].

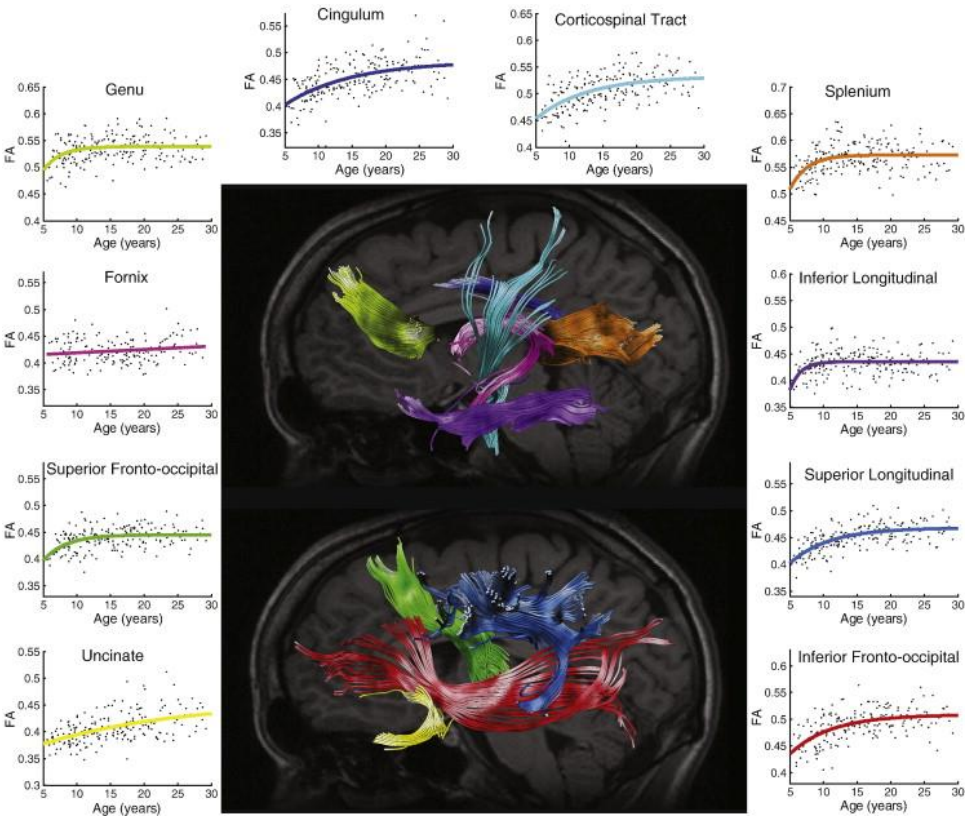

FIGURE 1.4

Age-related fractional anisotropy increases measured by tractography in 202 individuals across 10 tracts, between the ages of 5 and 30. Taken from, Lebel, C et al., 2008. NeuroImage, 40, 1044-1055 [249].

Relating WM differences to cognitive ability, Qiu et al., (2008) found that on top of age related changes, measures of FA were positively associated with reading ability in a number of brain regions [252]. Changes in FA thus likely reflect numerous biological processes that occur during development and sub-serve different cognitive functions. Notably, these changes in white matter may be activity-dependent as well as age-dependent.

1.2.3. Brain Development In ASD

One of the most consistent neurobiological findings in ASD is the observation of an altered neurodevelopmental trajectory of brain maturation relative to typically developing controls. We know that in typical development, the brain slowly matures throughout childhood and adolescence, reaching maximum size between the ages of 10 and 12 [253]. Furthermore, as outlined above, some maturational processes also continue through adolescence and adulthood [212, 213, 217, 220, 230]. The autistic brain is of normal size at birth [254], but undergoes a different pattern of development during early childhood, which involves a short lived period of accelerated growth between 2 and 5 years of age [210, 255, 256], with maximum size reached by 3-5 years [257], followed by decelerated growth throughout later childhood [258-261]. Despite this early 'over-growth' of the brain, individuals with ASD have an average or slightly smaller brain volume once adolescence is reached [210], with no significant differences in total brain volume typically observed during adulthood [262, 263]. This anomalous pattern has been established using traditional head circumference measurements [148] as well as MRI studies of brain volume [151, 264, 265].

However, it has also been shown that not all brain regions are equally affected by the atypical developmental trajectory. Rather, much of the increased brain volume is attributable to enlarged cerebral white matter (Figure 1.5 b), in particular, the dorsolateral and medial frontal and occipital areas [149, 257, 266-268]. In addition, regions associated with the severely affected 'social brain', are found to be abnormally large in autistic children [254]. For

example, neuroanatomical differences have been related to phenotypic domains of ASD in (1) the fronto-striatal system, which has been linked to repetitive and stereotyped behaviours [269, 270]; (2) fronto-temporal regions and the amygdala, which are associated with abnormalities in socio-emotional processing [271-273]; and (3) speech and language regions that may underlie impaired social communication and language [274]. Thus, it seems likely that the abnormal pattern of brain growth is associated with deficits in neural organization, and the connectivity of key brain systems.

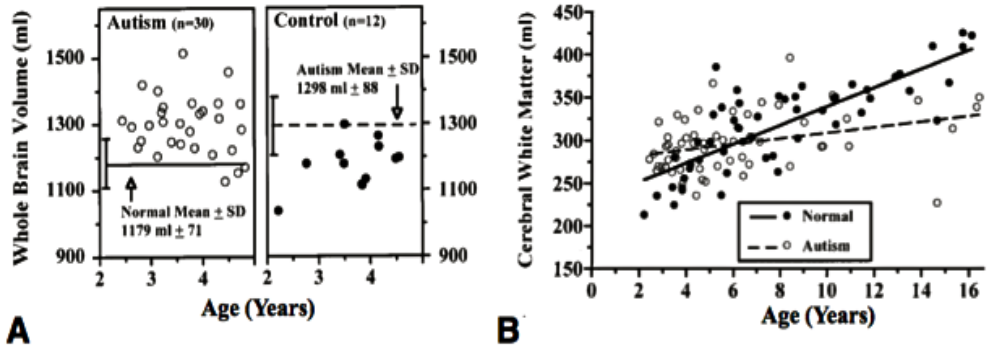

FIGURE 1.5

Comparison of (a) Whole brain volume and (b) Cerebral white-matter volume in healthy and autistic toddlers, children and adolescents. (Taken from: [259]).

As previously mentioned, a core feature of typical brain development is the hierarchical progression from lower-order somatosensory and visual cortices, to more complex higher-order association cortices, which integrate more developed functions. Thus differences in the underlying developmental time-course that characterises ASD would limit the subsequent development of higher-order association areas and their corresponding abilities. This is particularly acute in relation to social behaviour, language and communication, which are atypical in ASD, as the development of each depends greatly on the capacity of earlier developing components [275, 276]. The development of these

high-order association cortices and their neural circuits is thus of great interest when studying ASD.

GREY-MATTER DEVELOPMENT IN ASD

A number of studies have reported differences in cortical thickness in both children [277, 278] and adults with ASD [279, 280], and these differences seem most prominent in frontal, parietal and temporal lobes. In typical development the frontal and parietal lobes reach their maximum size in early adolescence, while temporal lobe grey matter plateaus later on in adolescence [212]. In children with ASD, cortical thickness in these regions mainly seems to be increased relative to typically developing individuals [277, 278], while decreased cortical thickness in ASD is typically observed in adult populations [279, 280]. Thus, differences in cortical thickness in ASD seem to be reflective of the particular age group under investigation. This has prompted investigation into age-related differences, for example, Wallace et al., (2010) investigated differences in cortical thickness in individuals with ASD and age matched typically developing controls ranging from 12 to 24 years of age, and noted extensive temporal and parietal cortical thinning in ASD while controlling for age [281]. Comparable findings of age-related cortical thinning were reported between 10 and 60 years of age [282], and 20 to 55 years of age [283]. While these studies were important first steps in determining age-dependent differences in a specific aspect of cortical pathology implicated in ASD, a major limitation to these studies is that only linear age effects were examined. As previously mentioned, grey matter (at least in typical development) is known to follow a more complex, inverted U-shaped developmental trajectory.

WHITE-MATTER DEVELOPMENT IN ASD

Increasingly, studies employing DT-MRI also investigate the micro-structural properties of white matter in ASD. Whilst some heterogeneity of results exists, these studies of individuals with ASD have generally reported abnormal development of WM micro-structural integrity in infancy and early

childhood [150, 284], which can persist into adulthood [285]. Recently, Wolf et al. (2012) demonstrated that the developmental rate of FA between 6 and 24 months is slower in infants who later received a diagnosis of ASD, despite these infants having increased FA at 6 months [150]. Other research has similarly shown increased FA in toddlers and very young children [284, 286]. The dominant pattern throughout childhood however is lower mean FA in ASD [241, 287]. This has been reported in the uncinate fasciculus, arcuate fasciculus, inferior fronto-occipital fasciculus, right cingulum and corpus callosum [288] as well as in the short-range association fibres of the frontal lobe [289]. The pattern of white matter abnormality becomes more diffuse and possibly even normalizes with age, with reduced FA values being reported in older children and adolescents in association fibres [287, 290-292], brainstem and cerebellar tracts [287, 293, 294] and projection fibres [287, 290, 293].

These deficits in white matter maturation are also often interpreted as deficits in brain connectivity in ASD, which will be discussed in the following section.

1.2.4. CONNECTIVITY

Abnormalities in brain connectivity have been suggested to underlie a number of neurological and psychiatric disorders (e.g. schizophrenia), and ASD in particular [295-299]. For example, ASD has also been termed a 'developmental disconnection syndrome' [276], which is associated with differences in anatomical [284, 300, 301], functional and electrophysiological connectivity (detected in electroencephalography) [302-305]. It is likely that abnormal brain connectivity is related to the atypical pattern of brain growth seen in ASD, as it coincides with the period when synaptogenesis, dendritic growth and myelination are at their peak [306] (reviewed in [210] and [307]). Furthermore, the development of higher-order cortical systems, such as the frontal and temporal lobes, is built upon the earlier maturation of lower-order systems (such as the somatosensory and visual cortices [217]). Thus, any developmental anomalies during development may not only affect the neural architecture and wiring of local brain regions, but also their global circuitry [210]. Concurrently, abnormalities in ASD are spatially distributed across large-scale cortical networks, and patterns of disrupted connectivity have been documented in both adults [301, 308] and children [301, 302, 309-311].

It has also been suggested that there is long distance under-connectivity and local over-connectivity of the frontal cortex [210, 312]. For example, reductions in long-range functional connectivity have been identified mostly in frontal regions using several functional MRI (fMRI) models, including executive functioning [313], working memory for faces [314] and facial-affect processing [315, 316]. Local over-connectivity has been a more difficult concept to prove, as it is difficult to measure using conventional neuroimaging approaches [317]. Nevertheless, these fMRI findings complement genetic findings, which also point towards atypical connectivity on the cellular level (that is, defective synapse formation and transmission), and may affect more widespread connectivity on the 'systems level' in ASD.

However, the differences in functional connectivity observed in ASD are hard to interpret and also rely on the specific experimental paradigm used to

stimulate the brain. Moreover, functional connectivity might be up-/down-regulated by different neurotransmitter systems, which will be discussed in the following paragraphs.

1.2.5. BRAIN CHEMISTRY

There is evidence to suggest that neurochemistry is altered in ASD. Despite the large heterogeneity in the neurochemical make-up between individuals, three main neurotransmitter systems have been associated with ASD. These are (1) the GABAergic system, (2) the glutamatergic system, and (3) the serotonergic system.

GAMMA-AMINOBUTIRIC ACID (GABA) NEUROTRANSMISSION

GABA plays differing roles throughout development. Early in life it acts as a trophic factor, affecting events from synaptogenesis and differentiation, to synaptic elimination and cell death - presumably, through both synaptic and non-synaptic mechanisms [318]. Naturally, developmental alterations in the GABAergic system would thus affect the brain in a variety of ways. In contrast, GABA acts as the primary inhibitory neurotransmitter in the mature brain. Crucially, GABAergic signalling and transmission at both of these levels (as a developmental signal and inhibitory molecule) has been implicated in ASD [319].

There is evidence that GABAergic inhibition is down-regulated in ASD [320], and might affect the excitation to inhibition (im)balance. GABA is synthesised from the excitatory neurotransmitter glutamate via the action of glutamate decarboxylase (GAD) enzymes, of which there are two main isoforms, GAD65 and GAD67. Both of these are reduced in the ASD brain [321], which may in turn reduce GABA synthesis and GABAergic inhibition. There are also a number of studies that point to deficits at the level of the GABA-A receptor in ASD. Examples include, (1) significant reductions in GABA-A receptor-binding in ASD [322, 323]; (2) reductions in GABA-A receptor density, as shown by a single-photon emission computed tomography study of both children and adults with ASD [324]; (3) and finally, reduced levels of GABA-A and GABA-A receptor-binding in limbic brain regions as visualised by positron emission tomography (PET) in adults with ASD [325]. Although direct quantification of GABA in vivo poses methodological challenges, recent work

has succeeded in showing reduced GABA concentration in the ASD brain. For example, an MR spectroscopy study of children (between 2 and 12 years of age) showed significantly reduced GABA concentration in the frontal cortex [326].

To sum up, the general picture in ASD is one of reduced GABAergic synaptic transmission, and region-specific variations in GABA-related receptor expression and transmitter concentration, all of which may contribute to or mediate the symptomatology of the condition. However, further studies are needed to clarify these findings and establish their biological origins. The differences observed could be of genetic or epigenetic origin, as several genetic variants strongly associated with ASD are amongst a number of genes involved with GABA receptors (for example, GABRB3 and GABRB5 [327]), or GABA synthesis (for example, GAD65 and GAD67 [328]).

GLUTAMATERGIC NEUROTRANSMISSION

Glutamate is the most abundant excitatory neurotransmitter in the human brain. In ASD, glutamatergic neurotransmission appears to be enhanced, as opposed to GABAergic neurotransmission, which is reduced [320]. This heightened excitatory neurotransmission forms the basis of the 'hyperglutamatergic hypothesis of autism' [320] and is well documented. One study recorded significantly increased serum glutamate levels in ASD, with serum glutamate levels being positively correlated with (ADI) sub-domain scores in subjects [329]. Increased glutamate receptor and transporter mRNA has been documented post-mortem [330], whilst in vivo magnetic resonance spectroscopy (MRS) studies have shown both increased mGluR expression [331] and increased glutamate concentration in the hippocampus and the auditory cortex of individuals with ASD [332, 333]. Studies investigating the opposing effects of GABA and glutamate have implicated excitatory / inhibitory imbalance as an important aspect of the pathophysiology underlying ASD [181]. This theory is compatible with the raised incidence of seizures and sensory hypersensitivity in ASD [181].

THE SEROTONERGIC SYSTEM

Serotonin (5-HT) is distinct from other neurotransmitters, such as GABA and glutamate, in that its biosynthesis requires a dietary source of the amino acid L-tryptophan (TRP). This of interest as it means that serotonin can to a degree, be impacted by extrinsic factors, such as diet and medication. TRP is able to cross the blood–brain barrier and enter the nervous system, where it is converted to 5-HT through a two-step metabolic pathway.

Abnormalities in the serotonergic system have consistently been implicated in ASD and it has been suggested that a significant fraction of individuals with ASD may suffer from 'hyperserotonemia' [334, 335]. For instance, a significantly greater concentration of 5-HT was observed in the blood of children with ASD and their relatives [336]. In vivo neuroimaging studies examining ASD, have brought to light some significant differences, in serotonin synthesis, receptor binding, and in the number of 5-HT transporters, especially in brain regions relevant to social communication (for example, the cingulate cortices) [337-339]. Of particular interest to brain development, is the difference in whole brain serotonin synthesis capacity in ASD compared to typical development. Chugani et al., found that up until the age of 5, in typical development, the capacity to synthesise serotonin was 200% greater than in adults. In contrast, serotonin synthesis capacity in children with ASD increased steadily from the age of 2 through to 15 years to values 1.5 times higher than the typical adult values [339]. This suggests that humans undergo a period of elevated brain serotonin synthesis during early childhood, and that this developmental process is disrupted in ASD at a time when ASD symptoms are most prominent.

Evidence for serotonergic involvement also comes from the genetic association between ASD and genetic polymorphisms for serotonin synthesis, transporters and receptors [340-342]. Furthermore, serotonin has a wide range of behavioural functions, most of which are affected in ASD, these include, circadian rhythms, the sleep-wake cycle, appetite, aggression, sexual behaviour, sensorimotor reactivity, pain sensitivity, and learning [343].

1.3. CONTROVERSIES SURROUNDING ASD

In recent years several controversial claims have been made about the possible causes of autism, in particular relating to the measles, mumps, and rubella (MMR) vaccination. Despite intense media attention, there is no scientific evidence to support these hypotheses [344-347]. Additionally, the 1998 Lancet paper by Wakefield et al., that first highlighted vaccines as a cause for concern was retracted in 2010 as the data presented were found to be fraudulent [348].

CHAPTER. 2

NEUROIMAGING TECHNIQUES

2.1. NUCLEAR MAGNETIC RESONANCE

In this section I will be referring to nuclear magnetic resonance (NMR). NMR enables us to observe the quantum mechanical properties of atomic nuclei. Many scientific techniques exploit this property of NMR in various forms. The phenomenon was first described in the 1938 when it was used to measure the magnetic moment of lithium isotopes in molecular beams [349], a feat for which physicist Isidor Rabi became Nobel Laureate. Eight years later, Felix Bloch and Edward Purcell began to develop the technique for use in liquids and solids, work which went on to earn them a Nobel Prize in Physics in 1952 [350]. Over half a century later, NMR is now widely utilized in medical physics, referred to clinically, as magnetic resonance imaging (MRI) - the 'N' was dropped to alleviate people's fears associated with the word 'nuclear'.

2.2. How Does It Work In Biological Tissues?

MRI is a medical imaging technique that enables us to investigate the anatomy and physiology of bodily tissues in vivo. MRI scanners use strong magnetic fields and radio waves to form images of the body without exposure to ionizing radiation. MRI works principally by creating a magnetic field around a subject, thus forcing changes to occur on a nuclear level within the body. Signal measurement in conventional MRI is based upon the detection of signal from protons in water and fat. Although MR is sensitive to many nuclei, the use of water is advantageous in biological tissues due to its abundance and adaptable properties depending on its biochemical environment [351].

Each water molecule contains two hydrogen nuclei ($^1\mathrm{H}$), or protons (which carry a positive electrical charge). All atomic nuclei possess a fundamental property referred to as spin angular momentum, or simply spin. Nuclei with a net spin (i.e. those that have an odd number of protons and/or neutrons) tend to orient themselves like tiny bar magnets, and the spin of each of these nuclei have an associated magnetic moment, μ.

In biological tissues the orientation of these magnetic moments is randomly distributed and no net magnetic moment is achieved. When placed in an external magnetic field $(\vec{B}_0)$, however, the spins independently align either with or against the direction of the $\vec{B}_0$ (defined later as the z axis). A small majority of spins align in the parallel (lower energy) direction compared to the (higher energy) anti-parallel direction, as illustrated below (Figure 2.1). This occurs because less energy is required to align with, rather than against the current. The summation of these new 'non-random' magnetic moments results in a net magnetic moment, $\vec{M}$ in the direction of $\vec{B}_0$. Although this change is tiny, it is sufficient for measurement.

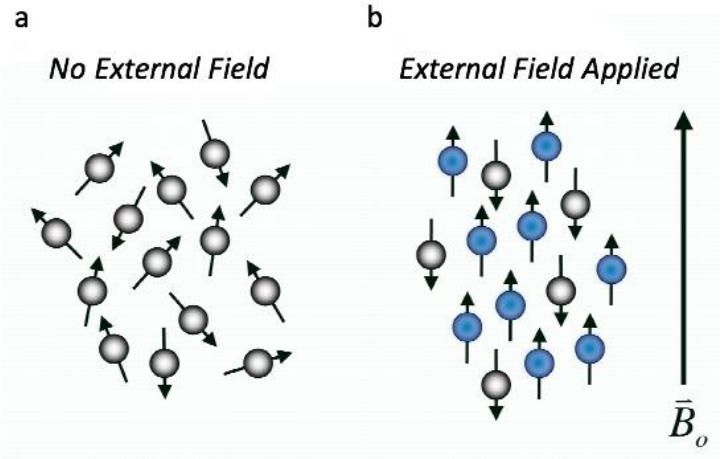

FIGURE 2.1

In free space (a), individual magnetic moments are randomly oriented and yield no net magnetic moment. When placed in an external magnetic field, however, a small majority of the moments align in parallel with the field rather than aligning in the anti-parallel orientation (b). This yields a net magnetic moment in the direction of the applied field and is the basis of the MR signal. Spins oriented parallel with the applied field are shown in blue.

In addition to aligning with or against the external magnetic field, the spinning nuclei behave like tiny gyroscopes and precess around the axis of the applied field with a specific frequency, named the Larmor frequency (ω) (Equation 1 and Figure 2.2).

$$\omega_0 = \gamma B_0$$

EQUATION 1

Larmor Frequency. Where $\boldsymbol{\omega_0}$ is the angular frequency, $\boldsymbol{\gamma}$ is a constant called the gyromagnetic ratio, and B_0 is the external magnetic field.

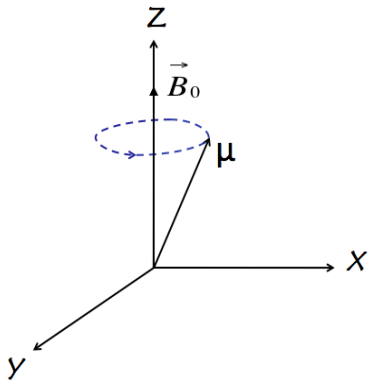

FIGURE 2.2

Nuclear spins placed in an external magnetic field will begin to precess about the axis of the applied field at the specific Larmor frequency, which can be calculated from the above equation (Equation 1).

In order to generate a detectable NMR signal we need to quantify and manipulate these nuclear properties within the biological tissue of interest. Firstly we need to know the number of spins aligning in the parallel, N_P, versus anti-parallel, N_{ap} direction. This can be predicted by the Boltzmann distribution (Equation 2), which is affected by Boltzmann's constant K_B, the magnitude of the applied field, $|B_0|$, and the absolute temperature in Kelvin, K.

$$\frac{N_p}{N_{ap}} = e^{\frac{-\gamma\hbar|B_0|}{2\pi k_B K}}$$

EQUATION 2

Boltzmann Distribution. Where $\hbar$ is Planck's constant, $|B_0|$ is the magnitude of the external magnetic field, K_B is Boltzmann's constant (1.3805 x 10¡23 J/Kelvin) and K is the absolute temperature (in Kelvin).

As mentioned previously the summation of individual magnetic moments produces a net magnetization $\vec{M}$ in the direction of the external magnetic field. This can be estimated according to Curie's law (Equation 3), which states that the strength of the resulting net magnetic moment is related to the number of excess spins (spin density), the gyromagnetic ratio of the nuclei, the strength of the applied magnetic field and the temperature.

$$\vec{M} = N.\gamma^2\left(\frac{\hbar}{2\pi}\right)\frac{\vec{B}_0}{4k_BK}$$

EQUATION 3

Curie's law, where *N* is the number of excess parallel spins.

At equilibrium $\vec{M}$ exists only along the direction of the applied magnetic field, $\vec{B}_0$. This is because the x and y components of $\vec{M}$ cancel each other out as a result of the random distribution in their orientation, or phase as it is generally termed. To generate a detectable NMR signal, it is necessary to manipulate $\vec{M}$ by rotating a portion of it into the xy-plane. This is achieved by perturbing the spin system with a small radio frequency pulse (RF) or magnetic field $\left(\vec{B}_1\right)$ at the Larmor frequency, applied perpendicular to $\vec{B}_0$.

This causes the magnetization to be rotated away from the direction of the main field into the transverse xy plane, before it slowly returns to equilibrium, or relaxes. How far the net magnetisation deviates from $\vec{B}_0$ depends on the specified flip angle of the pulse [352] (depicted in Figure 2.3, below). To describe this, Bloch formulated two relaxation time constants, T_1 and T_2 (Equation 4) [353].

$$\frac{d\vec{M}}{dt} = \vec{M}\times\gamma\vec{B} - \frac{M_x\vec{x} + M_y\vec{y}}{T_2} - \frac{(M_z - M_0)\vec{z}}{T_1}$$

EQUATION 4

Bloch's equation and describing relaxation times, T_1 and T_2

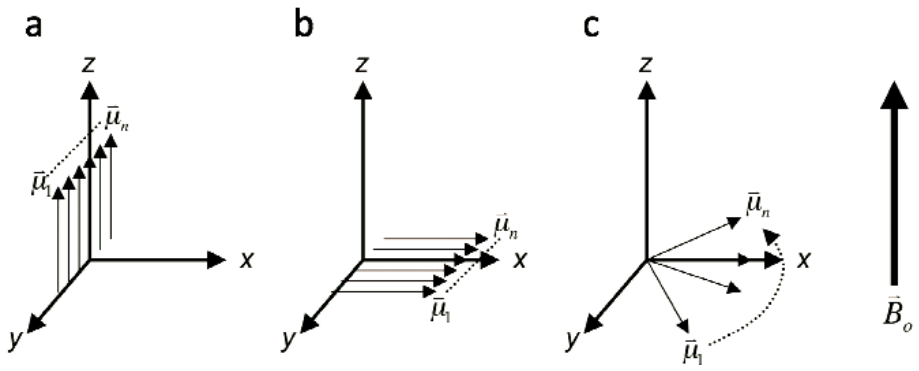

FIGURE 2.3

A 90° RF pulse tips the equilibrium magnetization (a) into the xy-plane (b). Over time, the individual magnetic moments fan-out in the xy-plane (c) resulting in a decrease in the magnitude of the transverse magnetization.

LONGITUDINAL AND TRANSVERSE RELAXATION TIME: T_1 AND T_2?

These energy changes that are produced by a sequence of RF pulses tell us about the tissue environment. T_1 and T_2 are the fundamental quantum mechanical parameters in MR that reflect what is happening in a tissue in relation to the binding and mobility of the nuclei within it.

T_1 refers to the longitudinal (also known as spin-lattice) relaxation time. In the context of the 90° RF pulse depicted in Figure 2.3, that is the time constant for the decay of longitudinal magnetization along the z axis (along $\vec{B}_0$) and following excitation [354] to re-establish equilibrium longitudinal magnetization, M_0. The basic physical mechanism of T_1 relaxation is the exchange of energy between individual spins and the surrounding lattice; hence, the name spin-lattice relaxation. As such, the structure and molecular composition of the surrounding lattice influences the rate of T_1 relaxation.

In water for example, the recovery of equilibrium longitudinal magnetization is influenced by fluctuating magnetic fields at the proton resonance frequency caused by neighbouring atoms; in this case, due to its proximity, the dominant source of fluctuation is usually the adjacent proton on the water molecule. Because of the mobility of water molecules, there tends to be little energy at the proton resonance frequency, resulting in slow T_1 relaxation. Similarly, molecules in lipids are influenced by their adjacent protons. However, because protons in lipids are less mobile, there tends to be more energy at the proton resonance frequency, resulting in a shorter T_1 for lipids. Thus, T_1 arises from molecular motion and is directly influenced by the local biophysical structure and biochemical environment, rendering it a good indicator of change associated with disease, or other biological processes.

The same properties are true of T_1. However, T_2 refers to the transverse relaxation time. In the context of a 90° RF pulse depicted (Figure 2.3), this is the time constant for the decay of transverse magnetization along the xy a plane (perpendicular to $\vec{B}_0$). Immediately following the application of an RF pulse, energy is transferred between neighbouring spins, as they share similar precessional frequencies and phase; this is often termed spin-spin relaxation, in contrast to spin-lattice in T_1. Inter- and intra- molecular interactions bring about small deviations in the precessional frequencies, resulting in the irreversible loss of phase coherence. This results in the 'fanning out' of individual magnetic moments in the xy-plane, as depicted in Figure 2.3.

In addition to these spin-spin interactions, macroscopic heterogeneities in the static magnetic field $(\vec{B}_0)$ also induce dephasing of the transverse magnetization. When these effects are considered, the transverse magnetization relaxes with an overall time constant T_2^*.

Differences in the T_1 and T_2 values between the various body tissues (e.g. grey and white matter), along with proton density (PD), form the main bases of image contrast in MRI. By varying the amount of T_1 and T_2 relaxation allowed before acquiring the MR signal, different contrast between biological tissues can be obtained (see Figure 2.4).

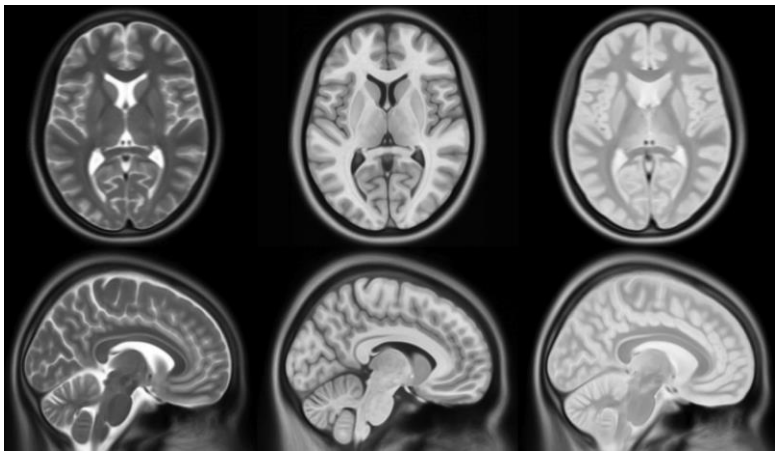

FIGURE 2.4

An example of the three primary image contrasts, (from left to right) T_2 weighted, T_1 weighted and proton density weighted (PD) images (modified from [355]).

CLINICAL AND RESEARCH IMPLICATIONS

The differences between T_1 and T_2 relaxation rates can be manipulated in our imaging sequence to generate anatomically meaningful contrast. Consequently, it is easy to see how abnormalities could be detected qualitatively using these measures in a clinical setting. From a research perspective however, comparing T_1 and T_2 weighted images quantitatively between groups is problematic. T_1 and T_2 weighted images do not provide physically meaningful values, only anatomically meaningful contrast. The direct estimation of quantitative T_1 and T_2 values (T_1 and T_2 mapping) has gradually become a more feasible option, with shortened acquisition times but retention of high spatial resolution (for example, [356]). The difference between conventionally acquired qualitative images and quantitative T_1 and T_2 maps is illustrated in Figure 2.4, and discussed in the subsequent section.

By convention, the analysis of neuroanatomy from T_1 and T_2 weighted MR images for research typically necessitates identification and

compartmentalization of substructures within the brain. Traditionally this has required the manual tracing of regions of interest by human experts. This approach is straightforward for subcortical structures with defined boundaries, but becomes subjective for cortex, where the boundaries between structures are more variable between individuals.

More recently automated computer programs have been used to gauge regional volumes (e.g. [357-360]). Automated techniques, such as voxel-based morphometry (VBM) (for example, [262, 268]), take a probabilistic approach towards the problem. Images are probabilistically classified into tissue "types" or "classes" (usually white matter, grey matter and cerebro-spinal fluid) and statistical analyses are carried out on the resulting probabilistic images (voxel-by-voxel), after aligning the images to a common space (so that each voxel in one image approximately corresponds with the same voxel in another image). These probability images are then interpreted in terms of either tissue concentration or volume.

By compartmentalizing the brain into different regions such as the major lobes, cortical regions, and sub-cortical nuclei, studies have generated growth curves, establishing norms as to how different brain regions change with age (for a notable example see, [212]). As well as showing age effects, notable gender differences have also been described using this methodology [221, 361, 362].

2.3. Quantitative Magnetic Resonance Imaging

In conventionally acquired MR images, the grey-scale value of each pixel is a consequence of differences in tissue properties such as M_0, T_1 and T_2, as well as extrinsic parameters such as scanner hardware characteristics. As such, the intensity at any pixel can only be interpreted qualitatively. In the absence of a quantitative metric for absolute interpretation of signal intensities (independent of scanner hardware and sequences) it is difficult to perform comparisons of MR images across groups of subjects or longitudinally in the same subject. To compare information on tissue structure and function in a quantitative manner, we first need to uncouple contributions of different contrast mechanisms from the MR signal. Quantitative MRI refers to the measurement of biophysical parameters through uncoupling the different contrast mechanisms that contribute to the overall MR signal [363].

Unlike qualitative images, in which the signal intensity of a particular tissue can vary depending on the acquisition technique used, the intrinsic T1 and T2 values obtained from quantitative maps (such as those shown in Figure 2.5 c and d), should not vary between scanners (as long as the data is acquired from the same subject at the same field strength). As a result of this stability, quantitative imaging can be used to measure fundamental tissue parameters and investigate deviations of these values from the norm. Thus, revealing underlying differences in tissue structure and composition.

Quantitative MR imaging can be divided into two categories; physical parameters and MR-intrinsic parameters. Physical parameters are those that exist independently of an applied magnetic field, such as diffusion. MR-intrinsic parameters on the other hand, require an induced magnetic field, such as T1, T2, and magnetization transfer. The following sections outline the principles and uses of these exemplified MRI parameters.

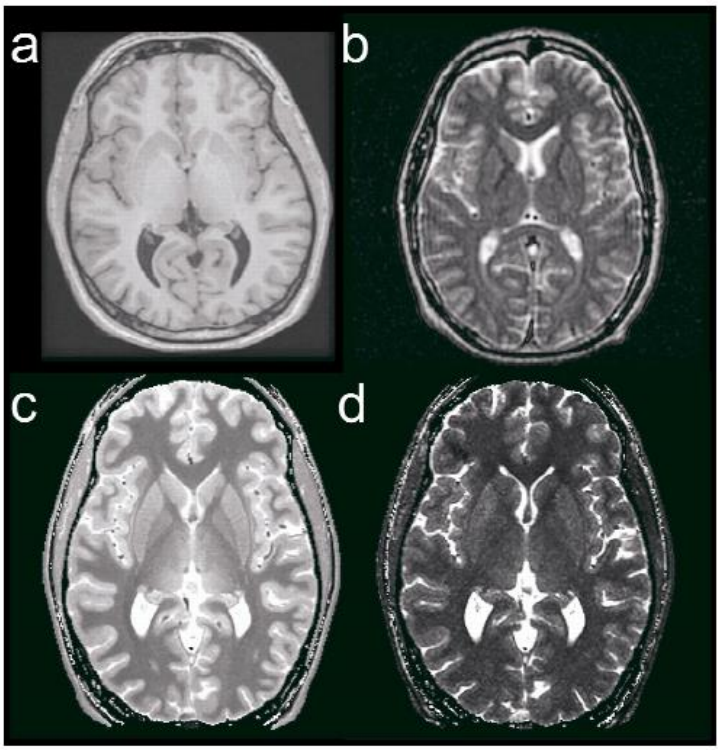

FIGURE 2.5

Typical MR images are qualitative in nature where the grey-scale value in each pixel is 'weighted' by an intrinsic MR parameter. Examples of T_1 and T_2 weighted images are shown in (a) and (b), respectively. In a quantitative image, or 'map', the grey-scale value in each voxel is indicative of the MR parameter (e.g. T_1 relaxation time in seconds). Examples of T_1 and T_2 maps are shown in (c) and (d), respectively.

DIFFUSION WEIGHTED IMAGING:

Image contrast in conventional MRI is derived from differences in the physical properties of water $^{1}\mathrm{H}$ in different chemical environments. For example, the T_2 relaxation time constant for water $^{1}\mathrm{H}$ in cerebrospinal fluid (CSF) is relatively large, in comparison to adjacent white matter, so CSF appears brighter. Similarly, the differing T_2 values for white and grey matter deliver clinically valuable contrast between these tissues on T_2-weighted images. Diffusion weighted MRI sequences are also sensitive to water, but more specifically the displacement or diffusion of water within biological tissues. The rate of diffusion at a fixed temperature follows Einstein's equation:

$$< \mathrm{r}^2 > = 6\mathrm{Dt}$$

EQUATION 5

Einstein's equation describing diffusion.

Where the mean squared displacement of the water molecules $< r^2 >$ is directly proportional to the time (t), and D is the diffusion coefficient for the substance being measured [364]. In free water at (37°C), D is approximately $3 \times 10^{-3}\ \mathrm{mm}^2 s^{-/1}$ [365]. In biological tissues however, the diffusion of water is influenced by a number of factors, such as the presence of cell membranes, myelin, vessel walls, intracellular filaments, and thus the diffusion coefficient in brain tissue is smaller than that in free water. Nevertheless, the simple Einstein model is retained, but the value calculated is termed the apparent diffusion coefficient (ADC), in acknowledgement of the confounds involved [366].

Using this concept, in diffusion MRI the physical location of water molecules is encoded through the application of a diffusion-sensitizing magnetic field gradient pulse, followed by a second pulse which is typically applied ~50ms later to measure the displacement of water molecules [351]. For motionless molecules the effect of these two gradient pulses cancels out, but the motion of water molecules between these pulses leads to attenuation in

the received MR signal and this attenuation can be used to estimate the diffusion coefficient of water in the imaged tissue. With the two images collected at two different b-values (a summary scalar that describes the degree of diffusion weighting with which an image is collected), it is possible to calculate the diffusion coefficient [367].

ISOTROPIC VS ANISOTROPIC DIFFUSIVITY:

In tissues that are physically homogenous, the diffusion of water is equally restricted (or unrestricted) in all directions, and so occurs with no directional bias. This type of diffusivity is isotropic in nature and occurs within the grey matter of sub-cortical nuclei or the cerebral cortex for example. In other tissues, for example white matter, the displacement of water in the direction parallel to myelinated axons is greater than water displacement in the perpendicular direction. This is because the axonal membranes and myelin bilayers here pose a physical barrier to water moving in the perpendicular direction (Figure 2.6.a). Consequently the ADC measured along the direction of these fibres is always greater than that measured in the perpendicular direction.

THE DIFFUSION TENSOR:

The ADC alone is insufficient for the characterisation of diffusion in the presence of anisotropy [368] as the ADC is sensitive to the direction of water displacement. In diffusion MRI the signal is typically sensitized to water displacement in a chosen direction, which would in turn mean that the ADC values within any given voxel would differ according to this pre-chosen direction. As diffusion is a three-dimensional process, an accurate mathematical description of anisotropic water displacement would have to be measured in three–dimensional space [369].

To account for this, the diffusion tensor is used. The diffusion tensor relies on at least 6 diffusion weighted images (with b-values greater than 0), collected along non-collinear directions, as well as one non-diffusion weighted image (b=0) [364], and is represented by the tensor D:

$$D = \begin{bmatrix} \mathbf{D_{xx}} & D_{xy} & D_{xz} \\ D_{yx} & \mathbf{D_{yy}} & D_{yz} \\ D_{zx} & D_{zy} & \mathbf{D_{zz}} \end{bmatrix}$$

This represents the molecular mobility along (in bold) and between each direction in the x, y and z planes. The diffusion tensor can also be visualized as a diffusion ellipsoid where the 3D geometrical profile of water displacement can be calculated from the diffusion coefficient values (eigenvalues) and orientations (eigenvectors) of its three principle axes [370]. Where diffusion is isotropic, this ellipsoid corresponds to a perfect sphere, as the ADC is equal in all directions. However, in the case of anisotropic diffusion, as would be expected along an axonal bundle in the brain, the shape becomes an elongated ellipsoid (see figure 2.6.b).

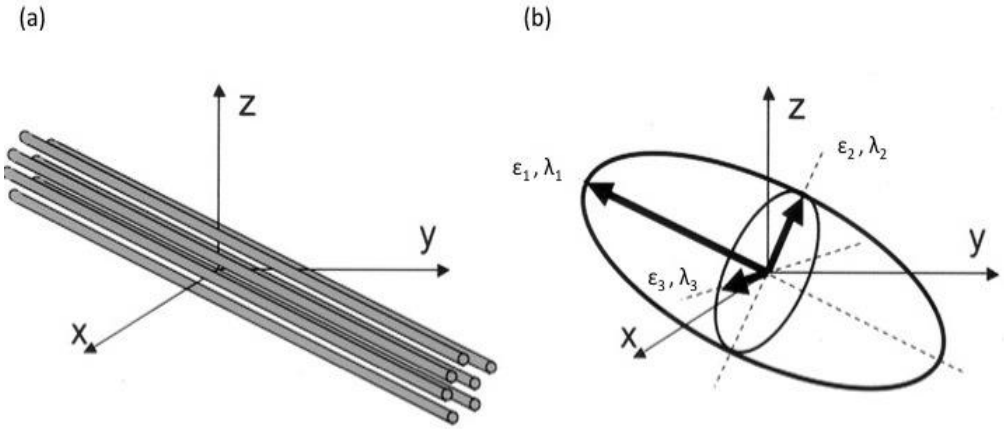

FIGURE 2.6

Tracts have an arbitrary orientation with respect to scanner geometry (x, y, z axes) and impose directional dependence (anisotropy) on diffusion measurements [371].

3D diffusivity modelled as an ellipsoid whose orientation is characterized by three eigenvectors (ϵ1, ϵ2, ϵ3) and whose shape is characterized by three eigenvalues (λ1, λ2, λ3). The eigenvectors represent the major, medium, and minor principle axes of the ellipsoid, and the eigenvalues represent the diffusivities in these three directions, respectively [371].

By means of this tensor model, a variety of quantitative parameters can be estimated, such as mean diffusivity (MD) and fractional anisotropy (FA). MD is a quantitative index describing the average diffusivity of water within an observed voxel. This is rotationally invariable and is calculated as the mean of the three eigenvalues, shown in Equation 6.

Calculation of FA is slightly more complex (Equation 7) and indicates the degree of directionality of intra-voxel diffusivity. In this case, λ1, λ2 and λ3 correspond to the three eigenvalues in order of magnitude. A high FA value indicates that one of these eigenvalues is greater than the other two, giving information about the microstructural organization of the imaged tissue. FA generally reduces in pathological tissue (in the presence of oedema or demyelination for example), rendering it a commonly used index (albeit indirect) of microstructural integrity [370].

$$MD = \frac{Tr(\bar{D})}{3} = \frac{\lambda_1+\lambda_2+\lambda_3}{3} \qquad FA = \frac{\sqrt{3((\lambda_1-\bar{\lambda})^2+(\lambda_2-\bar{\lambda})^2+(\lambda_3-\bar{\lambda})^2)}}{\sqrt{2(\lambda_1^2+\lambda_2^2+\lambda_3^2)}}$$

EQUATION 6 AND 7

Equations describing mean diffusivity and fractional anisotropy, respectively.

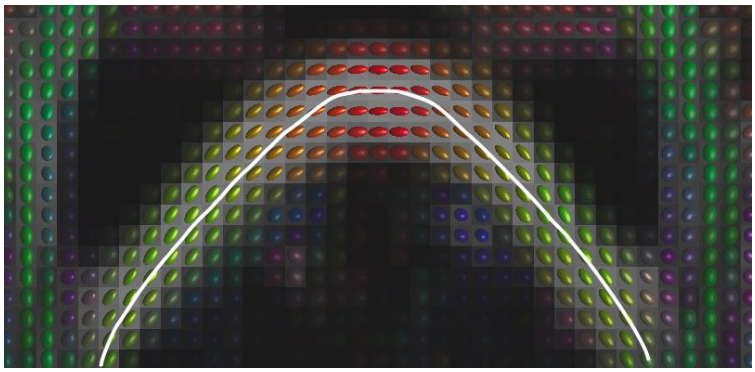

FIGURE 2.7

Deterministic tractography - The preferred diffusion directions are indicated by the elongated axes of diffusion ellipsoids. The colour of the ellipsoid codes for the preferred diffusion direction where red denotes left-right, green denotes back-front and blue indicates up-down (out of the image plane). The white line shows the streamline obtained by connecting voxels based on their preferred directions. (Image modified from the Humanconnectome.org ([372]).

A major adjunct of DTI has been that of fibre tractography, the technique of tracing and visually reconstructing white matter brain pathways in vivo, using diffusion data [373]. This method relies on estimates of anisotropy in white matter, and assumes rather simply, that only one fibre occurs per unit voxel. A software algorithm is set to follow the primary eigenvector (corresponding to the dominant eigenvalue, λ1) from a "seed" voxel or location to its neighbouring voxel, producing a "streamline" between the two locations. This continues along the preferred direction until a new anisotropic measurement is reached and a new direction is taken [374]. This streamline technique is also referred to as deterministic tractography, depicted in Figure 2.7. The resultant streamline is thought to represent the dominant fibre bundle along a pathway in the brain and has been shown to generate anatomically feasible results (as seen in Figure 2.8).

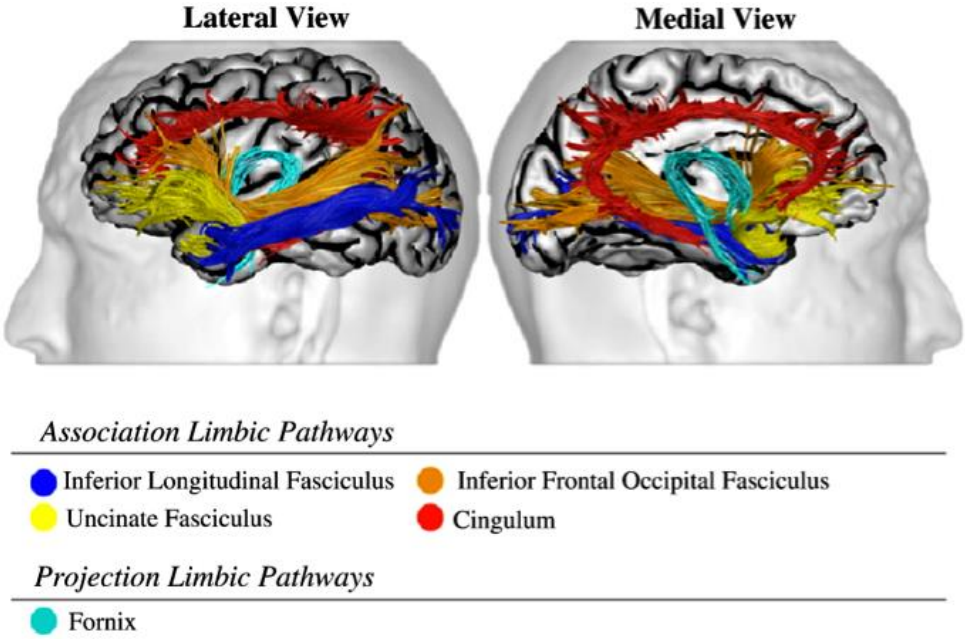

FIGURE 2.8

The results of a study using deterministic tractography for outlining the known, major white matter bundles of the limbic system [375].

However, white matter fibres in the brain have a diameter of approximately 1µm versus a voxel which visualizes around 1-2 mm. Consequently, thousands of axons are captured in one voxel and not all of these axons are oriented in the same direction, posing a problem for accurate streamline propagation. Further, the likelihood of error in streamline propagation increases with the length of the streamline [374]. To overcome this problem, the probabilistic method estimates the most likely fibre orientations by repeatedly sampling the orientation distribution in a voxel (commonly using Monte Carlo Markov Chain methods [376]). The result of this is an image that represents thousands of streamlines from a seed region, and can be thresholded to only include those streamlines that occur consistently.

QUANTITATIVE RELAXOMETRY OF T_1 AND T_2

Quantitative T_1 and T_2 relaxometry can facilitate improved characterization of tissue, by enhancing image contrast (as shown in Figure 2.5), and providing a more direct link between the observed signal changes that affect T_1 and T_2 and the micro-anatomical alterations under investigation. Further, it permits more meaningful interpretation of signal intensity, allowing comparison between groups and across longitudinal time points.

Quantitative relaxometry has been shown to have a number of clinical applications; one of the most successful illustrations is in the context of multiple sclerosis (MS) [377-379]. Characterized by focal lesions of demyelination in the brain and spinal cord, MS is both a neurodegenerative and inflammatory disorder, where the lesions are dispersed over space and time. From an MR perspective, MS pathology results in complex focal signal decay, which benefits from quantitative characterization [378, 380]. The utility of quantitative T_1 and T_2 mapping has also been demonstrated in epilepsy, Alzheimer's disease [381, 382], and normal ageing [223, 306, 383].

T_1 MAPPING:

The gold standard for T_1 mapping is the inversion recovery experiment (Figure 2.9.a), which involves inverting the longitudinal magnetization and measuring the MR signal as it recovers (according to the Bloch equation described earlier).

Two RF pulses are applied in sequence (at 180° and then 90°). The first pulse inverts longitudinal magnetization, and as it recovers (T_1 relaxation time) the second pulse is applied, tipping the recovered longitudinal magnetization into the transverse plane. This sequence is repeated several times, with varying $\boldsymbol{T_I}$, to sample the recovery curve (Figure 2.9.b). The experimental data can be fitted to an equation relating the measured signal to the total possible or available signal at full longitudinal magnetization (Equation 8).

While inversion recovery offers accurate and precise T_1 measurements, the technique is too time consuming to be used in clinical practice; it requires long inter-pulse time (TR) and acquires only one phase encode step per TR. To speed up the acquisition, several modifications have been proposed (for example, [356, 384-386])

$$S(\boldsymbol{T_I}) = S_0(1 - 2e^{-\frac{\mathbf{TI}}{T1}})$$

EQUATION 8

Equation describing the measurement of T1 using an inversion recovery experiment. Where S(TI) represents the signal measured at a certain TI and S0 represents the signal that would be available at full longitudinal magnetization (i.e. TI = 0).

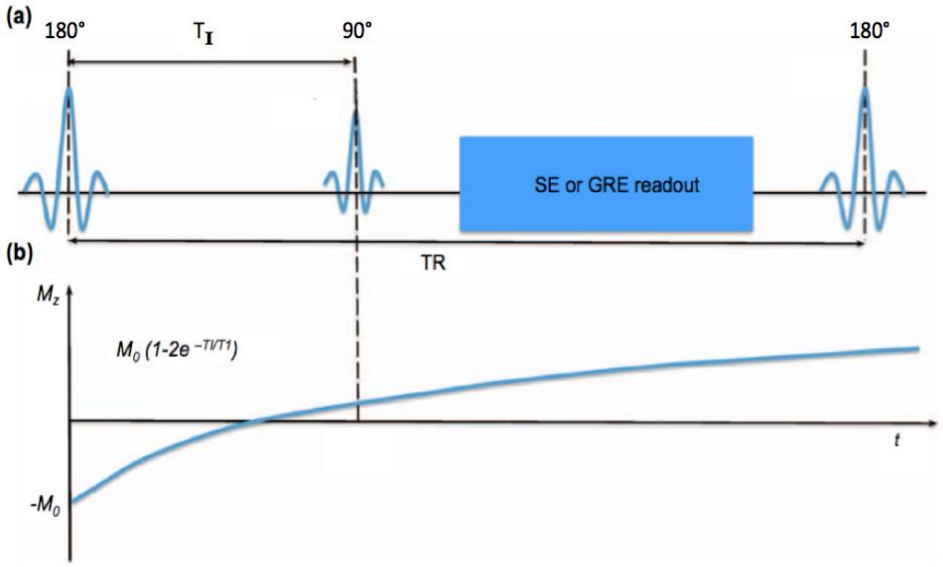

FIGURE 2.9

A schematic representation of an inversion recovery sequence (a) and (b) the T1 relaxation curve. The relaxation curve is sampled at several TI times, and then fitted to a single exponential. (Figure modified from Cheng et al., 2012 [363])

T_2 MAPPING:

To quantify T_2 relaxation a Carr-Purcell-Meiboom-Gill (CPMG) sequence is typically used (depicted in Figure 2.10). This involves taking measurements at different echo times in an "echo train", which allows us to sample the T_2 decay curve. A 90° pulse is applied followed by a succession of 180° refocusing pulses, the signal is then measured between these refocusing pulses. Whilst the number of echoes sampled is determined by the imaging time available, it is necessary to acquire at least two echoes. More echoes increase the accuracy and precision of single component T_2 mapping.

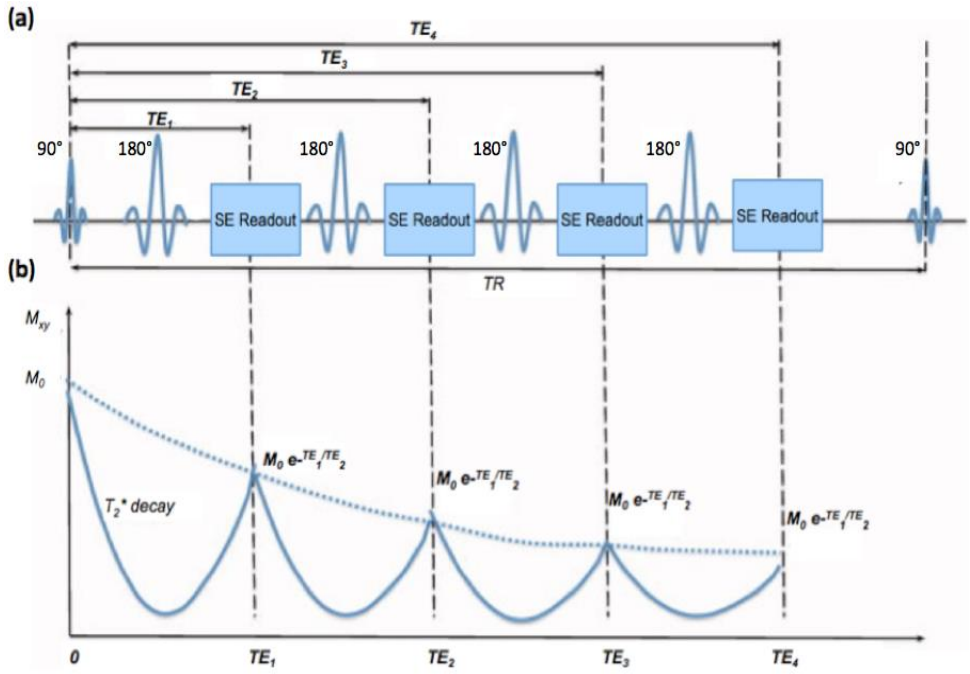

FIGURE 2.10

A schematic representation of a multi-echo spin echo sequence (a) and the resultant T_2 relaxation curve (b) (Figure modified from Cheng et al., 2012 [363]).

As two or more TEs are sampled, T_2 may be derived by using the relationship between signal, TE, and T_2 relaxation rate as described in Equation 9. Images

with varying TE may then be fit to this equation on a pixel-by-pixel basis to generate a parametric T_2 relaxation map. As with T_1 mapping, several accelerated methods have been proposed for T_2 measurement (for example, [387-389]).

$$S(t) = S_0 e^{-TE/T2}$$

EQUATION 9

Deriving T_2 from a multi-echo experiment.

MAGNETIZATION TRANSFER IMAGING:

T_1 and T_2 mapping also form the foundations of an advanced quantitative MR technique termed quantitative magnetization transfer (qMT). In qMT, the T_1 and T_2 parameters are entered into a two-pool tissue model [390] which relies on the transfer of magnetization from relatively restricted protons that are bound to macromolecular structures in a semisolid pool (e.g., myelin and cell membranes) to more mobile protons in free water [391]. The quantity of magnetic transfer differs between tissue types and is larger where more macromolecular structure is present [392]. The exchange of magnetization between these pools is measured in qMT experiments [393].

Magnetization transfer experiments involve the application of customised RF pulses in order to selectively saturate (or partially saturate) the short T_2 semisolid spins without any direct effect on the liquid components [394, 395]. If the magnetization state of the semisolid pool is selectively altered (e.g., saturated), exchange of magnetization between the pools produces an observable change in the magnetization of the liquid spins. This yields the magnetization transfer ratio (MTR), which describes the proportion of MRI signal reduction that is attributed to the presence of macromolecules.

A number of clinical applications have been suggested for qMT, notably it has been used to examine the evolution of acute demyelinating lesions in multiple sclerosis [390]. Within white matter, the motionally restricted myelin-

bound water disproportionally contributes to this measure, rendering MTR an index of myelination [396]. Further discussion of qMT is beyond the scope of this chapter, but it is important to remark that this contrast mechanism provides an additional motivation for T_1 and T_2 quantification.

MT has also been used for indirect evaluation of myelin (described later in Chapter 8: Preface).

CHAPTER. **3**

AIMS AND OBJECTIVES

PRIMARY HYPOTHESES

There is strong evidence to suggest that brain development is altered in ASD and that the atypical neurodevelopmental trajectory affects brain structure and functioning. Whilst neuroimaging studies have employed a multitude of different methodologies to examine different aspects of cortical anatomy and connectivity, no comprehensive study has yet been carried out with the aim of integrating findings across imaging modalities. Therefore, the present work conducts an investigation into brain development in ASD, utilizing a variety of MR metrics that are applied to the sample of children and adolescents with ASD and matched controls.

Overall, the proposed work aims to test the primary hypothesis (1) that children and adolescents with ASD present an altered pattern of brain development relative to typically developing (TD) controls, and the subsidiary hypothesis (2) that the observed differences will occur in brain regions consistent with observed clinical deficits and symptoms.

Based on the heterogeneity of previous findings, no hypothesis with regards to the direction of these differences is made.

TO TEST THE PRIMARY HYPOTHESIS, I PLAN TO CONDUCT FIVE INVESTIGATIONS:

STUDY 1. THE EFFECT OF AGE, DIAGNOSIS, AND THEIR INTERACTION ON VERTEX-BASED MEASURES OF CORTICAL THICKNESS AND SURFACE AREA IN AUTISM SPECTRUM DISORDER

In this cross-sectional neuroimaging study using high-resolution structural T1-weighted volumetric imaging, I aim to examine age-related differences in cortical thickness and surface area, between childhood and young adulthood. For this study the dataset described in Chapter 4, is combined with a compatible pre-existing dataset of adults.

Hypotheses:

a) There are age-related differences in cortical thickness and surface area between individuals with ASD and TD controls.

b)
   i. The neurodevelopmental trajectories of cortical thickness and surface area are atypical in individuals with ASD relative to typically developing controls.
   ii. The observed differences occur in brain regions consistent with observed clinical deficits and symptoms of ASD.

STUDY 2. AGE RELATED DIFFERENCES IN WHITE MATTER DIFFUSION MEASURES IN AUTISM SPECTRUM DISORDER

In this cross-sectional study utilizing Diffusion Tensor Imaging, I aim to examine age-related differences in white matter microstructure across childhood and adolescence in ASD and typical development.

Hypotheses

a) Trajectories for measures of diffusion are atypical in children and adolescents with ASD relative to typically developing controls.
b) Differences in diffusion measures are correlated with the severity of autistic symptoms (ADI-R and ADOS).

STUDY 3. GYRIFICATION AND ITS RELATIONSHIP WITH STRUCTURAL CONNECTIVITY IN AUTISM SPECTRUM DISORDER

In this multi-modal neuroimaging study, I aim to examine age-related differences in DTI diffusion measures alongside vertex-based measures of gyrification (lGI), across childhood and adolescence in ASD versus typical development.

Hypotheses:

a) There are differences in gyrification between individuals with ASD and TD controls.
b) There are significant group differences, and group by age interactions on measures of gyrification.
    i. The observed differences occur in brain regions consistent with observed clinical deficits and symptoms of ASD.
    ii. The same regions show significant group differences and group by age interactions for DTI diffusion measures.
c) Gyrification is correlated with underlying white matter architecture as measured by DTI (across groups).

STUDY 4. MAPPING WHITE MATTER DEVELOPMENT IN CHILDREN AND ADOLESCENTS WITH AUTISM SPECTRUM DISORDER

In this cross-sectional study utilizing the quantitative relaxation mapping technique mcDESPOT (multicomponent driven equilibrium single pulse observation of T_1 & T_2), I aim to examine age-related differences in myelin content across childhood and adolescence in ASD versus typical development.

Hypotheses:

a) Myelin water fraction (MWF) trajectories are altered in children and adolescents with ASD relative to typically developing controls.
b) Differences in MWF correlate with diagnostic measures (ADI-R and ADOS).

STUDY 5. LONGITUDINAL DIFFERENCES IN WHITE MATTER MATURATION IN CHILDREN AND ADOLESCENTS WITH AUTISM SPECTRUM DISORDER

In this longitudinal study utilizing mcDESPOT, I aim to examine trajectories of MWF, quantitative T_1 (qT_1), and quantitative T_2 (qT_2) across childhood and adolescence in ASD versus typical development.

Hypotheses:

a)

i. The developmental trajectory for myelin is altered in children and adolescents with ASD relative to typically developing controls.
ii. The observed differences will occur in brain regions consistent with observed clinical deficits and symptoms of ASD.

b)

i. The developmental trajectory for qT1 and qT2 is altered in children and adolescents with ASD relative to typically developing controls.
ii. The observed differences will occur in brain regions consistent with observed clinical deficits and symptoms of ASD.

CHAPTER. **4**

GENERAL METHODOLOGY

4.1. OVERVIEW OF DATA COLLECTION

The following chapter outlines the general protocol employed in data collection across a three-year period. More specific descriptions of individual samples and protocols are provided for each of my planned investigations in empirical chapters 5 – 9.

The planned protocol combined cross-sectional and longitudinal investigations of ASD, including a large cohort of children and adolescents with ASD and typically developing controls, followed up longitudinally for three years. Participants were required to return for testing on three occasions (at twelve monthly intervals). On each visit individuals received a structural MRI scan and underwent a battery of neuropsychological testing.

4.2. Recruitment And Sample Selection

I began recruitment in June 2010 as the sole researcher working on the study full-time. Males with ASD and typically developing (TD) male controls between the ages of 6 and 16 were invited to take part in the study. Individuals with autism were recruited through advertisements in the National Autistic Society's quarterly magazine and on the website (http://www.autism.org.uk/about-autism/our-publications/your-autism-magazine.aspx) [397]. Specialist schools and the Parents of children who had taken part in previous research at the Institute of Psychiatry were also contacted in order to pool participants. TD controls were recruited using advertisements placed in the local press and via email circulars within King's College, London. Parents who responded to the adverts were screened over the telephone in order to establish whether their children met inclusion criteria (for advertisement wording and screening questionnaires refer to the Appendix). A total of 251 individuals (195 ASD : 56 TD controls) went through the screening process, of which 157 (101 ASD : 49 TD controls) were eligible for participation.

All eligible participants were male, right handed, and native English speakers. Participants were excluded for the following reasons:

- Pre-existing medical conditions or complications (e.g. head trauma, epilepsy).
- Other psychiatric conditions or learning disability (IQ<70).
- Physical illness or medication, which would affect brain function.
- Chromosomal abnormalities associated with ASD (e.g. fragile X, Tuberous Sclerosis, VCFS).
- Any of the MRI contraindications on the standard South London and Maudsley NHS Trust radiography screening questionnaire (e.g. metallic implants, claustrophobia etc.).

For the autistic group, inclusion in the study was based on a clinical diagnosis of ASD as assessed by ICD-10 criteria. The ASD group underwent a second round of screening, whereby the clinical diagnosis was confirmed using the

Autism Diagnostic Interview and the Autism Diagnostic Observation Schedule. Participants were allowed to fall below threshold by a maximum of one point in a single ADI domain. Where subjects fell below threshold in the ADOS (by a maximum of a single point in a single domain) the ADI score was used as the deciding factor for inclusion. Participants that did not meet these criteria were excluded from the study. A total of 71 Autism Diagnostic Interviews were carried out, during which 9 individuals were excluded for not reaching the diagnostic thresholds.

Scanning for the study commenced in November 2010 and continued to December 2013. Myself, and one fellow researcher were responsible for gathering all the data for this study. Subject demographics through three study time-points are outlined briefly in Table 4.1. More specific subject demographics are provided alongside empirical investigations in Chapters 5 – 9.

TABLE 4.1. Subject demographics through three study time-points

	ASD INDIVIDUALS			CONTROLS			SIGNIFICANCE		
	YEAR 1	YEAR 2	YEAR 3	YEAR 1	YEAR 2	YEAR 3	YEAR 1	YEAR 2	YEAR 3
	n = 62	*n = 51*	*n = 35*	*n = 47*	*n = 33*	*n = 23*	*p-value*		
Age (SD) in Years	11.5 (3.2)	12.5 (3.3)	13.5 (3.4)	12.0 (2.9)	13.4 (2.5)	14.2 (2.6)	0.36	0.28	0.34
IQ (SD)	99 (15.5)		110.5 (10.0)	111 (13.3)		114.3 (8.7)	0.42		0.13
ADI Sub-Domain Scores									
Reciprocal Social Interaction	18.6 (4.9)	-	-	-	-	-	-	-	-
Communication	16.1 (4.1)	-	-	-	-	-	-	-	-
Restricted, Repetitive, and Stereotyped Behaviour	5.6 (2.5)	-	-	-	-	-	-	-	-
ADOS Sub-Domain Scores									
Communication	4.0 (1.7)	-	3.6 (1.6)	-	-	-	-	-	-
Reciprocal Social Interaction	8.7 (3.1)	-	6.6 (2.8)	-	-	-	-	-	-
Imagination/Creativity	0.9 (0.8)	-	0.5 (0.8)	-	-	-	-	-	-
Stereotyped Behaviours and Restricted Interests	1.9 (1.9)	-	1.3 (1.5)	-	-	-	-	-	-

4.3. Accounting For Attrition

A high rate of attrition was expected over the three-year time frame of the study. Naturally, attrition due to post-recruitment changes in health or medication could not be avoided. However, measures were taken to lessen the impact of participants leaving the study. For example, recruitment of participants continued to twenty per cent greater than the initial target sample size. Additionally, we also provided incentives to improve chances of participants returning for testing whilst they remained eligible. For instance, participants received an image of their own brain and a brief (non-clinical) report of their performance on some of the tests. In addition to payment and reimbursement of costs, the children also received small thank you gifts, in the form of t-shirts or mugs with the study logo (pictured below).

Attrition from the study was also expected due to test-related stress. MRI scanners are very noisy and individuals with ASD are known to be particularly sensitive to noise. The unfamiliar environment and enclosed space can also be daunting. In addition, participants are required to lie still under these conditions for almost one hour to be scanned. To buffer these stress-factors, maximum care was taken to create a comfortable environment and to allow participants to familiarise themselves with their surroundings. Participants

were sent photos and videos of what to expect, and 'mock' scanning visits were arranged in which participants could acquaint themselves and their parents with the scanner size and associated sounds (using an MR simulator identical to the actual scanner in all ways but omitting the magnetic field). To minimise discomfort during the MRI, participants were advised to bring their favourite DVD, which could be played to them during the structural scan. Last, they were allowed contact with the radiographers via a microphone and headphones throughout the scan, and were given a 'panic' button to alert the clinical research team and stop the scan at any time. Despite these efforts a small number of participants who were uncomfortable in the scanner were excluded from the study at this stage.

4.4. Diagnostic And Screening Measures

Individual clinical and neuropsychological tests were selected based on the following criteria:

- Brief administration time.
- Previously investigated in relation to autistic and/or typical neurodevelopment.
- Relevant to impairments hypothesised to occur in ASD.
- Reliable and published scoring criteria.

Selected tests included the Edinburgh Handedness inventory [398] (repeated yearly), the Autism Diagnostic Interview [52] (acquired at time-point 1), the Autism Diagnostic Observation Schedule [53] (acquired at time-points 1 and 3) and the Wechsler Abbreviated Scale of Intelligence [399] (acquired at time-points 1 and 3). The uses of these tests are outlined below.

- **The Edinburgh Handedness inventory** [398] is a brief questionnaire assessing an individuals handedness preference for 10 everyday tasks, such as, writing, drawing, or opening containers. For each task, the individual is required to respond as to whether they are right-handed, left-handed, or have no preference (ambidextrous). Individuals are

then scored 1 for every 'right-handed' response, 0 for every 'left-handed' response, and 0.5 if they indicate no preference. An overall score of ten therefore indicates a strong right-handed preference whilst an overall score of zero indicates a strong left-handed preference.

- **The Autism Diagnostic Interview** (ADI) is a standardised interview of established reliability and validity [52], focusing primarily on the key diagnostic characteristics of ASD (outlined previously, in Chapter 1. Section 1.1.5.)
- **The Autism Diagnostic Observation Schedule** (ADOS) [53] is a semi-structured assessment of communication, social interaction and behaviour (outlined previously, in Chapter 1. Section 1.1.5.).
- **The Wechsler Abbreviated Scale of Intelligence** (WASI) provides a brief and reliable measure of intelligence in clinical, educational and research settings [399].

4.5. MRI Data Acquisition:

All imaging for this study was performed on the latest generation General Electric 3T MR system. The high signal-to-noise ratio (SNR) associated with 3T, compared with lower field strengths (i.e. 1.5T) allowed us to make use of advanced parallel imaging techniques, facilitating shorter acquisitions whilst maintaining high image quality. All subjects were imaged using volumetric, DTI, and optimized mcDESPOT protocols (mcDESPOT is described later in Chapters 8 and 9). Specific imaging protocols and parameters were as follows:

Volumetric Imaging: For volumetric analysis of white and grey matter, a high-resolution sagittally-oriented 3D (IR-SPGR) image was acquired. Nominal acquisition parameters: Field of View (FOV): 24cm2 x 16cm, matrix; 240 x 240 x 160, echo time (TE) / repetition time (TR)/ inversion time (TI); 2.1ms/6ms/450ms and flip angle: 35°. Total imaging time was less than 7 minutes.

Diffusion Tensor Imaging: Diffusion tensor MRI scans were acquired with a spin-echo echo-planar imaging (SE-EPI) double refocused sequence providing whole head coverage with isotropic image resolution (2.4 x 2.4 x 2.4) mm^3. 32 diffusion-weighted volumes with different non-collinear diffusion directions with b-factor 1300 sec/mm^2 and 6 non-diffusion-weighted volumes; 60 slices; no slice gap; TE 104.5 msec; TR 20 R-R intervals; 128 x 128 acquisition matrix; FOV = 30.7 cm2; peripherally gated (Parameters compatible with [270]). Total acquisition time for complete DTI data was less than 12 minutes.

mcDESPOT: Sagittally-oriented whole-brain mcDESPOT data was acquired with a spatial resolution of (1.5 x 1.5 x 1.5) mm^3. Nominal acquisition parameters: FOV 24cm2 x 15cm, matrix: 160 x 160 x 100, TE/TR = 4.1ms/9.3ms for SPGR, and 4.2ms/8.4ms for SSFP. Eight SPGR flip angles (3,4,5,6,7,9,11,13 and 18) degrees and 8 SSFP flip angles (11,14,19,24,28, 34, 41, 51 and 67) degrees were acquired. To correct for potential main magnetic (B0) field in-homogeneities, a second set of 8 SSFP images were acquired with the same flip angles but an offset RF phase-cycling pattern. Further, to account for transmit magnetic field (B1) or flip angle in-homogeneities, a reduced resolution IR-SPGR image was acquired and DESPOT1-HIFI processing was used to calibrate the B1 field. To reduce acquisition times, parallel imaging, with a reduction factor of 2 and partial k-space acquisitions were used. Total acquisition time for the mcDESPOT data was less than 15 minutes.

The preparation and processing of all imaging data is outlined alongside specific study methodologies in Chapters 5 – 9.

4.6. Consent And Confidentiality

As many of the recruited participants were under the age of consent, informed consent was obtained from their parents or guardians. Prior to obtaining consent, volunteers and their parents received an information sheet detailing all aspects of the study, including aims, methodology and benefits. The study was explained in detail to parents and guardians during telephone

screening and on the initial visit. At all points throughout recruitment and the study, participants and their parents were encouraged to ask questions and air any concerns that might help clarify their role as a participant. All volunteers were reminded that participation in the study was voluntary and that they were free to withdraw at any time. Participants and their families were given a copy of the information sheet and signed consent form to keep (examples of these, and all other documents given to participants, are included in the Appendix).

All data for this study were stored anonymously. Clinical and questionnaire information collected for this study is safely stored in a locked filing cabinet and only those involve in the study will have access to these files. Participant's names were never stored along with their brain images or other medical information. Contact details were kept separately in an encrypted computer file and on paper in a locked filing cabinet.

According to clinical requirements, a trained radiologist reviewed all structural brain images acquired in the study. In the event of any significant abnormalities the participant's GP was contacted.

4.7. Ethical Approval

Ethical approval for this study was sought and approved in March 2010 (REC reference number: 09/H-720/115).

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EMPIRICAL FINDINGS

GUIDANCE FOR READERS

In this section I present 5 chapters, written in paper format.

The first (chapter 5) is a research article that was published in the Journal of Neural Transmission earlier this year. This paper, for which I hold joint first authorship with Dr Christine Ecker, is the result of combining our two compatible datasets. This combination of datasets allowed us to examine age-related differences in cortical morphology in a cross-sectional sample spanning from 7 to 25 years of age. In all subsequent chapters I describe findings that are based on the examination of the child/adolescent dataset described in chapter 4. These chapters are written in the conventional order: Abstract, Introduction, Materials and methods, Results, Discussion, Supplementary material (where applicable) and References.

In chapters 8 and 9, I describe the use of a novel multi-component relaxometry, myelin-mapping technique entitled mcDESPOT. Therefore, to aid the reader, I have included a brief preface to chapter 8, with the aim of summarising early myelin mapping endeavours and elucidating my rationale for using mcDESPOT.

CHAPTER. **5**

THE EFFECT OF AGE, DIAGNOSIS, AND THEIR INTERACTION ON VERTEX-BASE MEASURES OF CORTICAL THICKNESS AND SURFACE AREA IN AUTISM SPECTRUM DISORDER

J Neural Transm (2014) 121:1157–1170
DOI 10.1007/s00702-014-1207-1

The effect of age, diagnosis, and their interaction on vertex-based measures of cortical thickness and surface area in autism spectrum disorder

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Received: 29 November 2013 / Accepted: 26 March 2014 / Published online: 22 April 2014

Abstract Autism spectrum disorder (ASD) is a lifelong neurodevelopmental condition that is accompanied by an atypical development of brain maturation. So far, brain development has mainly been studied during early childhood in ASD, and using measures of total or lobular brain volume. However, cortical volumetric measures are a product of two distinct biological neuroanatomical features, cortical thickness, and surface area, which most likely also have different neurodevelopmental trajectories in ASD. Here, we therefore examined age-related differences in cortical thickness and surface area in a cross-sectional sample of 77 male individuals with ASD ranging from 7 to 25 years of age, and 77 male neurotypical controls matched for age and FSIQ. Surface-based measures were analyzed using a general linear model (GLM) including linear, quadratic, and cubic age terms, as well as their interactions with the main effect of group. When controlling for the effects of age, individuals with ASD had spatially distributed 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. We also observed significant group × age interactions for both measures. However, while cortical thickness was best predicted by a quadratic age term, the neurodevelopmental trajectory for measures of surface area was mostly linear. Our findings suggest that ASD is accompanied by age-related and region-specific reductions in cortical thickness and surface area during childhood and early adulthood. Thus, differences in the neurodevelopmental trajectory of maturation for both measures need to be taken into account when interpreting between-group differences overall.

Keywords Autism · Neuroanatomy · Cortical thickness · Surface area · Neurodevelopment

C. Ecker and A. Shahidiani contributed equally to the manuscript.

Electronic supplementary material The online version of this article (doi:10.1007/s00702-014-1207-1) contains supplementary material, which is available to authorized users.

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Introduction

Autism spectrum disorder (ASD) is a lifelong neurodevelopmental condition characterized by a triad of symptoms in (1) impaired social communication, (2) deficits in social reciprocity, and (3) repetitive and stereotypic behavior (Wing 1997). These 'core' symptoms of ASD typically manifest before the age of 2 years and are accompanied by developmental differences in brain anatomy and connectivity (Geschwind and Levitt 2007; Amaral et al. 2008; Ecker et al. 2013b). However, the wider neural systems underlying ASD are complex and involve abnormalities in multiple, spatially distributed neurocognitive

systems (Ecker et al. 2012). This makes the neuroanatomy of ASD inherently difficult to describe in vivo.

So far, most existing structural neuroimaging studies in ASD have examined either children or adults with ASD in order to determine the set of brain regions that differ most from typical controls in terms of their neuroanatomy. For example, neuroanatomical differences have consistently been reported in (1) the fronto-striatal system, which has been linked to repetitive and stereotyped behaviors (Langen et al. 2007, 2011); (2) fronto-temporal regions and the amygdala, which are associated with abnormalities in socio-emotional processing (Waiter et al. 2004; Schumann et al. 2009; Nordahl et al. 2012); and (3) speech and language regions that may underlie impaired social communication and language (Redcay and Courchesne 2008). However, to date, there are few neuroimaging studies examining neuroanatomy in ASD across different age-groups in order to characterize age-dependent anatomical variations between individuals with ASD and healthy controls. Moreover, the few existing studies mainly focus on cortical development through early infancy into late childhood. For instance, there is evidence to suggest that the brain is enlarged in toddlers with ASD (between 2 and 5 years of age) (Courchesne and Pierce 2005; Schumann et al. 2010; Hazlett et al. 2011), while no significant differences in total brain volume are typically observed during late childhood or adulthood (McAlonan et al. 2005; Hardan et al. 2009). However, it is largely unknown how the brain develops during adolescence in ASD, and there is thus a need for neuroimaging studies to also examine neurodevelopmental trajectories from late childhood into adulthood.

Furthermore, previous structural neuroimaging studies in ASD have largely used volume-based approaches and focused on measures of global or regional differences in brain volume (e.g., Waiter et al. 2004; Carper and Courchesne 2005; McAlonan et al. 2005). However, cortical volume is by definition a product of cortical thickness and surface area and can therefore be fractionated into different morphometric sub-components, which have distinct genetic determinants (Panizzon et al. 2009), contrasting phylogeny (Rakic 1995), and differing developmental trajectories (Raznahan et al. 2011). It is therefore important to examine cortical thickness and surface area in isolation in order to better understand the neurobiological mechanisms associated with brain abnormalities in ASD.

Differences in cortical thickness have been reported in children (Hardan et al. 2006; Mak-Fan et al. 2011) and adults with ASD (Hyde et al. 2010; Ecker et al. 2013a), and seem most prominent in temporal, parietal, and frontal lobes. In children with ASD, cortical thickness in these regions mainly seems to be increased relative to controls, while decreased cortical thickness in ASD is typically observed in adult samples. Thus, the sign of between-group differences in cortical thickness (i.e., increased or decrease) in ASD seems to be dependent on the particular age-group under investigation, which prompted several studies to also examine age-related differences in cortical thickness in ASD. For example, Wallace et al. (2010) investigated differences in cortical thickness in individuals with ASD and matched controls ranging from 12 to 24 years of age, and noted extensive temporal and parietal reductions in cortical thickness in ASD while controlling for age, and a more accelerated age-related decline in cortical thickness over time (Wallace et al. 2010). Similar findings of an accelerated cortical thinning in ASD were reported in individuals ranging from 10 to 60 years (Raznahan et al. 2010) and 20 to 55 years of age (Scheel et al. 2011). While these studies were important first steps in determining age-dependent differences in a specific aspect of cortical pathology implicated in ASD, a major limitation to these studies is that only linear age effects were examined. Studies of typical gray matter maturation suggest that there is considerable regional variation in complexity of the normal developmental trajectory of cortical thickness across the cerebral cortex, including cubic, quadratic, and linear effects (Shaw et al. 2006). As noted previously by Wallace et al. (2010), the cubic and quadratic developmental trajectories (i.e., inverted U-shapes) of gray matter maturation pose a challenge for comparisons across cross-sectional studies as a precocious or delayed maturation in a particular subject group could lead to a significantly positive difference at one age and a negative difference at a different age (Wallace et al. 2010). It is therefore important to examine a variety of statistical models in order to find the model best suited to examine age-related differences in cortical thickness in ASD. Moreover, while the typical neurodevelopmental trajectories are well established for measures of cortical thickness, there is currently no comparable data for vertex-based measures of surface area.

Differences in cortical surface area remain relatively unexplored in ASD, particularly on the local (i.e., vertex) level, and results seem to be in disagreement. Raznahan et al. (2010) reported that there was no main effect of group nor a group × age interaction in lobar-level surface area when comparing individuals with ASD to neurotypical controls ranging from 10 to 60 years (Raznahan et al. 2010). Similarly, Mak-Fan et al. (2011) found no significant between-group differences but reported a significant group × age interaction in occipital lobe surface area in older children with ASD (Mak-Fan et al. 2011). There are also two studies examining vertex-level differences in surface area in ASD. A recent study by Wallace et al. (2013) reported no group differences in vertex-wise estimates of surface area, and no interaction between age and group, in a sample of individuals with ASD and matched

controls ranging from 12 to 24 years of age (Wallace et al. 2013). Also, Doyle-Thomas et al. (2013) investigated a larger age range of individuals with ASD (7–39 years of age), reporting that surface area in the right cingulate was significantly different between groups and decreased more rapidly with age in ASD compared to controls. There is thus a need for replicating these findings in a large and well-characterized sample of individuals with ASD and matched neurotypical controls, and to contrast differences in the neurodevelopmental trajectory of cortical thickness with age-dependent variations in surface area.

Here, we employed a cross-sectional design to examine age-related differences in cortical thickness and surface area in the same group of individuals with ASD and matched healthy controls between 7 and 25 years of age. We examined linear, quadratic, and cubic age effects on both measures in order to find the most 'parsimonious' model (i.e., model with the smallest number of parameters) that allowed us to examine between-group differences in cortical thickness and surface area in ASD in the presence of significant age effects, as well as age × group (age 'by' group) interactions.

Materials and methods

Participants

Seventy-seven (77) right-handed male individuals with ASD and 77 controls aged 7–25 years were recruited by advertisement and subsequently assessed at the Institute of Psychiatry, King's College, London. Both groups were matched for gender (all male), age, full-scale IQ, and handedness (all right-handed). Exclusion criteria for all participants included a history of major psychiatric disorder, head injury, genetic disorder associated with autism (e.g., fragile × syndrome and tuberous sclerosis), or any other medical condition affecting brain function (e.g., epilepsy). We excluded potential participants with a history of substance abuse (including alcohol) and individuals taking antipsychotic medication, mood stabilizers, or benzodiazepines. All participants with ASD were initially assessed according to the International Statistical Classification of Diseases, 10th Revision (ICD-10) research criteria. Diagnosis for individuals with ASD was then confirmed using the Autism Diagnostic Interview–Revised [ADI-R, (Lord et al. 1994)] to ensure that all participants with ASD met the criteria for childhood autism. All cases of ASD reached ADI-R algorithm cutoff values in the three domains of impaired reciprocal social interaction, communication, and repetitive behaviors and stereotyped patterns, although failure to reach cutoff in one of the domains by one point was permitted (see Table 1 for details). Current symptoms were assessed using the Autism Diagnostic Observation Schedule [ADOS, (Lord et al. 1989)], but were not used as inclusion criteria. Overall intellectual ability was assessed using the Wechsler Abbreviated Scale of Intelligence (Wechsler 1999) in all participants. All participants fell within the high-functioning range of the spectrum, defined by a full-scale IQ higher than 70. All participants gave informed written consent in accordance with ethics approval by the National Research Ethics Committee, Suffolk, England.

Table 1 Subjects Demographics

	ASD ($n = 77$)	Control ($n = 77$)
Age, years	17 ± 4 (7–25)	16 ± 4 (8–25)
Full-scale IQ, WASI	107 ± 14 (70–140)	110 ± 10 (84–134)
ADI-R social	19 ± 5 (9–28)	–
ADI-R communication	15 ± 4 (7–24)	–
ADI-R repetitive behavior	9 ± 3 (2–20)	–
ADOS total	9 ± 3 (3–19)	–

Data expressed as mean ± standard deviation (range). There were no significant between-group differences in age or iq, $p < 0.05$ (two tailed)

MRI data acquisition

All participants were scanned at the Centre for Neuroimaging Sciences, Institute of Psychiatry, London, UK, using a 3-T GE Signa System (General-Electric, Milwaukee, WI). High-resolution structural T1-weighted volumetric images were acquired with full-head coverage, 196 contiguous slices (1.1 mm thickness, with 1.09 × 1. 09-mm in-plane resolution), a 256 × 256 × 196 matrix, and a repetition time/echo time (TR/TE) of 7/2.8 ms (flip angle = 20 in., FOV = 28 cm). A (birdcage) head coil was used for radiofrequency transmission and reception. Consistent image quality was ensured by a semiautomated quality control procedure.

Cortical reconstruction using FreeSurfer

All individual T1-weighted scans were initially screened by a radiologist to exclude images with visible clinical abnormalities or large-scale movement artifacts. Scans of insufficient quality were excluded from the analysis (dropout <2 %). The FreeSurfer analysis suite (vFS5.3.0 release, http://surfer.nmr.mgh.harvard.edu/) was used to derive models of the cortical surface in each T1-weighted image. These well-validated and fully automated procedures have been extensively described elsewhere (e.g., Fischl et al. 1999b, a; Dale et al. 1999; Ségonne et al.

2004). In brief, a single filled white matter volume was generated for each hemisphere after intensity normalization, skull stripping, and image segmentation using a connected components algorithm. Then, a surface tessellation was generated for each white matter volume by fitting a deformable template. This resulted in a triangular cortical mesh for gray and white matter surfaces consisting of approximately 150,000 vertices (i.e., points) per hemisphere. Following standard FreeSurfer preprocessing, each reconstructed surface was then visually inspected for reconstruction errors, and images that did not reconstruct correctly (i.e., with visible anatomical abnormalities) were further excluded from the statistical analysis (dropout 5 %).

Measures of cortical thickness were computed as the closest distance from the gray and white matter boundary to the gray matter and cerebrospinal fluid boundary at each vertex on the tessellated surface. For each participant, we also computed mean cortical thickness across the entire brain. Vertex-based estimates of surface area were derived as outlined by (Winkler et al. 2012). Here, the individual's native surface is initially transformed into a spherical representation, which preserves vertex identities (e.g., total numbers) and original areal quantities, and subsequently registered to a common atlas/template. This registration does not change areal quantities but shifts vertex positions to match the template. Finally, areal quantities are transferred to a common grid via areal interpolation. Here, the final amount of 'area' each face receives on the new grid depends on the overlap between the original source face and the target (i.e., common grid) face. In this way, the fixed target surface is redistributed across one of more source faces and can be used as weighting factor to account for inter-individuals differences in surface reconstructions. We also computed total surface area and mean cortical thickness (across both hemispheres) for each participant. To improve the ability to detect population changes, each parameter was smoothed using a 10-mm surface-based smoothing kernel.

Statistical analysis

Statistical analysis was conducted using the SurfStat toolbox (http://www.math.mcgill.ca/keith/surfstat/) for Matlab (R2010b; MathWorks). To determine developmental trajectories at each vertex, we initially tested for linear, cubic, and quadratic age effects on measures of cortical thickness and surface area, in addition to the main effect of group. Here, an F test for nested model comparisons was used at each vertex employing a step-up model selection procedure. Initially, the linear (i.e., most reduced) model was compared to a more complex quadratic model in order to determine whether the addition of a quadratic age effect significantly improved the goodness of fit. If the quadratic model performed significantly better, it was then compared to the full cubic (i.e., most complex) model, which contained a linear, quadratic, and cubic age term. This allowed us to identify the most parsimonious model at each vertex, i.e., most simple plausible model that explains variations in measures of brain morphology with the smallest set of predictors. Parameter estimates for CT and SA (Y_i) were estimated separately by regression of a general linear model (GLM) at each vertex i and subject j, with (1) group (G) as categorical fixed-effects factor, (2) linear, quadratic, and cubic terms for age as well as their interactions with group, and (3) FSIQ as continuous covariate.

Thus, the cubic model was formalized as: $Y_i = \beta_0 + \beta_1 G_j + \beta_2 \ \mathrm{Age}_j + \beta_3 \ \mathrm{Age}_j^2 + \beta_4 \ \mathrm{Age}_j^3 + \beta_5 \ (\mathrm{Age}_j \times \mathrm{Group}) + \beta_6 \ (\mathrm{Age}_j^2 \times \mathrm{Group}) + \beta_7 \ (\mathrm{Age}_j^3 \times \mathrm{Group}) + \beta_8 \ \mathrm{IQ}_j + \varepsilon_i$, where ε denotes the residual error. The quadratic model lacked the cubic age term, so that: $Y_i = \beta_0 + \beta_1 G_j + \beta_2 \ \mathrm{Age}_j + \beta_3 \ \mathrm{Age}_j^2 + \beta_4 \ (\mathrm{Age} \times G_j) + \beta_5 \ (\mathrm{Age}_j^2 \times \mathrm{Group}) + \beta_6 \ \mathrm{IQ}_j + \varepsilon_i$. The linear model lacked cubic and quadratic age terms, so that: $Y_i = \beta_0 + \beta_1 G_j + \beta_2 \ \mathrm{Age}_j + \beta_3 \ \mathrm{Age}_j \times G_j + \beta_4 \ \mathrm{IQ}_j + \varepsilon_i$. Subsequently, we examined between-group differences in the neurodevelopmental trajectory of cortical thickness and surface area using the most parsimonious model resulting from the nested model comparison.

Age-related differences in cortical thickness and surface area were firstly examined based on the fixed-effect coefficient β_1 normalized by the corresponding standard error, which indicated significant between-group differences while controlling for the effects of age. Secondly, we examined the interactions between group and each corresponding age term. Thus, for the linear model, we examined the interaction between age × group; for the quadratic model, we examined the interactions between age × group and age^2 × group; and for potential cubic growth curves, we examined the interactions between age × group, age^2 × group, and age^3 × group. This approach allowed us to examine between-group differences and age × group interactions for both linear and more complex age terms. Corrections for multiple comparisons across the whole brain were performed using random-field theory (RFT)-based cluster-corrected analysis for non-isotropic images using a $p < 0.05$ (two tailed) cluster significance threshold (Worsley et al. 1999).

Results

Subject demographics

There were no significant differences between individuals with ASD and controls in age [$t(152) = 1.32$, $p = 0.186$]

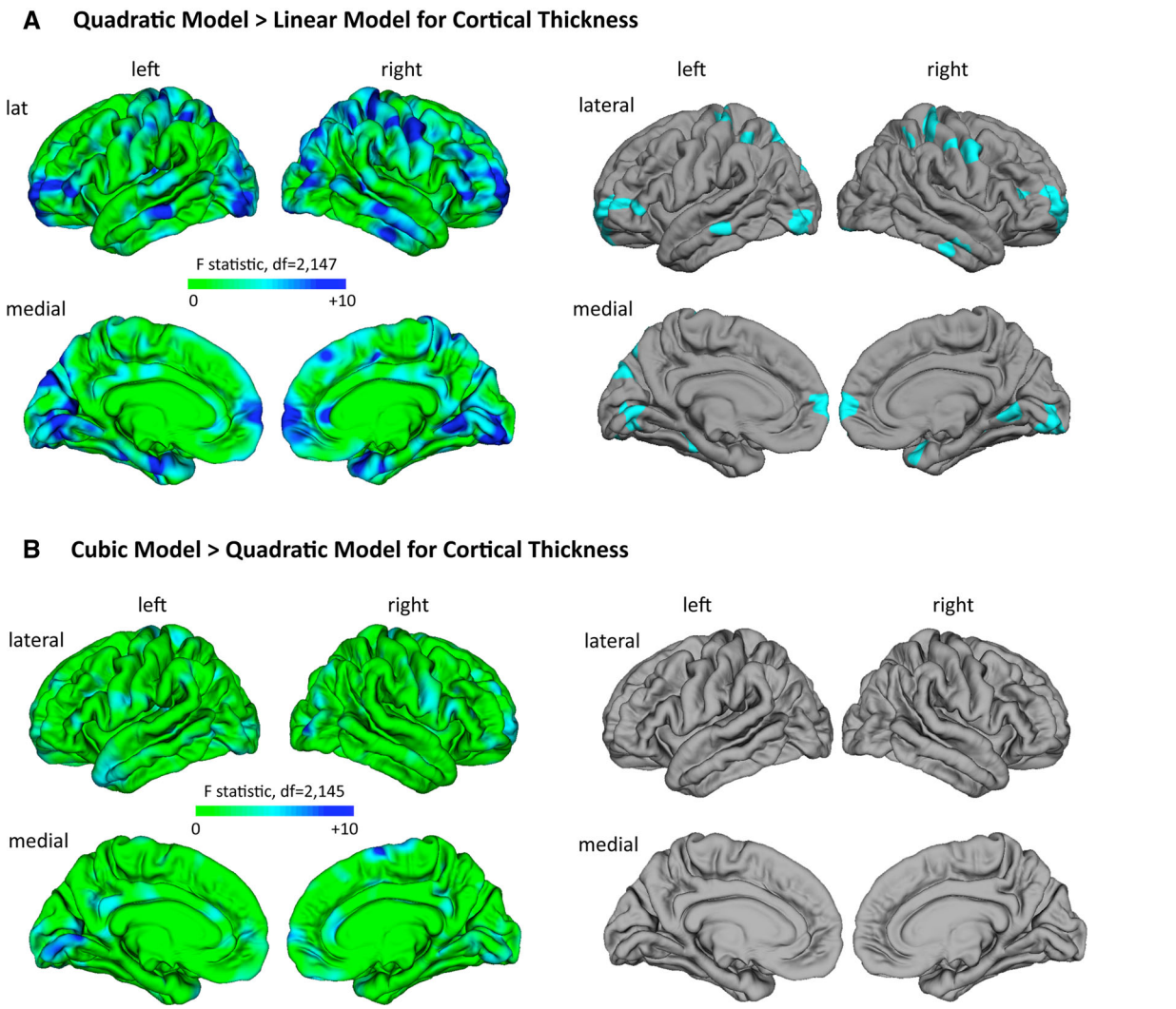

Fig. 1 Nested model comparisons for cortical thickness. **a** Linear vs. quadratic model. **b** Quadratic model vs. cubic model. *Left panel* shows the difference map resulting from the model comparison (*F* statistic, unthresholded). *F* values (*green* to *blue*) indicate voxels where the more complex model fits better than the more reduced model. *Right panel* indicates random-field theory (RFT)-based, cluster-corrected ($p < 0.05$) difference maps indicating regions where the more complex model provides a significant better goodness of fit than the simpler model

or full-scale IQ [$t(152) = -1.73$, $p = 0.085$]. There were also no significant between-group differences in mean cortical thickness [$t(152) = -0.91$, $p = 0.361$] or total surface area [$t(152) = 0.23$, $p = 0.813$]. We therefore did not covary for total brain measures in the statistical analysis of cortical thickness and surface area.

Nested model comparisons

For measures of cortical thickness, we found that the quadratic model provided a significantly better goodness of fit than the linear model in several spatially distributed clusters across the cortex (see Fig. 1a for individual regions). However, there was no significant improvement in fit when comparing the quadratic with the more complex cubic model, and no clusters survived correction for multiple comparisons (RFT-based, cluster-corrected, $p < 0.05$) (Fig. 1b). Thus, we selected the quadratic model as the most parsimonious model for examining between-group differences in cortical thickness (i.e., model with the smallest number of predictors), which also allowed us to investigate age × group interactions for the linear and quadratic age term.

For vertex-based estimates of surface area, we found that neither quadratic nor cubic age term significantly increased the goodness of fit overall, and there were no clusters in which the more complex models (quadratic or cubic) provided a significantly better fit than the linear model (Fig. 2). Thus, we selected the linear model as the most parsimonious model for examining between-group

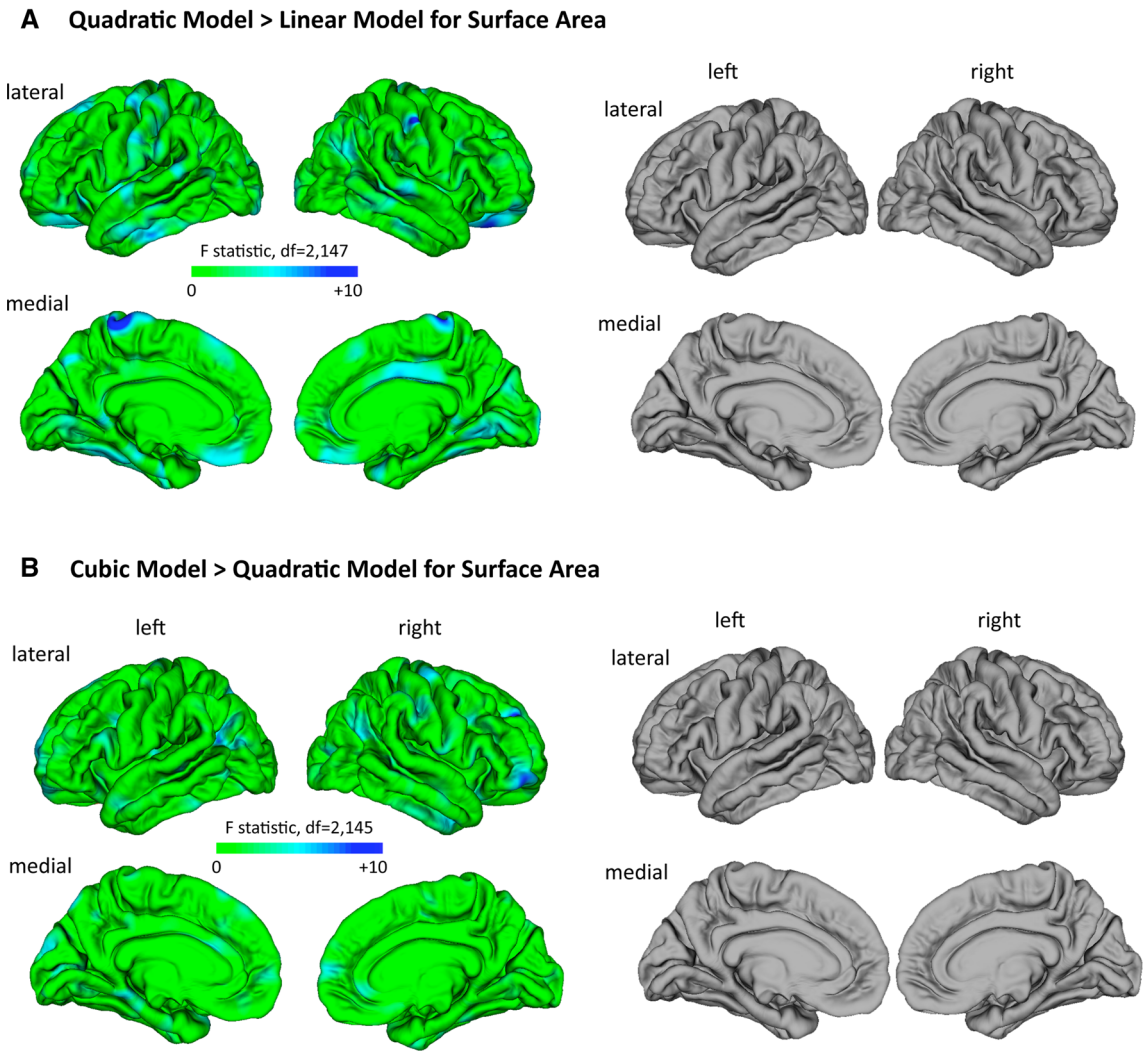

Fig. 2 Nested model comparison for surface area. **a** Linear vs. quadratic model. **b** Quadratic model vs. cubic model. *Left panel* shows the difference map resulting from the model comparison (*F* statistic, unthresholded). *F* values (*green* to *blue*) indicate voxels where the more complex model fits better than the more reduced model. Right panel indicates random-field theory (RFT)-based, cluster-corrected ($p < 0.05$) difference maps indicating regions where the more complex model provides a significant better goodness of fit than the simpler model

differences in surface area and the interaction between age × group.

Between-group differences in cortical thickness and interactions with age terms

Based on the outcome of the nested model comparison, a quadratic model was used to examine the effects of group, age, and their interactions on measures of cortical thickness. We found significant reductions in cortical thickness in ASD across the cortex when controlling for the effects of age (linear and quadratic terms), as well as for interactions between group and age terms. Relative to controls, individuals with ASD had significantly reduced cortical thickness (Table 2) in several large frontal lobe clusters (RFT-based cluster-corrected, cluster threshold $p < 0.05$), which included (1) the right medial orbitofrontal and rostral middle frontal lobe (approximate Brodmann area [BA] 10/11/12/46), (2) medial and lateral superior frontal regions (BA8/9), and (3) in the pars triangularis of the dorsolateral prefrontal cortex (BA44/45). Furthermore, we observed reduced cortical thickness in ASD in a cluster located in the bilateral postcentral gyrus (BA6), the bilateral superior parietal cortex (BA7), the right lingual gyrus (BA19/37), the right inferior and middle temporal lobe (BA20/21), and in the left precentral gyrus (BA4). There were no brain regions in which individuals with ASD showed a significant increase in cortical thickness relative to controls (Fig. 3).

Four out of 10 clusters with a significant between-group difference in cortical thickness also displayed a significant

Table 2 Clusters of significant reductions in cortical thickness in ASD relative to controls while controlling for age effects

Region	Side	BA	Vertices	*t*	*p*	Age (age^2) × group
Medial orbitofrontal and rostral middle frontal	R	10–12/46	4,447	3.86	0.00002	Age (age^2)
Middle and inferior temporal	R	20/21	4,447	3.79	0.00009	Age (age^2)
Superior frontal	R	8/9	2,717	4.75	0.00081	Age
Pars triangularis	R	44/45	2,779	3.56	0.0026	–
Postcentral gyrus	L	6	3,656	4.76	0.0006	Age (age^2)
Postcentral gyrus	R	6	3,046	3.96	0.0021	Age (age^2)
Precentral gyrus	L	4	2,143	4.19	0.031	–
Superior parietal	R	7	3270	3.33	0.0008	–
Superior parietal	L	7	2,939	3.79	0.0038	Age
Lingual gyrus	R	19/37	2,717	3.81	0.0085	–

BA Brodmann area, *L* left, *R* right, *Vertices* indicate the number of vertices within the cluster, *t value* maximum *t* statistic within cluster, *p* cluster-corrected *p* value, *age/age^2* existence of significant age × group interaction for linear and quadratic term

linear and quadratic interaction effect of age × group (i.e., age × group, age^2 × group, respectively). The clusters in the right superior frontal lobe and in the left superior parietal cortex showed only a significant linear, but not quadratic, age × group interaction. No significant age × group interactions were observed in the right pars triangularis, the left precentral gyrus, the right superior parietal lobe, and in the right lingual gyrus. There were no brain regions with a significant age × group interaction term, but no significant between-group difference in cortical thickness.

In regions with significant age × group interactions, individuals with ASD tend to have reduced cortical thickness during childhood, but increased cortical thickness in adulthood relative to controls (Fig. 5a, b).

Between-group differences in surface area and age × group interactions

Based on the outcome of the nested model comparison, a linear model was used to examine the effects of group, age, and their interactions on vertex-based measures of surface area. Across groups, we found that vertex-based measures of surface area increased significantly with increasing age (i.e., from 7 to 25 years) overall, with strongest correlations being observed in the bilateral anterior inferior temporal lobes, the medial orbitofrontal cortex, the anterior cingulate cortex, and the medial prefrontal cortex (see Figure Supplementary Material). There were no significantly clusters where measures of surface area decreased with age.

Individuals with ASD had significant reductions in surface area relative to controls, when controlling for the effect of age and for the interaction between age × group. Regions of reduced surface area in ASD included a large frontal cluster ($t_{max} = 3.07$, $N_{vertices} = 4{,}217$, $p_{cluster} = 0.005$), including the right anterior cingulate gyrus (BA32/33) and the right medial orbitofrontal and rostral middle frontal lobe (BA10/12). We also observed reduced surface area in ASD in the left temporal pole (BA38) ($t_{max} = 3.04$, $N_{vertices} = 3{,}864$, $p_{cluster} = 0.023$). There were no brain regions in which individuals with ASD showed a significant increase in surface area relative to controls (Fig. 4).

Furthermore, none of the regions with a significant between-group difference in surface area also displayed a significant age × group interaction. Instead, a significant linear age × group interaction was observed in the postcentral gyrus (BA6) of the left ($t_{max} = 4.27$, $N_{vertices} = 10{,}007$, $p_{cluster} = 0.0013$) and right hemisphere ($t_{max} = 3.10$, $N_{vertices} = 4{,}535$, $p_{cluster} = 0.0025$). In these regions, individuals with ASD show reduced measures of surface area during childhood and increased surface area during adulthood compared with controls (see Fig. 5c).

Discussion

We report the results of a cross-sectional structural neuroimaging study examining age-related differences in cortical thickness and surface area in a large and well-characterized sample of male adults with ASD, and matched neurotypical controls, between 7 and 25 years of age. Based on prior knowledge of the typical developmental trajectory of gray matter maturation, we examined linear, quadratic, and cubic effects of age in order to identify the statistical model that best predicted developmental trajectories in our sample. We found that measures of cortical thickness were best predicted by a quadratic model, which included a linear and a quadratic age term, while a simple linear model was best suited to predict measures of surface area. When controlling for age effects, individuals with ASD showed significant reductions in cortical thickness across the cortex, and particularly in fronto-temporal regions. In most of these regions, we also observed

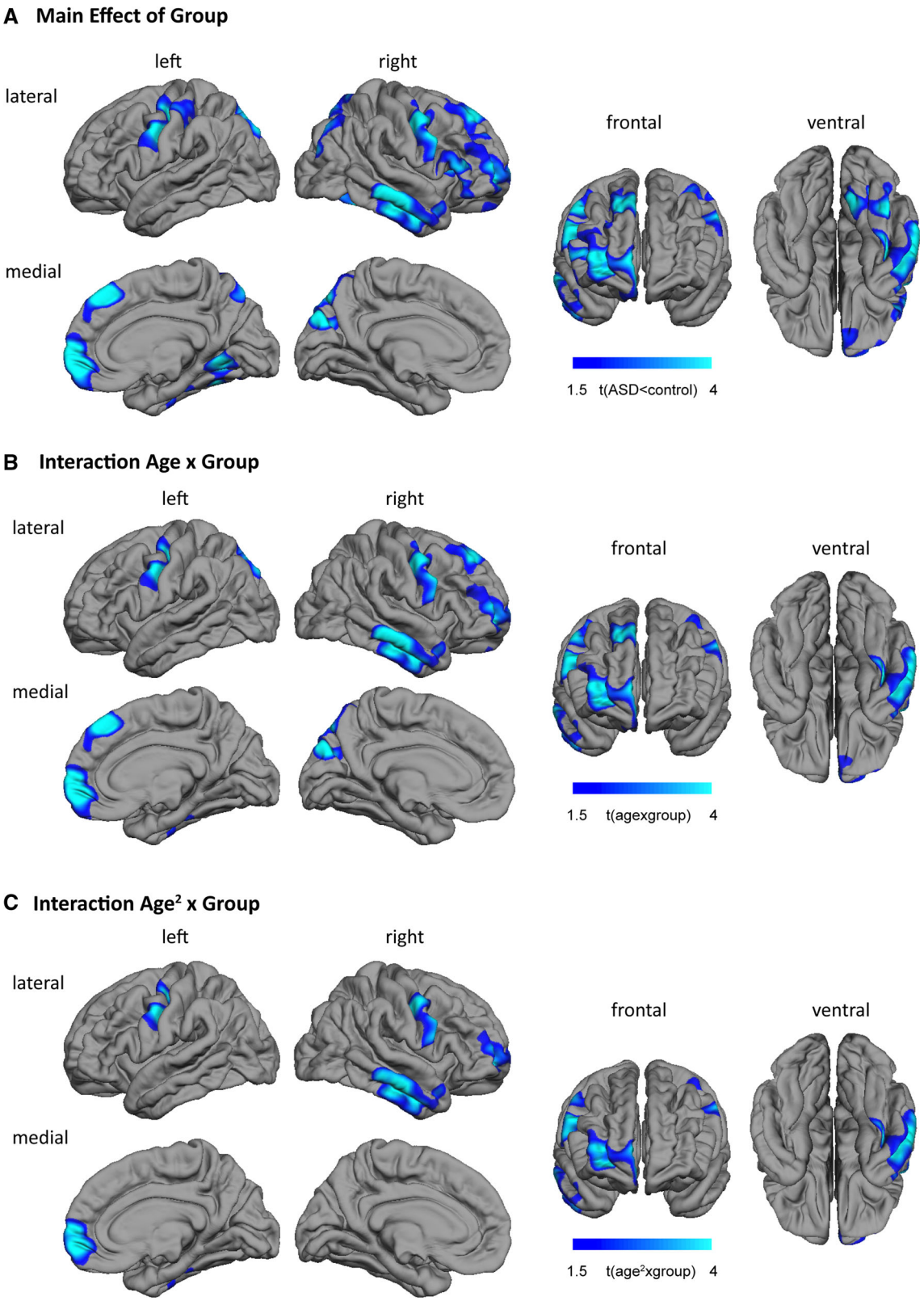

Fig. 3 Between-group differences and age-by-group interactions for measures of cortical thickness. **a** Clusters with significantly reduced cortical thickness measures (RFT-based, cluster-corrected, $p < 0.05$) in ASD compared to controls while controlling for the effects of age and age-related interactions (i.e., main effect of group). **b** Clusters with significant interactions between age × group (RFT-based, cluster-corrected, $p < 0.05$). **c** Clusters with significant interactions between age^2 × group (RFT-based, cluster-corrected, $p < 0.05$)

significant group × age and group × age^2 interactions, indicating that individuals with ASD tend to have thinner cortices during childhood but increased cortical thickness during adulthood. Last, we found that individuals with ASD had significant reductions in surface area, in addition to a significant age × group interaction. Thus, our study confirms that there are region-specific between-group differences in cortical thickness and surface area in ASD in addition to age-related interactions and that the direction of differences between groups (i.e., increased or decreased in ASD) heavily depends on the particular age-group of the investigated sample.

Our finding of reduced cortical thickness in ASD—when controlling for age-related effects—agrees with previous studies employing a similar approach in comparable samples (e.g., Raznahan et al. 2010; Wallace et al. 2010; Scheel et al. 2011). However, while previous studies report that age-related cortical thinning is mostly restricted to temporal and parietal regions, our study extends these findings by also reporting extensive reductions in cortical thickness in several areas of the frontal cortex. For example, we found reduced cortical thickness in ASD in the medial and rostral dorsolateral prefrontal cortices, which play a crucial role in the typical development of social cognition and empathy (Lombardo et al. 2007; Blakemore 2008). Moreover, these regions have also been linked to atypical theory of mind (ToM) (Castelli et al. 2002) and self-referential cognition in ASD (Lombardo et al. 2010). One aspect that sets our study apart from others is that we also considered quadratic interactions between group and

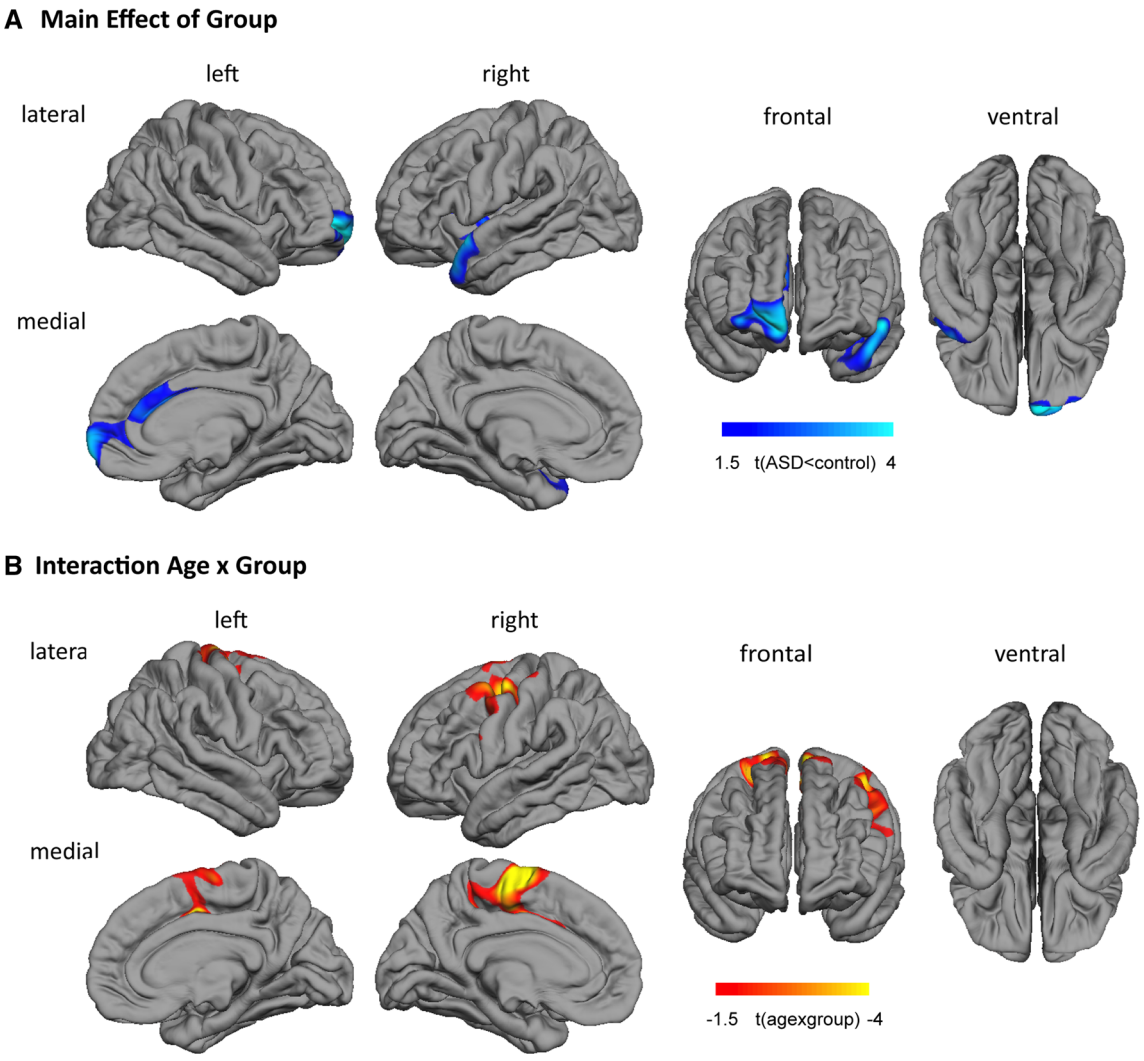

Fig. 4 Between-group differences and age-by-group interactions for vertex-based measures of surface area. **a** Clusters with significantly reduced surface area (RFT-based, cluster-corrected, $p < 0.05$) in ASD compared to controls while controlling for the effects of age and age-related interactions (i.e., main effect of group). **b** Clusters indicating significant interactions between age × group (RFT-based, cluster-corrected, $p < 0.05$)

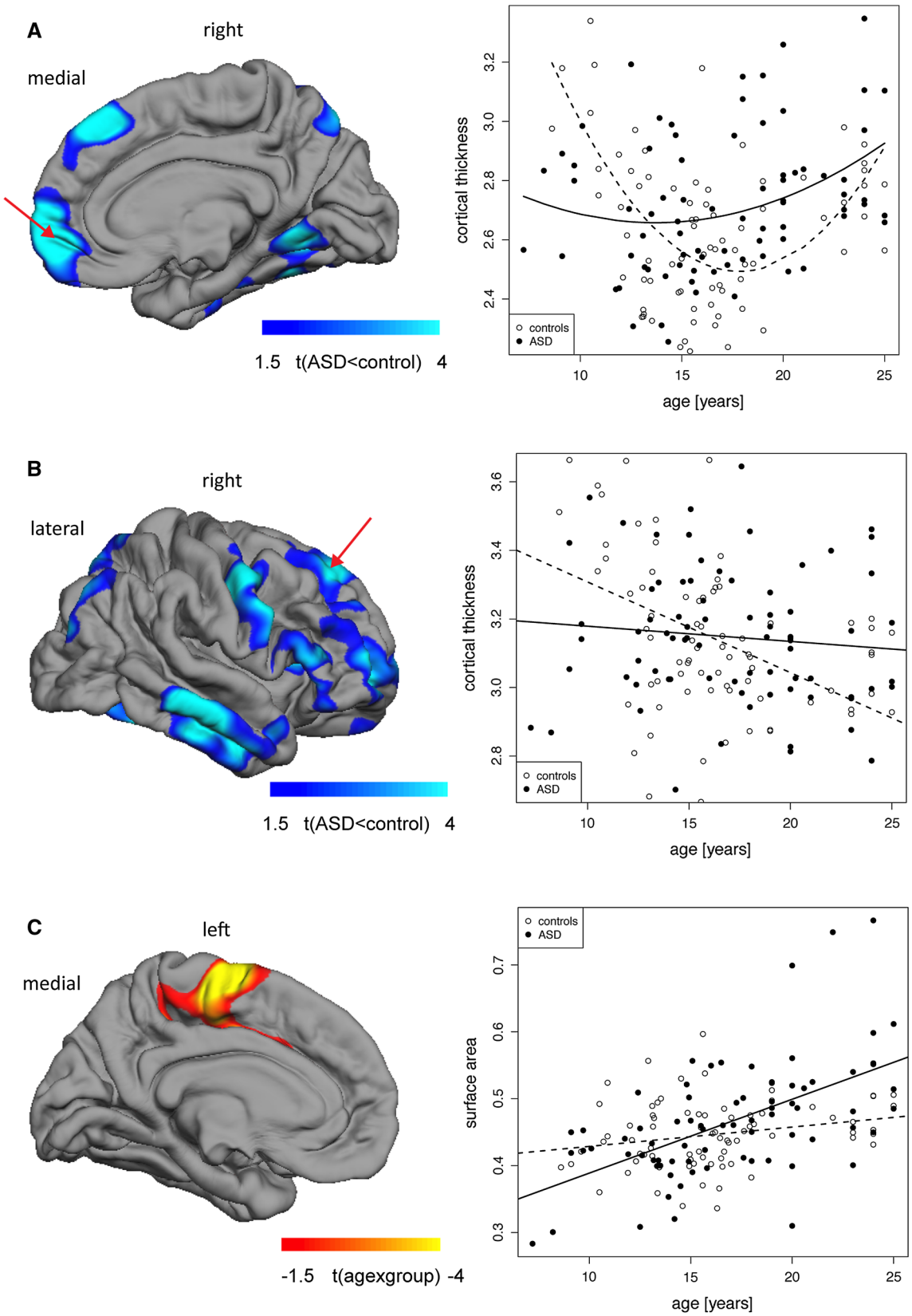

Fig. 5 Different types of age × group interactions. **a** Quadratic interaction between age × group for cortical thickness in left medial orbitofrontal cluster, extending into the rostral middle frontal lobe. **b** Linear interaction between age × group for cortical thickness in the left superior frontal lobe. **c** Linear interaction between age × group for surface area in the right postcentral gyrus

age in our statistical model, while previous investigations modeled linear effects exclusively (Raznahan et al. 2010; Wallace et al. 2010). It is known from longitudinal studies examining brain maturation in healthy controls that the neurodevelopmental trajectory of cortical thickness is nonlinear in most regions across the cortex, but also includes complex age terms (i.e., cubic and quadratic) resulting in a typical inverted U-shape (Shaw et al. 2006). Thus, our quadratic model, which included a quadratic and linear age term, is expected to be statistically more powerful in comparison with the simple linear model and may explain our finding of cortical thinning in the frontal lobe in ASD. Also, our sample of 77 individuals per group ($N_{\text{total}} = 154$) that are well matched in terms of age, FSIQ, and gender offers increased statistical power in comparison with existing studies, which mostly investigated smaller samples of individuals. Due to the large sample size, we were also able to examine complex interactions between group $\times$ age and group $\times$ age^2. Such complex age $\times$ group interactions were predominantly observed in the anterior temporal and prefrontal lobe where individuals with ASD tent to have reductions in cortical thickness during childhood, but increased cortical thickness during adulthood. Thus, it is important to consider such complex age $\times$ group interactions when interpreting between-group differences in neuroanatomy in ASD.

Furthermore, we observed a significant reduction in vertex-based measures of surface area in the medial orbitofrontal and anterior temporal lobe in ASD. Anterior temporal lobe abnormalities have previously been linked to the core symptoms of ASD, which center on deficits in language, emotional, and social behavior. For instance, the temporal pole is crucial for high-level social cognitive processes, such as mentalizing (e.g., ToM) and semantic processing (Patterson et al. 2007). Functional MRI studies on ASD also suggest that the recruitment of the anterior temporal lobe is atypical across social cognitive tasks with mentalizing demands such as irony processing (Wang et al. 2009), emotional introspection (Silani et al. 2008), attributing mental states to geometric shapes (Castelli et al. 2002), and language tasks with semantic demands (Gaffrey et al. 2007). Also, we observed reductions in surface area of the anterior cingulate in ASD, which is part of the extended neural system processing emotions (Pessoa 2008), and also plays a major role in general executive functioning that mediate the capacity to shift attention between social and non-social goals and representation (Mundy et al. 2010). Our finding of reduced surface area in these brain regions thus further corroborates their importance in mediating autism-related neurocognitive impairments, particularly in the social domain.

However, while the typical neurodevelopmental trajectory of cortical thickness is well established across the early human life span (see Shaw et al. 2006), there is currently no comparable data indicating the growth trajectory of vertex-based measures of surface area. Here, we found that age $\times$ group interactions for surface area were best modeled by a simple linear model, thus suggesting that the neurodevelopmental trajectory of surface area may be different from the neurodevelopmental trajectory of cortical thickness. This agrees with the previous notion that differences in cortical thickness and surface area in ASD may represent neurobiologically distinct mechanisms that are mediated by different sets of genes (Panizzon et al. 2009), distinct phylogenies (Rakic 1995), and also relate to different aspects of the neural architecture. For instance, it has been suggested that both measures originate from different types of progenitor cells, which divide in the ventricular zone to produce glial cells and neurons. Cortical thickness has been related primarily to intermediate progenitor cells (neurogenic transient amplifying cells in the developing cerebral cortex) (Pontious et al. 2008), which divide symmetrically at basal (non-surface) positions of the ventricular surface and only produce neurons (Noctor et al. 2004; Miyata et al. 2004). These neurons then migrate along radial glial fibers to form ontogenetic columns (i.e., radial units). According to the radial unit hypothesis (RUH) (Rakic 1995), cortical thickness depends on the neuronal output from each radial unit—amplified by intermediate progenitor cells—and therefore reflects the number of neurons produced in each unit. On the other hand, surface area has mainly been related to radial unit progenitor cells, which divide at the apical (ventricular) surface. The early proliferation of radial unit progenitor cells leads to an increase in the number of proliferation units, which in turn results in an increase in SA (Pontious et al. 2008). In other words, surface area is related to the number of ontogenetic columns. Our findings therefore suggest that the brain in ASD differs from neurotypical controls in terms of neuronal numbers and the number of cortical minicolumns, which has also been demonstrated by histological studies (e.g., Courchesne et al. 2011; Casanova et al. 2006). In addition to these abnormalities that influence early brain maturation, atypical growth curves of the brain in ASD may also result from abnormalities to mechanisms mediating late brain maturation. For instance, it has been shown that the brain continues to mature until late childhood and/or early adolescence, particularly in temporal and frontal lobes (Giedd et al. 1999). Late brain maturation seems to be driven by a combination of progressive and recessive events including synaptic proliferation, synaptic pruning, and myelination (Huttenlocher and Dabholkar 1997; Paus 2005). Thus, atypical neurodevelopment in ASD may be driven by different aspects of pathology occurring during early and/or late brain maturation.

Notably, age × group interactions in surface area were not observed in regions where we also found a significant between-group difference. For example, we found significant age × group interactions in the bilateral postcentral gyrus (BA6) where surface area did not differ between individuals in ASD and controls overall. Our study is also the first study to report significant age × group interactions for vertex-based estimates of cortical thickness while others, using a similar approach, did not report any significant interactions (e.g., Raznahan et al. 2010; Wallace et al. 2013). This discrepancy may partially be due to differences in sample size and issues of matching and clinical characterization between groups. For instance, participants with ASD were not matched to controls on FSIQ in the study by Raznahan et al. (2010), and not all participants with ASD were diagnosed using ADOS or ADI-R diagnostic criteria (Raznahan et al. 2010). However, significant between-group differences in surface area and age × group interactions have previously been noted by Doyle-Thomas et al. (2013), who found that surface area in the right cingulate was significantly different in ASD, and decreased more rapidly with age in ASD than in controls (Doyle-Thomas et al. 2013). It is thus important for future studies to examine surface area at different stages of development in order to elicit reliable and interpretable differences in ASD.

Our findings should be interpreted in light of a number of methodological considerations. First, we investigated surface-based neuroanatomy in a sample of high-functioning male individuals with ASD (and neurotypical controls), whose diagnostic status was confirmed using the ADI-R. The ADI-R rather than ADOS scores were chosen as exclusion criteria because current symptoms assessed in adult samples can often be masked by coping strategies developed across the life span and can also be alleviated by treatments/interventions (e.g., social skills training). Hence, it is not uncommon for individuals to meet ADI-R (i.e., diagnosis of childhood autism) but not ADOS diagnostic criteria during adulthood. Our sample thus represents a subpopulation of the autistic phenotype, and our results may not generalize to other groups on the autism spectrum (e.g., individuals with intellectual disability) or females with ASD. Second, we employed a cross-sectional design to investigate age-related differences in brain anatomy between groups. While this design enabled us to investigate neuroanatomy across a relatively large age range, it did not allow us to determine neurodevelopmental trajectories for cortical thickness and surface area within individuals. Longitudinal studies are therefore needed to replicate our findings by also taking into account intra-individual variations, and to identify the individual growth trajectories for cortical thickness and surface area. Also, we did not covary for total brain volume as it is a rather 'convoluted' measure, which can be further subdivided into distinct neuroanatomical features, e.g., total gray matter volume is a product of total surface area and cortical thickness. Moreover, total brain volume is the sum of total gray and total white matter and thus contains a third component (i.e., white matter) that may not necessarily be correlated with the dependent variable (e.g., one would not expect a significant between-group difference in regional cortical thickness to be driven by differences in total white matter volume). Accordingly, including total brain volume as a covariate may remove not only global effects directly related to the dependent variable, but also eliminate unspecific and indirect effects that would alter the scientific question under investigation. Last, did we not compare the neurodevelopmental trajectories for cortical thickness and surface area directly, but rather indirectly via the nested model comparison. Future research is, however, required to directly compare the individual growth curves for both measures (e.g., via cross-correlation analysis) in order to establish their distinct genetic and neurobiological underpinnings.

To sum up, our cross-sectional study suggests that there are age-related changes in cortical thickness and surface area of the brain across childhood and early adulthood in ASD. We observed significant reductions in cortical thickness and surface area in ASD relative to controls when controlling for the effect(s) of age mainly in fronto-temporal regions. In these regions, we also found significant interactions between group and age terms (linear and quadratic) predominantly for measures of cortical thickness. Our findings thus support the hypothesis that the brain in ASD undergoes an atypical trajectory of brain maturations and that the regions maturing last during typical brain development are also the regions most affected in ASD.

Acknowledgments This work was supported (1) by the Medical Research Council UK (G0400061 and G0800298), (2) by the Dr. Mortimer and Theresa Sackler Foundation, (3) by the EU-AIMS project (European Autism Interventions—a Multicentre Study for developing New Medications) receiving support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no. 115300, which includes financial contributions from the EU Seventh Framework Programme (FP7/2007-2013), (4) by the NIHR Biomedical Research Centre for Mental Health at King's College London, Institute of Psychiatry, and (5) by the South London & Maudsley NHS Foundation Trust. We are also grateful to those who agreed to be scanned and who gave their time so generously to this study.

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CHAPTER. **6**

AGE RELATED DIFFERENCES IN WHITE MATTER DIFFUSION MEASURES IN AUTISM SPECTRUM DISORDER

ABSTRACT

It is well established that autism spectrum disorder is accompanied by developmental differences in brain anatomy and connectivity. However, the neural systems underlying ASD are complex and remain poorly understood. White matter differences in ASD have been widely studied with Diffusion Tensor Imaging but results are heterogeneous and vary across the age range of study participants. In order to characterize the neurodevelopmental trajectory of white-matter maturation, it is thus necessary to examine a broader age range of individuals with ASD and typically developing controls, and to dissect potential age x group interactions. Here, we employed a region-of-interest analysis and a spatially unbiased Tract-Based Spatial Statistics approach to examine age-related differences in white-matter connectivity in a sample of 41 individuals with ASD, and 41 matched controls between 7-17 years of age.

We found significant age-related difference between the ASD and control group in widespread brain regions resulting from both the regional and voxel –wise analyses. In these regions we observed that measures of fractional anisotropy (FA) significantly increased with age in both groups. However, the increase in FA with age was significantly larger within the ASD group relative to controls. Furthermore, we found that measures of radial diffusivity (RD) significantly decreased with age in both groups, and that the decrease in RD was stronger in the ASD group relative to controls. We also investigated between-group differences in lateralization of FA, and report FA values in the post-central gyrus to be significantly more left lateralized in ASD. Last, we examined the relationship between DTI measures and symptom severities in the ASD group. Here we found significant correlations between FA values and restricted and repetitive behaviour as identified by the ADOS in several large-scale white-matter regions in the brain.

Taken together, our findings suggest that individuals with ASD have an atypical trajectory of white matter maturation relative to controls. However, future histological validation and longitudinal analyses are required to further characterize the extent, time course and aetiology of these differences.

Autism Spectrum Disorder is a life-long neurodevelopmental condition characterized by impairments in social communication and reciprocity, and a propensity for repetitive and stereotyped behaviour [1]. These principal symptoms typically manifest before the age of 2 years and are accompanied by developmental differences in brain anatomy and connectivity [2-4]. The neural systems underlying ASD are complex and involve abnormalities in multiple, spatially-distributed neurocognitive systems [5]. Thus our understanding of the neuroanatomy of ASD remains incomplete.

Despite the high degree of phenotypic heterogeneity and complex aetiology of ASD, it is well accepted that individuals with ASD show an atypical trajectory of brain development. For example, neurodevelopmental differences in brain maturation have been observed on the global level, and age-related differences in overall brain growth of total grey- and white-matter volume have been reported [6-11]. More specifically, it has been suggested that individuals with ASD undergo a period of accelerated brain growth during early postnatal life [12-14], causing the ASD brain to be larger in early childhood relative to typically developing controls [9, 15-17]. This precocious increase in total brain volume is followed by a period of atypically slow or arrested growth throughout the remainder of childhood, so that no global differences are generally observed by adulthood [11, 18]. Increased white matter development has been suggested to contribute to the early brain overgrowth in childhood [9, 15-17], thus suggesting that brain maturation in ASD also seems to affect the development of brain connectivity.

There is strong evidence coming from structural and functional MRI studies [19-22], positron emission tomography [23], and electroencephalography studies [24-26], that ASD is associated with altered brain connectivity [27-29]. Atypical brain connectivity has been documented in both adults [27, 30] and children with ASD [19, 24, 25, 27, 31], and particularly in the neural networks that mediate autistic symptoms and traits. For example, abnormalities in functional connectivity of temporal-lobe networks [32] have been associated with

social/emotional behaviours, and abnormalities in fronto-striatal networks have been associated with ritualistic/repetitive behaviours in ASD [16, 33]. There is also evidence of atypical white-matter structural connectivity between these regions in ASD, which has been extensively studied using Diffusion Tensor Imaging [34, 35].

DTI studies report widespread differences in white matter microstructure among infants [36], children and adolescents [28, 37-46], and adults with ASD [47-50]. More specifically, increased mean fractional anisotropy (FA) has been reported in infants and toddlers with ASD, which may indicate increased tract coherence and axonal alignment in the condition [36, 45]. However, this pattern seems to be reversed (i.e. lower FA values in ASD) in childhood and adolescence [37-44, 46, 48, 51]. Fewer differences in diffusion measures have been described in later adolescence and adulthood [42], suggesting that between-group differences in white matter microstructure might disappear with increasing age (see Table 6.1, for a summary of relevant FA findings, ordered by age).

Differences have also been reported in mean diffusivity (MD) and radial diffusivity (RD), but there is some disagreement. MD indicates the degree of direction-independent average diffusivity, and has been shown to decrease over the course of healthy white matter maturation [52]. Studies have mainly report no difference or higher MD values in individuals with ASD relative to controls (see Table 6.1) [48, 53, 54] although reduced MD values have also been noted [55]. Last, there are reports of increases in RD in both children [53, 56] and adults with ASD [27, 57], which may reflect changes in underlying white matter properties including reduced myelination [58, 59].

Taken together, these findings contribute to a heterogeneous body of DTI literature regarding ASD. This heterogeneity may indicate that the pattern of white-matter abnormality varies across the investigated age range of participants, and affects widespread neural systems rather than isolated brain regions. Moreover, differences in the employed methodology, may affect the results. For example, the majority of DTI studies that have previously been conducted in ASD are based on a region-of-interest approach [60]. ROI-

approaches rely on *a priori* hypotheses regarding the specific white matter tract or region under investigation. As it is well established that ASD is related to diffuse and spatially-distributed white matter differences [5], an exploratory whole-brain approach such as TBSS is particularly well suited for examining this group of individuals.

A number of studies have previously used TBSS to investigate ASD (see Table 6.1) but these have generally been small scale, including a narrow age range of participants or a heterogeneous sample (including both right and left handed, males and females). A large scale TBSS analysis on a rigorously selected and homogenous sample spanning childhood and adolescence is yet to be carried out. Therefore, here we present an ROI and TBSS analysis that examines age-related differences in diffusion measures, spanning 10 years through childhood and adolescence, in a large, well-characterized group of male individuals with ASD and matched TD controls.

Table 6.1. Summary of Relevant DTI Literature

Studies are listed in order of study age-range, from infancy through to young adulthood. Arrows show statistically significant decreases (↓), and increases (↑) in mean FA, MD and RD, with respect to typically developing controls. Abbreviations**:** (alic) anterior limb of the internal capsule; (ATR) anterior thalamic radiation; (CC) corpus callosum; (sCC) splenium of the corpus callosum; (gCC) genu of the corpus callosum; (CST) cortico-spinal tract; (HFA) high functioning autism; (IFOF) inferior fronto-occipital fasciculus; (ILF) inferior longitudinal fasciculus; (L-) left lateralized; (mcp) middle cerebellar peduncle; (NS) reported as non-significant; (-) not reported; (PDD-NOS) pervasive developmental disorder not otherwise specified; (plic) posterior limb of the internal capsule; (R-) right lateralized; (ROI) region of interest; (scp) superior cerebellar peduncle; (SLF) superior longitudinal fasciculus; (SPM) statistical parametric mapping; (STG) superior temporal gyrus; (TBSS) tract based spatial statistics.

Study	Analysis	Diagnosis	Sample (*n*)	Age Range (SD)	Findings			Region
			ASD:Control	ASD:Control	FA	MD	RD	
Wolff et al. 2012 [36]	*Tractography*	*Autism*	28:64	0.57 (0.07):0.56 (0.07)	↑	-	-	*L-fornix, L-ILF, L-uncinate, CC, R-plic*
Bashat et al. 2007 [28]	*ROI*	*Autism*	07:18	1.8–3.3:0.25–23	↓ ↑	-	-	*Decrease: L-cortico-spinal tract-sl2. Increase: gCC, sCC, L-plic*
Weinstein et al. 2011 [45]	*TBSS, ROI & whole-brain*	*Autism*	21:26	1.5–5.8:1.5–5.8	↑	NS	↓	*gCC, body of the CC, L-SLF, cingulum*
Walker et al. 2012 [46]	*TBSS, whole-brain*	*Autism*	26:28	2.09-8.09:2.19-8.72	↓	↑	-	*gCC, sCC & body of CC*
Sundaram et al. 2008 [90]	*Tractography*	*Autism, PDD-NOS, Asperger's*	50:16	4.79 (2.43):6.84 (3.45)	↓	-	-	*Short association fibres*
Kumar et al. 2010 [91]	*TBSS, ROI*	*Autism, PDD-NOS, Asperger's*	32:16	2.5–8.9:2.5–8.6	↓	-	-	*R-uncinate, L-SLF, R-cingulum, CC*
Ke et al. 2009 [92]	*Whole-brain*	*Autism*	12:10	8.75 (2.26):9.40 (2.07)	↓ ↑	-	-	*Decrease: L-middle frontal gyrus, L-STG, L-inferior frontal gyrus* *Increase: R-middle temporal gyrus, R-sub-gyral frontal lobe*

Koldewyn et al. 2014 [69]	*Whole-brain, tractography*	*ASD*	40:43	8.98 (1.81):8.90 (1.87)	↓	-	-	*R-ILF*
Cheung et al. 2009 [40]	*Whole-brain*	*Autism*	*13:14*	*9.30 (2.60):9.90 (2.50)*	↓ ↑	-	-	*Decrease: L-frontal orbital cortex BA47, R-precentral gyrus BA4, frontal pole BA11, R-fusiform gyrus BA19, R-uncinate, L-middle temporal gyrus BA20. Increase: R-SLF*
Brito et al. 2009 [38]	*ROI*	*Autism*	*08:08*	*9.53 (1.83):9.57 (1.36)*	↓	-	-	*Anterior body of CC, R-cortico-spinal tract, R-plic, L-scp, mcp*
Cheung et al. 2009 [40]	*Whole-brain, VBM*	*Autism*	*13:14*	*9.9 (2.5):9.3 (2.6)*	↑ ↓	-	-	*Decrease: bilateral prefrontal and temporal regions, especially R- ventral temporal lobe adjacent to the fusiform gyrus. Increase: R- inferior frontal gyrus and L-occipital lobe*
Poustka et al. 2012 [44]	*ROI, tractography*	*ASD*	18:18	9.70 (2.10):9.70 (1.90)	↓	-	-	*R-SLF, uncinate*
Barnea-Goraly et al. 2011 [37]	*Whole-brain*	*ASD*	13:11	10.50 (2.00):9.60 (2.10)	↓	-	NS	*Medial prefrontal white matter, acr, gCC, body of CC, external capsule, SLF, cingulate gyrus, STG, temporo-parietal junction*
Jou et al. 2011 [49]	*Whole-brain*	*ASD*	15:08	10.90 (3.70):11.50 (2.60)	↓	-	-	*cingulum, IFOF, ILF, SLF, uncinate, ATR, cortico-spinal tract, f-major, f-minor*
Cheon et al. 2011 [93]	*ROI*	*Asperger's, PDD-NOS*	17:17	11.00 (2.10):10.20 (2.00)	↓	↑	↑	*ATR, CC, L-uncinate, ILF*
Ameis et al. 2011 [56]	*Whole-brain*	*Autism, Asperger's*	19:16	12.40 (3.10):12.30 (3.60)	NS	↑	↑	*Whole-brain*
Shukla et al. 2010 [53]	*TBSS, whole-brain*	*Autism, Asperger's*	26:24	9–20:9–19	↓	↑	↑	*Widespread WM (ILF, IFOF, SLF, cingulum, gCC, body of the CC, sCC, plic, alic, cortico-spinal tract, ATR)*
Cheng et al. 2010 [94]	*TBSS, whole-brain*	*ASD*	25:25	13.71 (2.54):13.51 (2.20)	↑ ↓	-	↑ ↓	*Decrease: R-SLF, L-plic, R-inferior cerebellar peduncle. Increase: R-SLF, L-insula, R-ATR, R-plic, R-IFOF, mcp*

Noriuchi et al. 2010 [43]	*SPM, whole-brain*	*ASD*	07:07	13.96 (2.68):13.36 (2.74)	↓	-	-	*WM around the R-anterior cingulate cortex, L-dorsolateral prefrontal cortex, R-temporal pole, R-amygdala, R-SLF, L-posterior superior-temporal sulcus, anterior CC, R-fronto-occipital fasciculus*
Schaer et al. 2013 [95]	*TBSS & tractography*	*ASD*	11:11	9.3-17.4:8.7-16.8	↓	-	-	*CC & WM of the right hemisphere*
Fletcher et al. 2010 [41]	*ROI*	*Autism*	10:10	14.25 (1.92):13.36 (1.34)	NS	↑	↑	*SLF*
Groen et al. 2011 [48]	*TBSS, whole-brain*	*Autism*	17:25	14.40 (1.60):15.50 (1.80)	↓	↑	-	*SLF, ILF, L-corona radiata*
Bode et al. 2011 [96]	*SPM, whole-brain*	*ASD*	27:26	14.70 (1.60):14.50 (1.50)	↑	NS	-	*optic radiation, R-IFOF*
Lee et al. 2007 [57]	*ROI*	*Autism, PDD-NOS*	43:34	16.20 (6.70):16.40 (6.00)	↓	↑	↑	*WM of the STG, temporal stem*
Alexander et al. 2007 [27]	*ROI*	*Autism, PDD-NOS, Asperger's*	43:34	16.23 (6.70):16.44 (5.97)	↓	↑	↑	*gCC, sCC, total CC*
Pardini et al. 2009 [97]	*Whole-brain & ROI*	*Autism (low functioning)*	10:10	19.70 (2.83):19.90 (2.64)	↓	-	-	*L-orbitofrontal cortex, anterior cingulate, medial frontal gyrus, inferior frontal gyrus, R-superior frontal gyrus*
Kleinhans et al. 2012 [42]	*TBSS*	*Autism, PDD-NOS, Asperger's*	25:25	21.29 (5.66):21.31 (7.27)	↓	↑	↑	*SLF, uncinate, cingulum, superior & inferior cerebellar peduncles, plic, alic, CC, external capsule, CST, thalamic radiation*
Gibbard et al. 2013 [98]	*TBSS*	*ASD*	25:25	24.50 (4.02):23.22 (4.05)	↓	↑	↑	*Widespread WM, including, SLF, uncinate, sCC, gCC, body of CC, thalamic radiation & fornix*
Langen et al. 2012 [65]	*Tractography*	*Autism*	21:22	25.57 (6.08):28.45 (6.39)	↓	-	NS	*putamen tract*
Catani et al. 2008 [99]	*Tractography*	*Asperger's*	15:15	18-49:18-49	↓	NS	-	*Superior cerebellar peduncle, cerebellum*

MATERIALS AND METHODS

PARTICIPANTS

Forty-one males with ASD (aged 7–17 years) and forty-one typically developing male controls (aged 8–17years) were recruited by advertisement and assessed at the Institute of Psychiatry, London. All participants were right handed (measured using The Edinburgh Handedness inventory [61]), and native English speakers. Exclusion criteria included; pre-existing medical conditions or complications (e.g. head trauma, epilepsy); use of medication affecting brain function; mental retardation; a history of major psychiatric disorder (e.g. psychosis); chromosomal abnormality (e.g. fragile X, Tuberous Sclerosis, VCFS); and any MRI contraindications. Intellectual ability was assessed using the WASI [62]. All participants had an IQ greater than 70 (within the high-functioning range of the autistic spectrum). For the autistic group, inclusion was based on a confirmation of autism diagnosis as required by the International Statistical Classification of Diseases, 10th Revision (ICD-10) research criteria and confirmed using the ADI-R [63] (all cases reached ADI-R algorithm cut-offs in the domains of impaired reciprocal social interaction, communication, and repetitive behaviours and stereotyped patterns, although failure to reach cut-off in a single domain by 1 point was permitted). Current symptoms were assessed using the ADOS [64], but not used as an inclusion criterion. All participants and their parents or guardians gave informed written consent in accordance with ethics approval by the National Research Ethics Committee, Suffolk, UK.

MRI

All imaging for this study was acquired on a General Electric 3T MR system. Diffusion tensor MRI scans were acquired with a spin-echo echo-planar

imaging (SE-EPI) double refocused sequence providing whole head coverage with isotropic image resolution (2.4 x 2.4 x 2.4mm), 32mm diffusion-weighted volumes with different non-collinear diffusion directions with b-factor 1300 sec/mm^2 and 6 non-diffusion-weighted volumes; 60 slices; no slice gap; TE 104.5 msec; TR 20 R-R intervals; 128 x 128 acquisition matrix; FOV = 30.7 cm^2; peripherally gated (parameters compatible with [65]). Total acquisition time was approximately 12 minutes. If participants were unable to tolerate scanning or obvious head movement was detected during the acquisition (due to anxiety or hyperactivity for example), they were invited to return for a second time - at which time scan quality was usually significantly improved.

Preparation of Data

All data was initially visually inspected by raters at the Institute of Psychiatry, London UK, and Brown School of Engineering, Rhode Island, to ensure inter-rater reliability. The data then went through a comprehensive correction pipeline using TORTOISE software (https://science.nichd.nih.gov/confluence/display/nihpd/TORTOISE) [66], which registers the volumes of a DTI dataset to reduce the effects of motion, and eddy current based deformations. Corrections were performed in the native space of each subject, and appropriate rotations were applied to the b-matrix [67, 68]. All deformations in TORTOISE were computed and applied in a single step to avoid multiple interpolations of the data.

After correction, expert raters manually inspected the data and removed any volumes with residual artefacts. A total of ten volumes (across seven subjects) were removed, accounting for 0.3% of the total number of volumes acquired across the cohort. Differences in subject motion have been shown to be an important consideration for group comparisons of DTI data [69, 70]. Therefore, to rule out the effect of age and between-group differences in head motion, a univariate analysis of variance was carried out to identify the main effect of age and group, as well as their interaction on the number of volumes removed and the amount of distortion and motion-correction applied

during processing. For this purpose, transformation data detailing the degree of distortion and motion correction for each participant was extracted using TORTOISE software.

Estimation of the diffusion tensor

Following the completion of quality control procedures, all subsequent analysis was carried out using the FMRIB Software Library (FSL, www.fmrib.ox.ac.uk/fsl). First, skull and non-brain tissue were removed using BET. Voxel-wise values of FA, MD, and RD were then calculated.

Voxel-wise analysis

Voxel-wise statistical analysis was performed on the FA, MD and RD data using TBSS [71]. TBSS tests for between-group differences in diffusion measures across a 'skeleton' of WM tracts across the whole brain. This procedure includes a number of steps. First, individual FA maps were non-linearly aligned to a standard space using a target image. In this study the target image was chosen to be the most representative FA image. This is the recommended option for studies of adolescents and young children. After image registration, a cross-subject mean FA image was calculated, which informed the generation of the WM tract 'skeleton', thresholded at FA >0.3 to include major WM pathways whilst excluding peripheral tracts that are more vulnerable to partial volume effects and/or inter-subject variability. Finally, each subject's FA, MD and RD values were projected onto the group skeleton and the resulting data was fed into voxel-wise analysis. For statistical analysis, we used the Randomize function within FSL to conduct permutation-based nonparametric statistics [72] with 10,000 permutations. Areas of significant difference were displayed as a P-value image, where $P<0.05$, corrected for multiple comparisons across space via threshold-free cluster enhancement [73].

In order to establish the most parsimonious model of age-related differences, we initially examined linear and quadric effects with regards to

age-related differences in diffusion measures. Applying a quadratic age term did not significantly increase the goodness-of-fit, and the more parsimonious linear model was thus favoured for examining WM integrity. The GLM used in the present study therefore included a main effect of group, a linear term for age, as well as the interaction between age and group.

LATERALIZATION

Due to the findings of a number of studies that suggest that ASD is associated with altered brain asymmetry [74, 75], we also tested between-group differences in asymmetry of diffusion characteristics using TBSS. Investigation of asymmetry of diffusion measures using TBSS required a second, symmetrical WM 'skeleton' to be created, on which to conduct voxel-wise analysis.

Creation of the symmetrical WM skeleton involved the following steps. First, the cross subject mean FA image was flipped and averaged to create a symmetrized mean FA image. This was then skeletonized to generate a symmetric skeleton (FA > 0.3). To ensure the skeleton is exactly symmetric, this was masked twice. Initially by the original skeleton, dilated by one voxel. Secondly, the symmetric skeleton was flipped and masked by the un-flipped version of itself. Following this, each subject's FA values were projected onto the symmetrical group skeleton to be fed into voxel-wise comparison. This data was then left-right flipped with the right side subtracted from the left, and the left half of the dataset zeroed. The resulting images were then fed into voxel-wise statistical analysis. Parameter estimates for lateralization indices (FA left – right) (Y_i) were estimated by regression of a general linear model (GLM) at each voxel *i* on the mean FA skeleton with diagnostic group *G* as categorical fixed-effects factor, so that:

$$Y_i = \beta_0 + \beta_1 G + \varepsilon_i$$

Where ε_i is the residual error. Between-group differences were estimated from the coefficient β_1 normalized by the corresponding standard error. Corrections for multiple comparisons across the whole brain were performed using

threshold-free cluster enhancement [73].

CORRELATION WITH SYMPTOM MEASURES

Finally, to investigate the relationship with the severity of autistic symptom and diffusion measures, correlation analyses were conducted between the diffusion maps and ASD severity measured by the ADI-R and the ADOS.

For all voxel-wise analyses, affected WM structures were identified with the John Hopkins University White Matter Atlas [76].

REGION-WISE ANALYSIS

For region-wise analysis, masks for 16 (a priori chosen) WM regions/pathways were generated, from which diffusion measures were extracted. These masks included the main ROIs that were highlighted to be of relevance to ASD, based on evidence coming from previous neuroimaging studies. Regions included the body, genu and splenium of the CC, the fornix, the anterior thalamic radiations, UF, sagittal striatum (including the ILF), SLF and the anterior and posterior limbs of the internal capsule, which were derived from the John Hopkins University white matter atlas [76]. The masks were co-registered with the diffusion data and mean FA, MD and RD values were obtained for each participant in each of the ROIs. For ROI analysis, a linear model of the form of $FA = \alpha \times age + \beta$, was fitted in each of the 16 ROIs to estimate the slope (α) and y-intercept (β) within each group. Here, the slope of the regression line indicates the age-trajectory of diffusion measures per ROI. To perform a group comparison of the slope of these trajectories, a wild bootstrap with residual re-sampling approach [77] with 500 re-samples was then used to derive an empirical distribution of the distribution of α and the y-intercept β. A p-value less than 0.05 (corrected for multiple comparisons) was used to test statistical significance. The same method was used for measures of MD and RD.

RESULTS

PARTICIPANTS

Participant Demographics are listed in Table 6.2.

Table 6.2. **Subjects Demographics.** Data expressed as mean ± standard deviation (range). There were no significant between-group differences in age (t(80) = 0.49, p=0.63), or IQ (t(80) = -0.30, p=0.76)

	ASD (n = 41)	Control (n= 41)
Age (years)	13.1 ± 2.7 (7 - 17)	12.9 ± 2.5 (8 - 17)
IQ (WASI)	110.1 ± 15.4 (70 - 140)	110.9 ± 11.3 (79 - 132)
ADI-R Reciprocal Social Interaction	17.7 ± 4.4 (10 - 26)	-
ADI-R Communication	16.3 ± 3.8 (8 - 23)	-
ADI-R Restricted, Repetitive, Stereotyped Behaviour	5.5 ± 2.3 (2 - 11)	-
ADOS Communication	3.7 ± 1.5 (1 - 6)	-
ADOS Reciprocal Social Interaction	7.4 ± 2.7 (3 - 13)	-
ADOS Imagination/Creativity	0.9 ± 0.7 (0 - 2)	-
ADOS Stereotyped Behaviours, Restricted Interests	1.5 ± 1.5 (0 - 6)	-

MOVEMENT

There was no significant effect of age (f(48) = 0.91, p=0.61), group (f(1)= 1.07, p=0.32), or group x age interaction (f(17)= 1.96, p=0.09) on the number of volumes removed during quality control. No significant group differences were found in the application of distortion and motion correction (t(82)= -1.72, p=0.09). And no significant effect of age (f(48)= 1.76, p=0.12),

group (f(1)= 0.87, p=0.37), or group x age interaction (f(17))= 1.96, p=0.08) on the degree of distortion and motion correction applied during processing.

TBSS Analysis

The TBSS analysis did not reveal any significant between-group differences in FA, RD or MD. There were, however, significant age x group interactions for both measures of FA and RD in the UF, corticospinal tract, ILF, IFOF, anterior thalamic radiation, SLF, and forceps major (Figure. 6.1). In these regions we found that measures of FA increased significantly with age in both groups; however, this increase was accelerated in the ASD group in comparison to controls. Measures of RD decreased with age in both groups; however, the decrease in RD was accelerated in the ASD group in these ROIs. The age x group interaction for measures MD approached statistical significance, but did not reach the statistical threshold of $p<0.05$.

The lateralization analysis revealed a significant between-group difference in lateralization for measures of FA, with individuals with ASD displaying an increased left-hemisphere lateralization in a region located in the post-central gyrus, which also included the WM of the SLF (Figure 6.2).

There were significant correlations between measures of symptom severity based on the ADOS domain of 'Stereotyped Behaviours and Restricted Interests' in spatially-distributed brain regions including the UF, ILF, IFOF, corticospinal tract, and the anterior thalamic radiation (Figure. 6.3). These regions also overlap significantly with the brain regions where we observed a significant age x group interaction in the TBSS.

REGION-WISE ANALYSIS

All of the regions we examined displayed significant differences in developmental trajectory of FA between the ASD and typical control groups. Both groups showed a positive correlation between age and FA, with the ASD group showing a stronger *positive* correlation compared to controls (i.e. a steeper uphill developmental slope). The only exception to this finding was the UF, where the control group showed a negative correlation for age and FA. Most of the pathways examined also showed significant differences in developmental trajectories of MD (with the exception of the body and genu of the CC and the left anterior limb of the internal capsule), and RD (with the exception of the splenium of the CC). For these measures, the ASD group showed a stronger *negative* correlation in comparison to controls (i.e. a steeper downhill slope). Again, the UF was an exception to this finding; here RD was positively correlated with age in the control group.

Results of the region-wise analysis, including statistical test parameters for each region are listed in Table 6.3. Scatter plots of raw data with superimposed mean linear development trajectories for three of the sixteen WM pathways we examined are displayed in Figure 6.4, for scatter plots of all 16 WM pathways refer to Supplementary Figure 6.5

Table 6.3. Results of region-wise analysis for FA, MD and RD

Region	Side	ASD	Control	*t-stat*	*p-value*
Fractional Anisotropy	***R/L***	***Slope***	***Slope***	***(df = 998)***	
Anterior Limb of Internal Capsule	L	0.0071	0.006	13.2001	<0.001
Anterior Limb of Internal Capsule	R	0.0085	0.0053	40.2026	<0.001
Body Corpus Callosum	-	0.0055	0.0033	23.5606	<0.001
Cingulum	L	0.0055	0.0044	10.1883	<0.001
Cingulum	R	0.0033	0.00067	25.7018	<0.001
Fornix	-	0.0053	0.00031	28.6249	<0.001
Genu Corpus Callosum	-	0.0061	0.0025	45.0209	<0.001
Posterior Limb IC	L	0.0059	0.0021	69.7153	<0.001
Posterior Limb IC	R	0.0068	0.0015	99.8842	<0.001
Sagittal Striatum	L	0.0035	0.0039	-2.806	0.005
Sagittal Striatum	R	0.008	0.0028	57.6581	<0.001
SLF	L	0.0063	0.0034	36.099	<0.001
SLF	R	0.0059	0.0042	22.8844	<0.001
Splenium Corpus Callosum	-	0.0066	0.0053	17.8415	<0.001
Uncinate	L	0.0056	-0.0023	55.2284	<0.001
Uncinate	R	0.0022	-0.0048	49.5538	<0.001
Mean Diffusivity		***Slope ($x10^{-6}$)***	***Slope ($x10^{-6}$)***		
Anterior Limb of Internal Capsule	L	-4.841	-4.714	0.02176	0.98
Anterior Limb of Internal Capsule	R	-3.474	-2.661	-11.6855	<0.001
Body Corpus Callosum	-	-6.607	-6.449	-2.4232	0.015
Cingulum	L	-7.644	-5.986	-20.808	<0.001
Cingulum	R	-6.895	-5.318	-17.7908	<0.001
Fornix	-	-19.479	3.743	-34.9082	<0.001
Genu Corpus Callosum	-	-10.174	-10.259	-0.14352	0.89
Posterior Limb IC	L	-5.547	-4.639	-0.14091	<0.001
Posterior Limb IC	R	-5.085	-3.055	-30.074	<0.001
Sagittal Striatum	L	-7.637	-13.162	12.7546	<0.001
Sagittal Striatum	R	-9.861	-5.77	-21.7122	<0.001
SLF	L	-9.056	-6.233	-27.1896	<0.001
SLF	R	-8.706	-5.971	-26.4998	<0.001
Splenium Corpus Callosum	-	-6.179	-8.678	20.9661	<0.001
Uncinate	L	-8.522	-7.964	-4.3038	<0.001
Uncinate	R	-3.429	-0.577	-20.7439	<0.001
Radial Diffusivity		***Slope ($x10^{-6}$)***	***Slope ($x10^{-6}$)***		
Anterior Limb of Internal Capsule	L	-7.604	-6.972	-7.7773	<0.001
Anterior Limb of Internal Capsule	R	-7.795	-4.997	-37.5394	<0.001
Body Corpus Callosum	-	-8.32	-6.601	-14.2154	<0.001
Cingulum	L	-9.213	-7.256	-20.9262	<0.001
Cingulum	R	-7.492	-4.844	-26.5918	<0.001
Fornix	-	-22.022	3.757	-34.2933	<0.001
Genu Corpus Callosum	-	-11.965	-9.295	-13.1483	<0.001
Posterior Limb IC	L	-7.217	-3.892	-55.7216	<0.001
Posterior Limb IC	R	-7.448	-2.591	-76.6294	<0.001
Sagittal Striatum	L	-8.195	-13.34	13.4305	<0.001
Sagittal Striatum	R	-12.541	-6.154	-37.2267	<0.001
SLF	L	-10.333	-6.686	-34.7121	<0.001
SLF	R	-9.973	-7.108	-28.2543	<0.001
Splenium Corpus Callosum	-	-8.937	-9.078	0.0272	0.304
Uncinate	L	-9.755	-4.643	-34.3965	<0.001
Uncinate	R	-3.729	2.788	-36.1965	<0.001

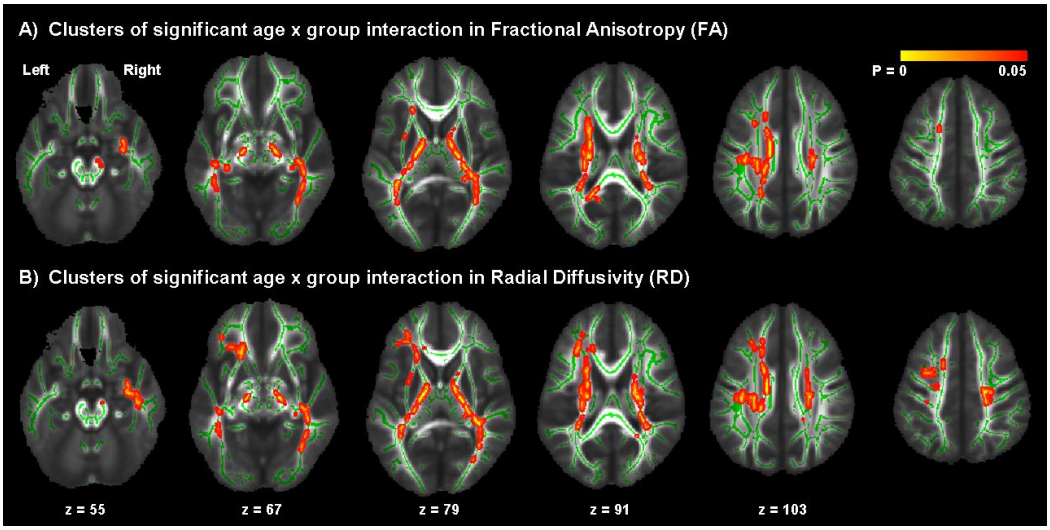


FIGURE 6.1 RESULTS OF TBSS ANALYSIS OF FA AND RD

Age x group interaction for (A) FA and (B) RD, overlaid mean FA computed from all subjects. FA increases with age in both groups but increases faster in the Autism Spectrum Disorder (ASD) group than in typical controls, where as RD decreases with age in both groups but decreases faster in the ASD group than in the control group. Statistically significant voxels are displayed in red-yellow; white matter skeleton voxels are displayed in green, overlaid onto mean FA computed from all subjects. Significance was set at p<0.05 corrected for multiple comparisons with family wise error. Group differences 'thickened' and images flipped in the right-left plane for visualization purposes.

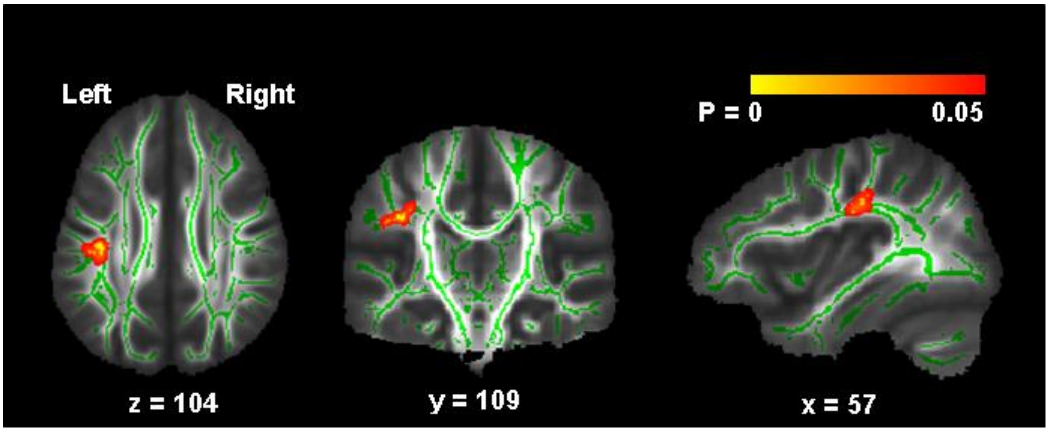


FIGURE 6.2 GROUP DIFFERENCES IN LATERALIZATION

Cluster of significant difference between the ASD and control group in asymmetry of FA (FA). Left-ward asymmetry is increased in the ASD group in this region. Statistically significant voxels are displayed in red-yellow; white matter skeleton voxels are displayed in green. Significance was set at p <0.05 corrected for multiple comparisons with family wise error. Group differences 'thickened' and images flipped in the right-left plane for visualization purposes.

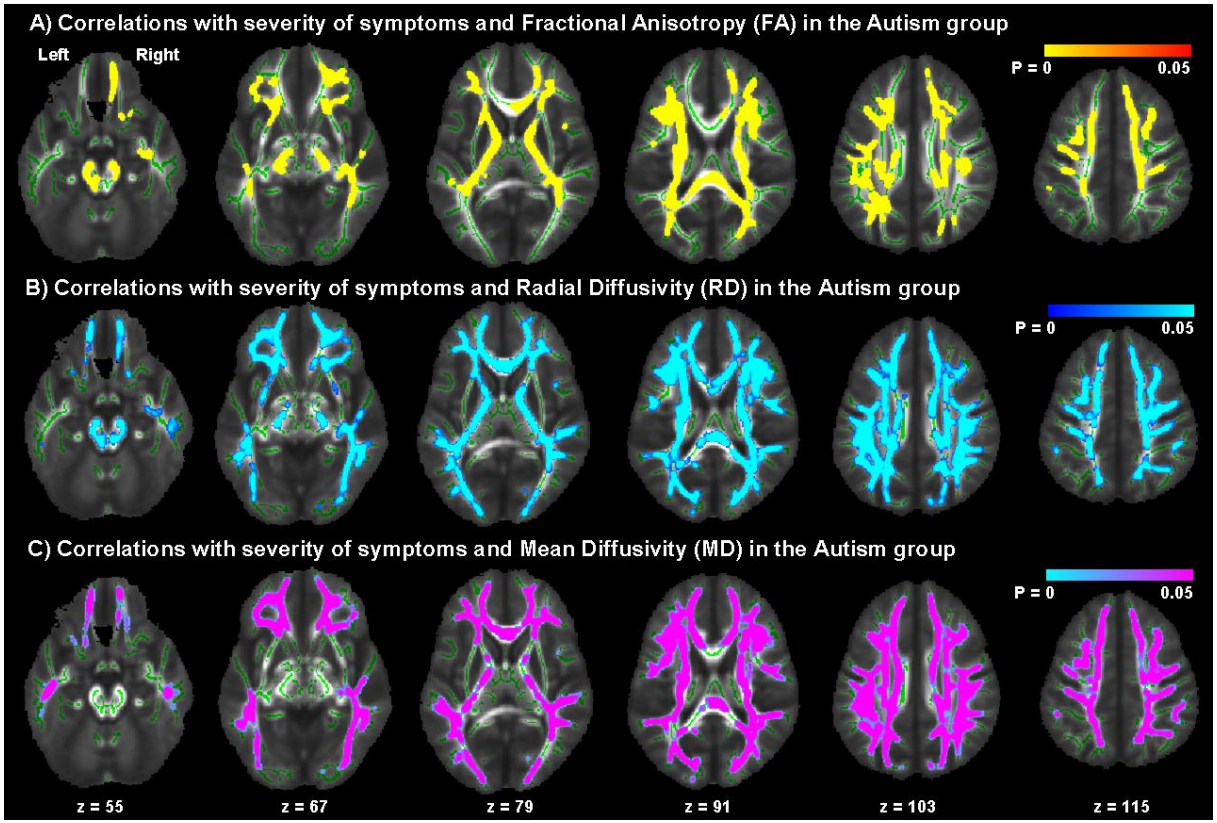
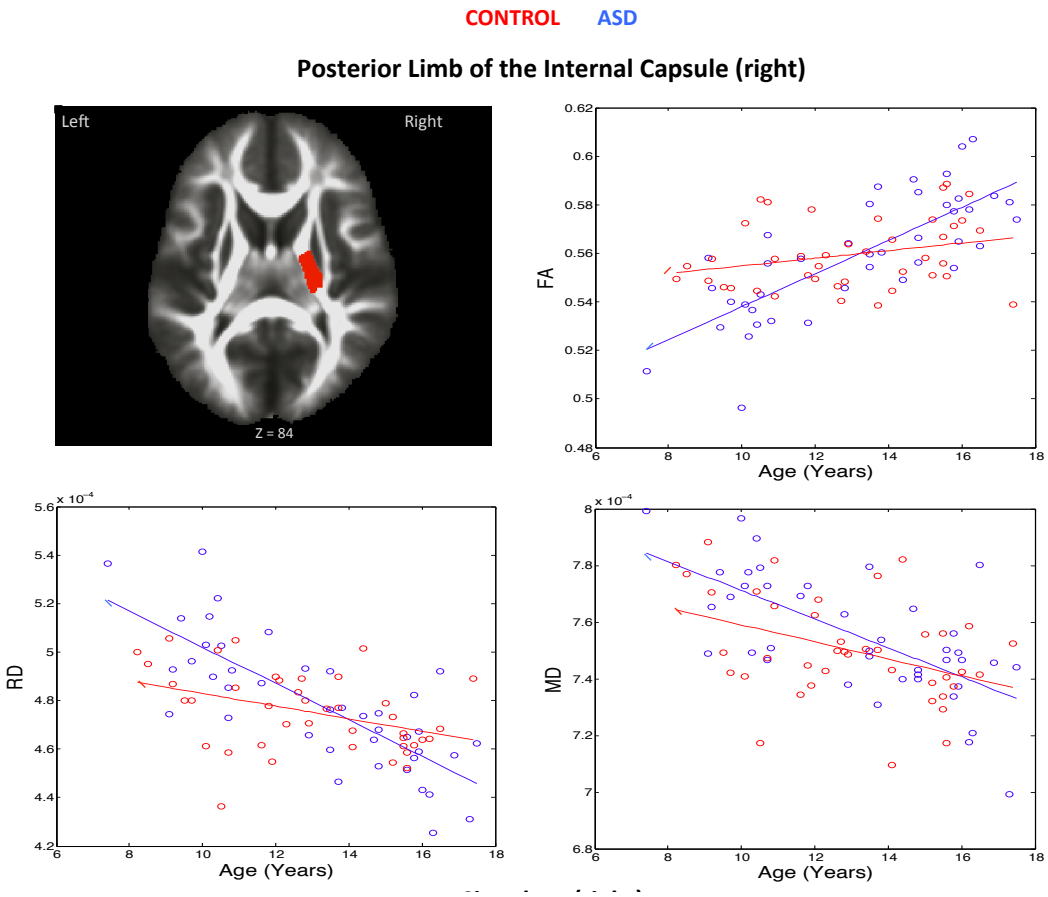


FIGURE 6.3 CORRELATIONS WITH ADOS-D SYMPTOM MEASURES

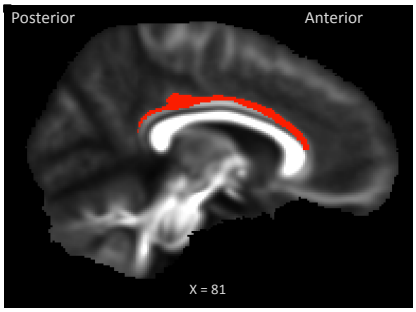
Clusters of significant correlation between the ADOS-D measures in the ASD group and (A) FA, (B) RD and (C) MD. Correlations were negative for FA and positive for RD and MD. Statistically significant voxels are displayed in red, blue and purple; white matter skeleton voxels are displayed in green. Significance was set at $p < 0.05$ corrected for multiple comparisons with family wise error. Group differences 'thickened' and images flipped in the right-left plane for visualization purposes.

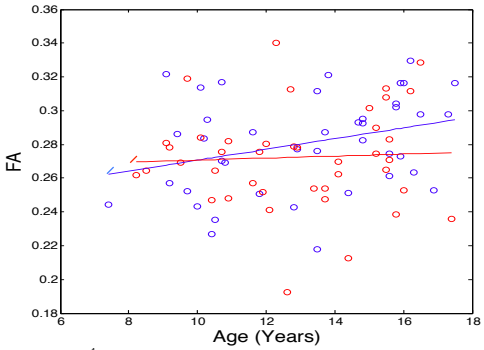
FIGURE 6.4 (CONTINUED OVERLEAF)

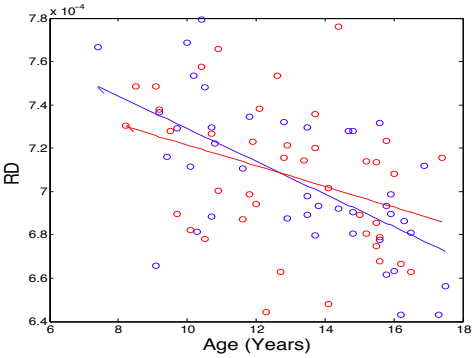
Examples of three regions of interest are shown (Results for all sixteen regions of interest are included in supplementary figure 1). Regions derived from the John Hopkins University white matter atlas are co-registered with mean diffusion data. For each white matter pathway, a plot of the raw data and mean linear trend-line can be seen for FA, MD and RD. Blue corresponds to participants with ASD, and Red with typically-developing controls. All three of these pathways had a significant ($p < 0.05$ corrected) difference in developmental trajectory. Images flipped in the right-left plane for visualization purposes.

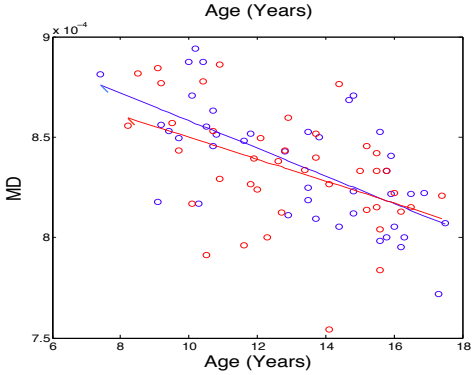


Cingulum (right)

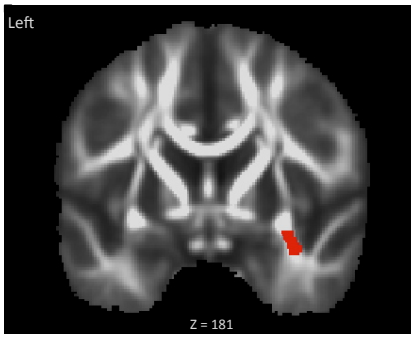


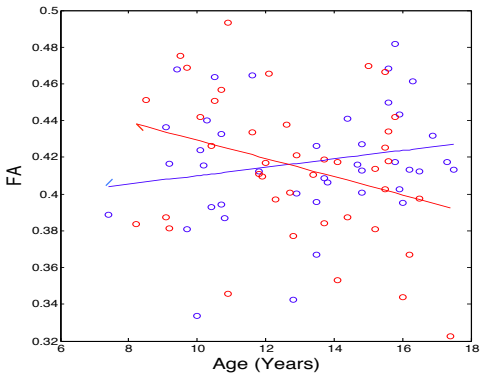


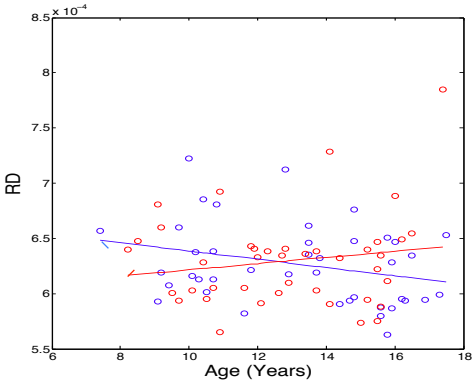


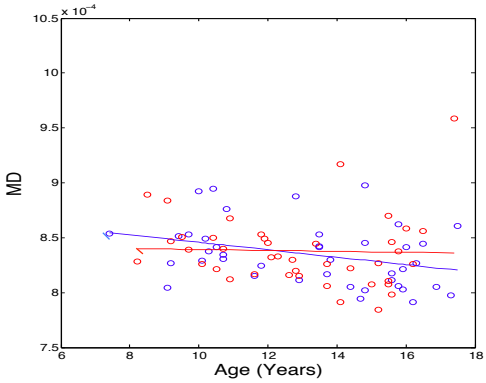


Uncinate (right)









MOy

DISCUSSION

We found significant differences in the trajectory of white matter maturation between children with ASD and typically developing controls. The developmental differences in white matter were observed in spatially distributed white-matter regions across the brain. In both groups, measures of FA increased significantly with age, while measures of both RD and MD decreased over time. These findings are in agreement with previous neuroimaging studies suggesting that neurodevelopmental changes in diffusivity accompany the general maturation of white matter connections in the brain [78, 79]. We further observed that individuals with ASD showed a more pronounced increase in FA with age, which was accompanied by a concomitant decrease in MD and RD, particularly in brain regions and white-matter tracks that have previously been implicated in the disorder. Additionally, we found that these DTI measures significantly correlated with the severity of stereotyped behaviours and restricted interests as measured by the ADOS (domain D) within the ASD group. On the whole, our findings corroborate with previous studies that have noted widespread white-matter differences in multiple brain areas in ASD and explicitly highlight the importance of aging in the disorder.

Whilst we have reported extensive age-related between-group differences at both the voxel- and region-wise levels, we did not observe any significant between-group differences per se. This could indicate that the most important effect, of aging, is overlooked in simple group comparisons. A few

recent DTI studies have indicated an altered developmental trajectory of white matter in ASD, in infancy [36], early childhood [46], and from adolescence to adulthood [42]. For example, Walker et al, 2012 showed that FA increased faster in typical controls than in young children with ASD (aged 2-8)[46]. Our results extend these previous observations, as we observed the strongest reductions in FA predominantly in the lower percentile of our age range (which overlaps with theirs). In addition, we have demonstrated that in later childhood and adolescence, FA increases more rapidly in the ASD group relative to controls.

Concurrently, we found that increased FA, and reduced RD and MD values correlated with fewer observations of stereotyped behaviours and restricted interests within the ADOS setting. This trend was observed across widespread brain areas. This pattern of correlations suggests that whilst the ASD group is characterized by a more exaggerated increase in FA, this is concomitant with a reduction of ASD symptomatology. Thus, the accelerated change in diffusion measures in the ASD group may in fact be adaptive. A number of studies have suggested that abnormalities in the ASD brain become less pronounced with age, with some studies reporting no significant differences in adulthood [11, 18, 42]. Our findings are in agreement with this trend.

We also report differences in white-matter lateralization in our sample, which has been noted in prior studies examining both grey and white matter [74, 75, 80]. Altered brain asymmetry has been suggested as a fundamental characteristic of the disorder [74], possibly relating to specific genetic and environmental effects [81, 82]. To our knowledge, no previous study has

investigated the asymmetry of white matter structural indices in ASD using the whole brain method of TBSS. We found a significant group difference in asymmetry of FA indices in the inferior region of the post-central gyrus, which was significantly more left-lateralized in the ASD group. The region of increased left lateralization in the ASD group in the present study included white matter of the superior longitudinal fasciculus. In typically developing individuals the superior longitudinal fasciculus is right-lateralized, and is involved in a right-hemispheric network which underpins visuospatial attention [83]. Abnormal left-lateralization of this tract in the ASD group may thus be indicative of abnormal hemispheric specialization of visuospatial abilities. Such abnormality could relate to reports of alterations in visuospatial processing in individuals with ASD, and may, for example, contribute to weak visuospatial coherence [84]. This would be of interest to investigate in further studies.

Overall, diffusion measures reflect a number of underlying biological processes, which need to be considered when interpreting the current findings. Alterations in FA, RD and MD may suggest differences in fibre organization and geometry, myelin formation and myelin remodelling, as well as inflammation and gliosis [85-89]. This lack of specificity of diffusion measures constitutes an inherent limitation of DTI. This is further compounded by the finding that DTI measures may also be impacted by extrinsic factors that render group-differences specious. An example of such an effect comes from group differences in head motion in the scanner. Increased in-scanner motion in one group has lead to significantly decreased FA and increased RD in comparison to a relatively motionless control group [69, 70]. These findings are

particularly salient when investigating children and individuals with ASD, who may display increased movement inside the scanner. In the present study the amount of in-scanner motion was not found to be different between the control and ASD groups, suggesting that group differences in motion are unlikely to be an explanatory factor in the present findings. In line with this, we did not find decreased FA and increased RD in the group comparison, which would have been predicted as a result of increased motion [69, 70].

To conclude, our findings indicate a distinct neurodevelopmental trajectory of white matter maturation in ASD. However, without further histological validation these differences cannot be attributed to a specific biological process or feature. As the differences we see in FA, MD and RD are widespread, our study confirms that ASD is a neural systems disorder with neurodevelopmental differences in white-matter brain connectivity.

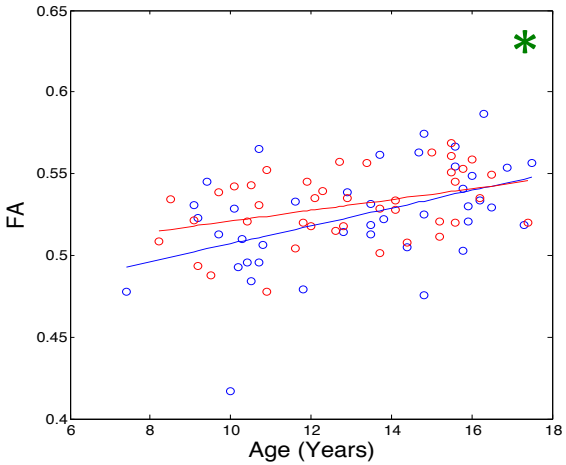
SUPPLEMENTARY FIGURE 6.5.

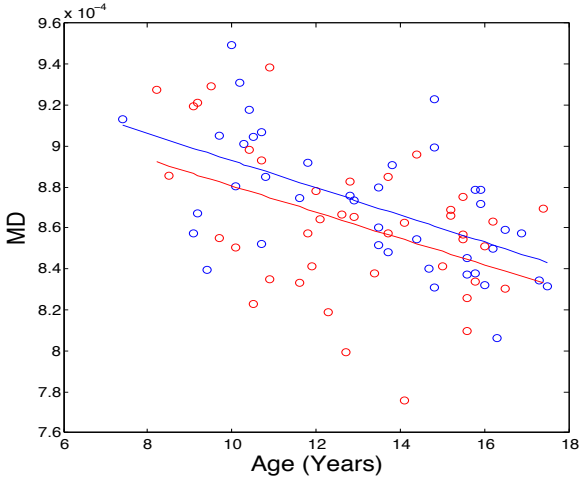
RESULTS OF REGION OF INTEREST ANALYSIS (DISPLAYED OVERLEAF)

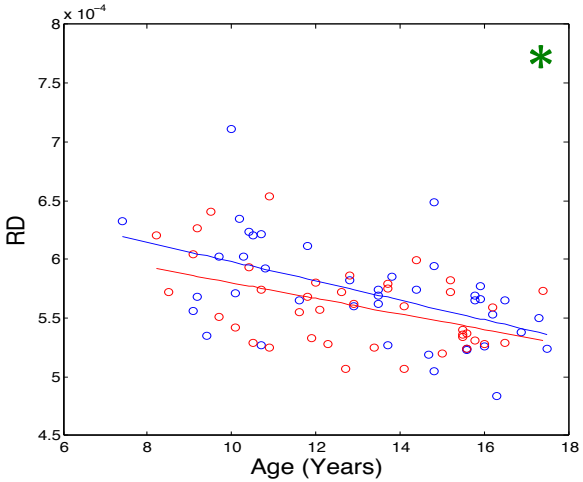
Scatter plots of raw data with superimposed mean linear development trajectories for sixteen white matter pathways. Blue corresponds to participants with ASD, and Red with typically developing controls. A star (*) denotes pathways with a significant ($p<0.05$ corrected) difference in developmental trajectory.

ASD : CONTROL

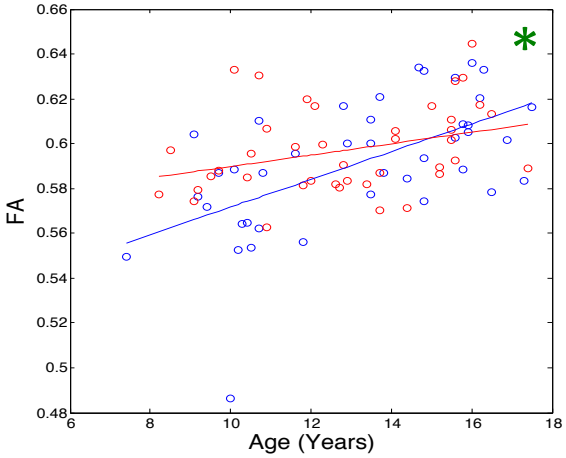
Body of the Corpus Callosum

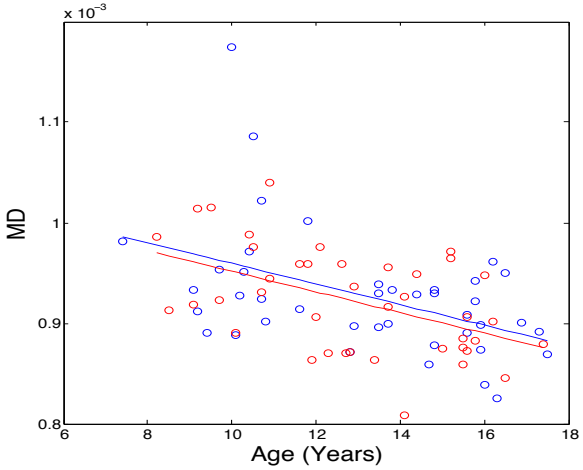


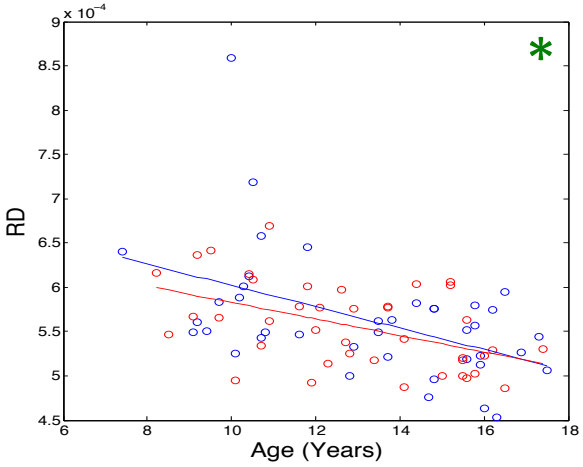




Genu of the Corpus Callosum



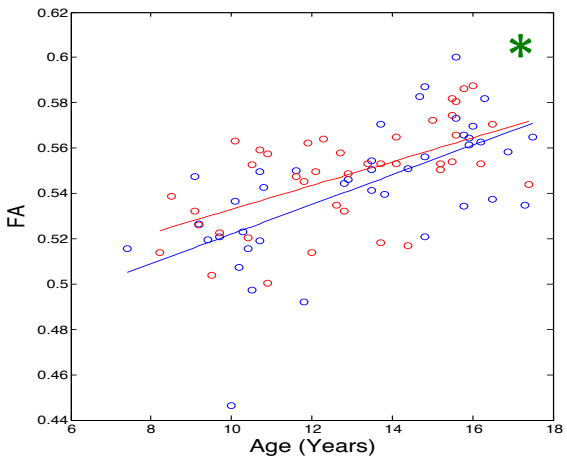


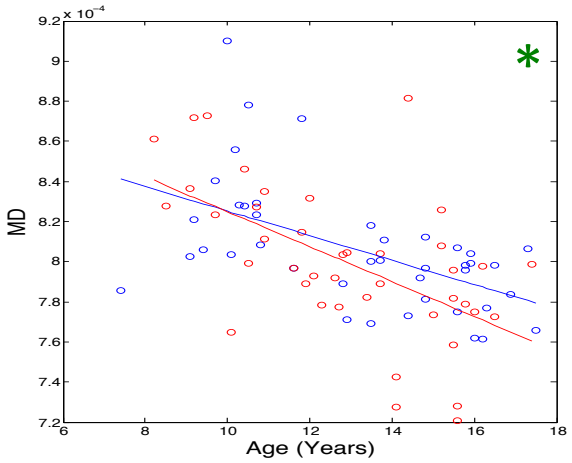


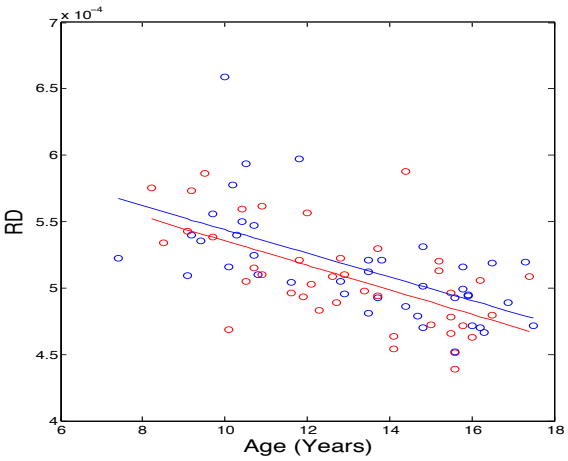
M43

ASD : CONTROL

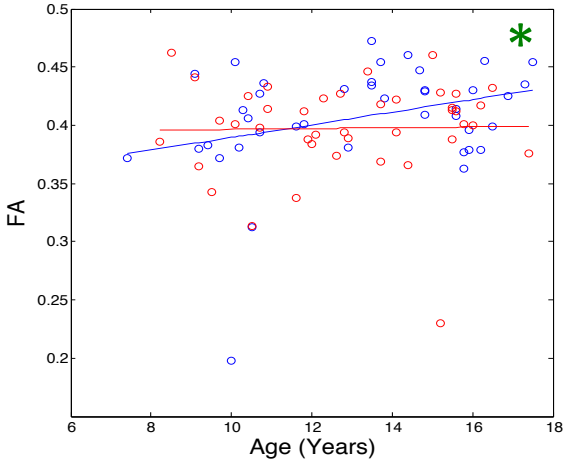
Splenium of the Corpus Callosum

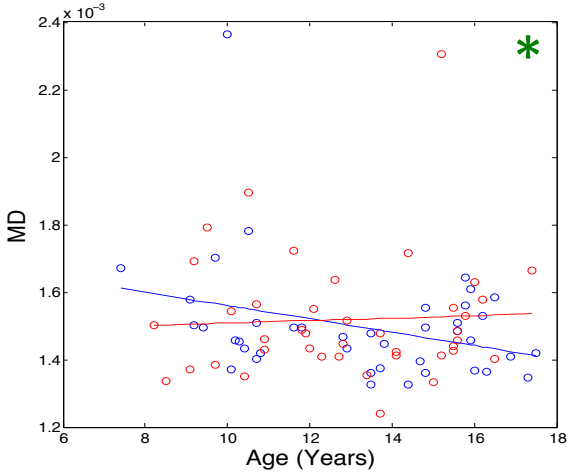


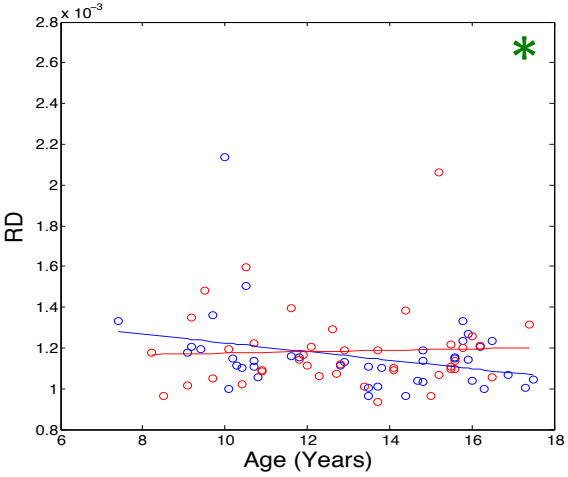




Fornix

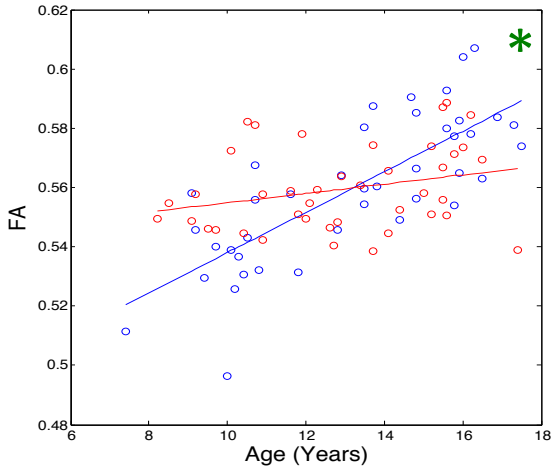


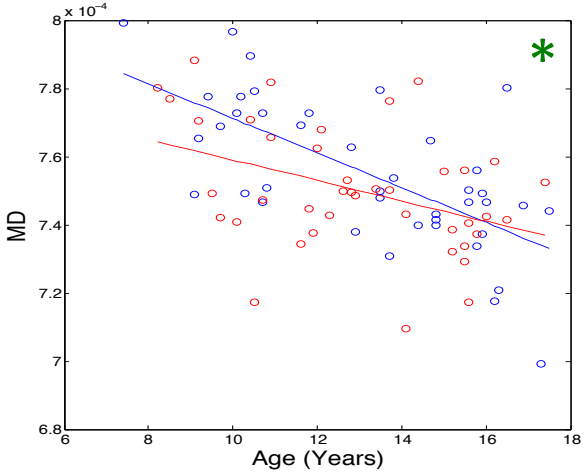


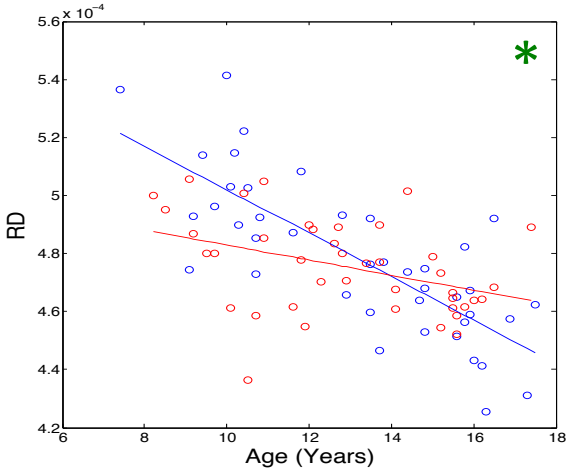


ASD : CONTROL

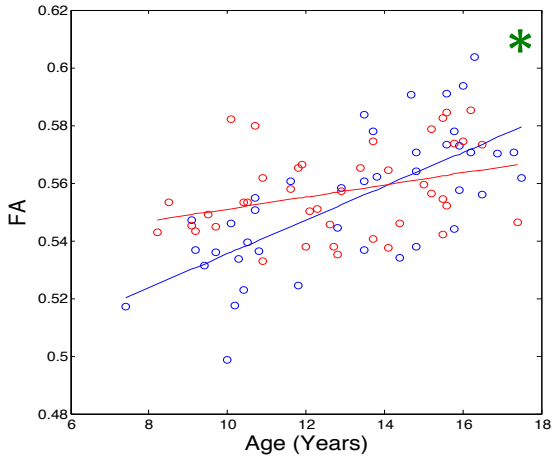
Posterior Limb of Internal Capsule (right)

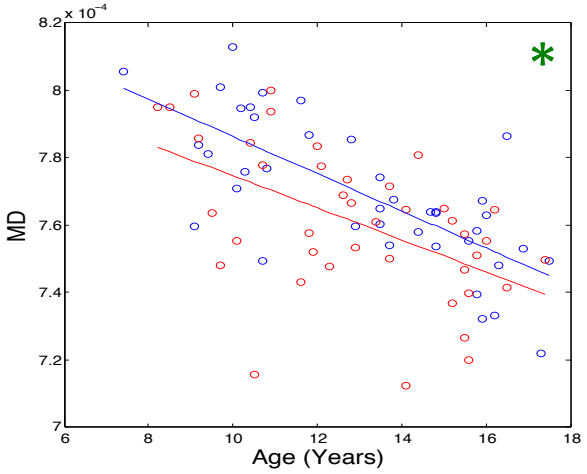


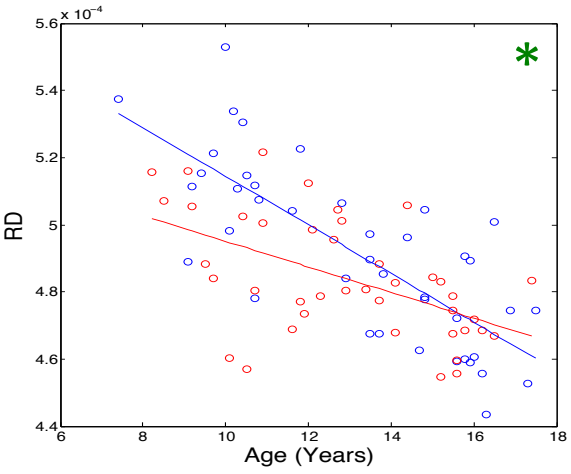




Posterior Limb of Internal Capsule (left)



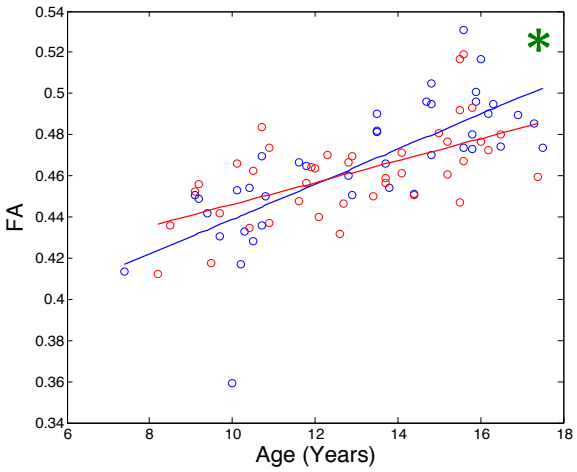


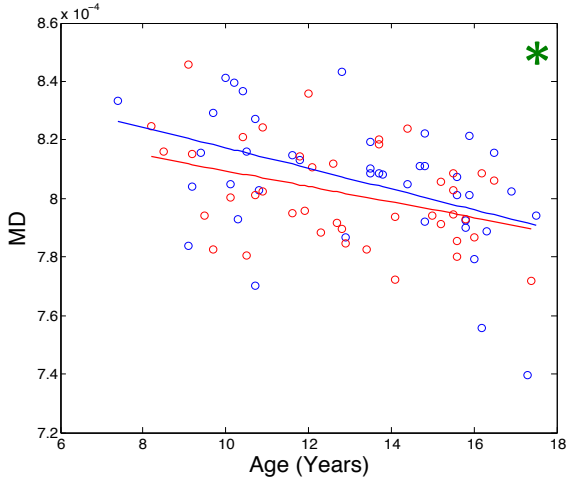


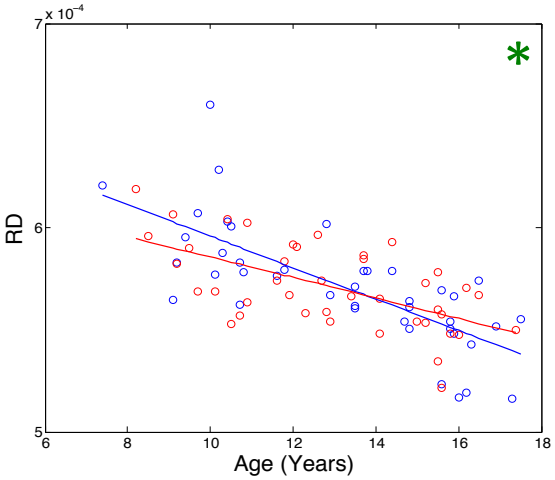
W45

ASD : CONTROL

Anterior Limb of Internal Capsule (right)







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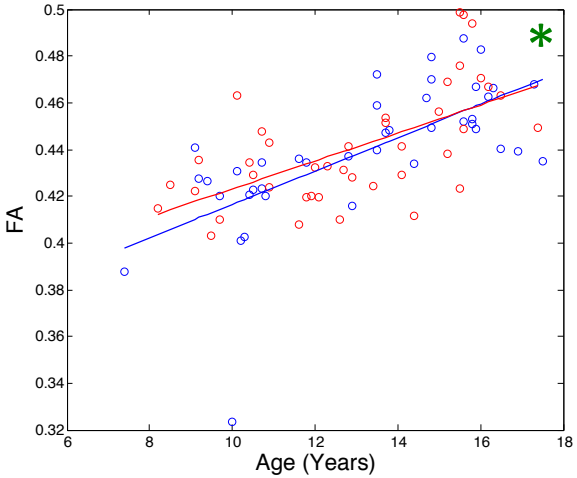
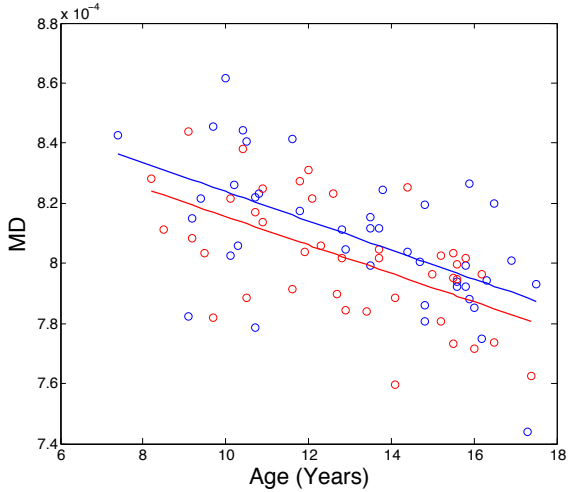
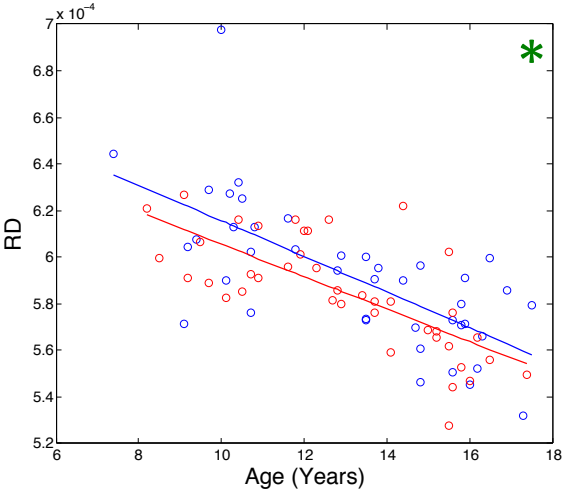
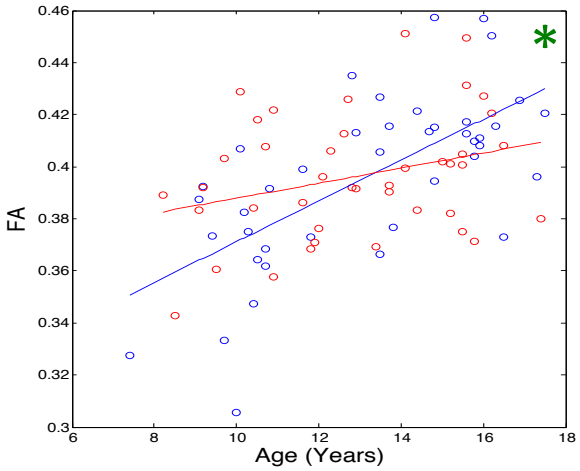


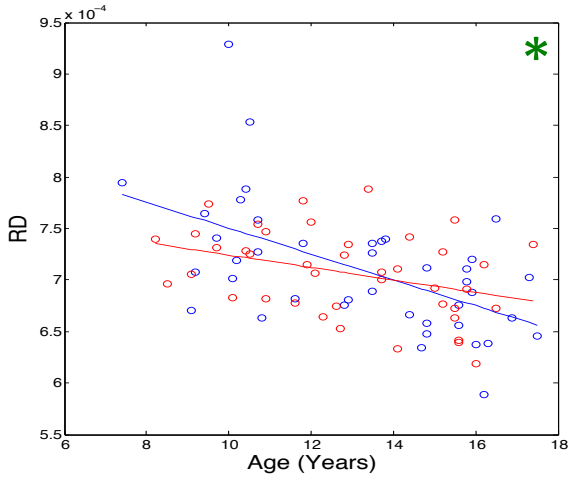


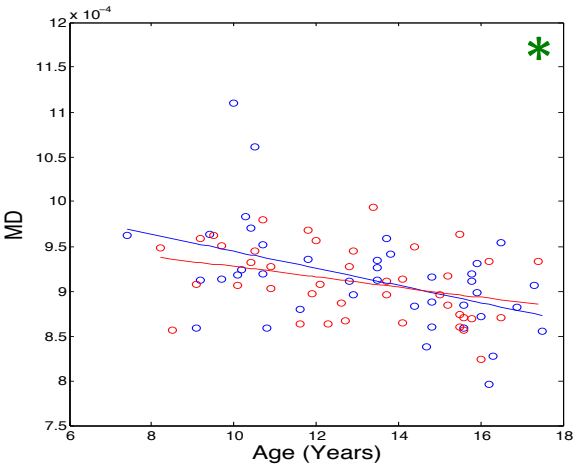


ASD : CONTROL

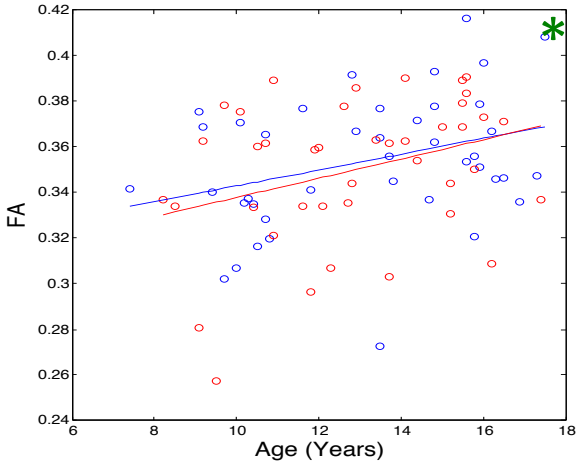
Sagittal Striatum (including ILF) (right)

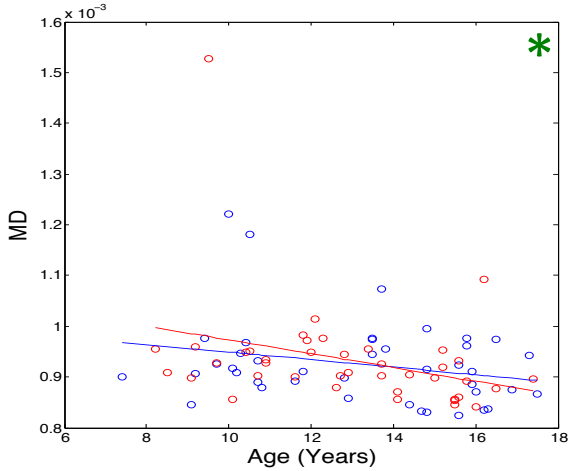


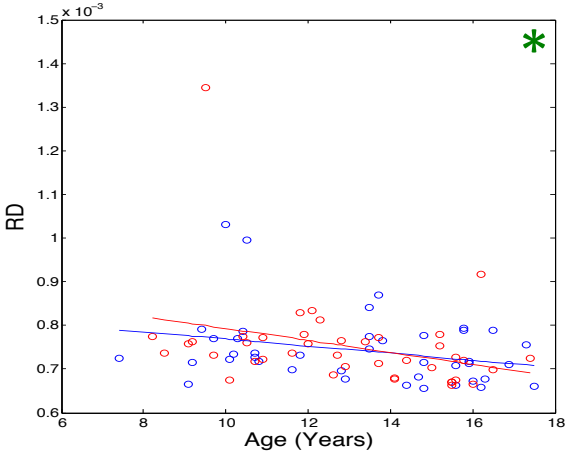




Sagittal Striatum (including ILF) (left)



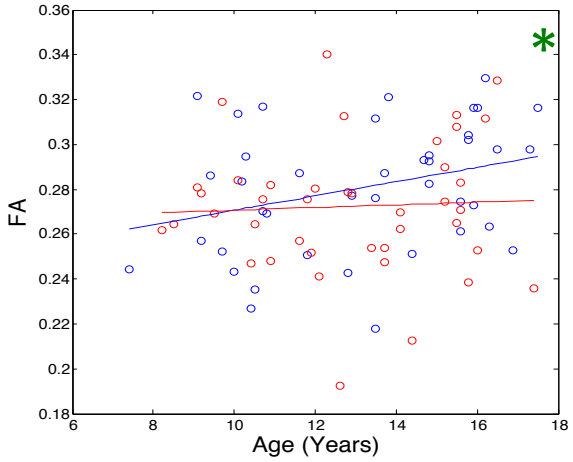


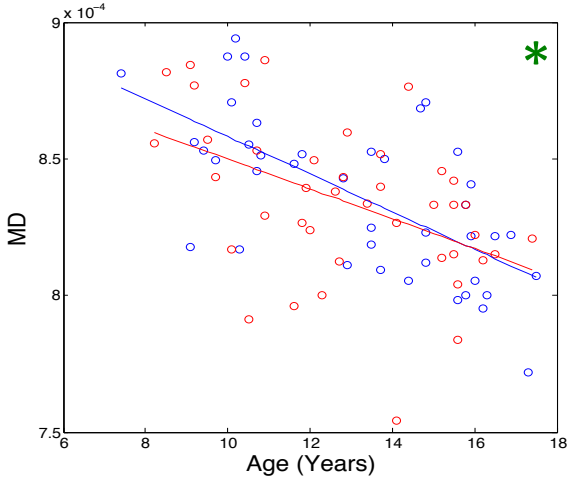


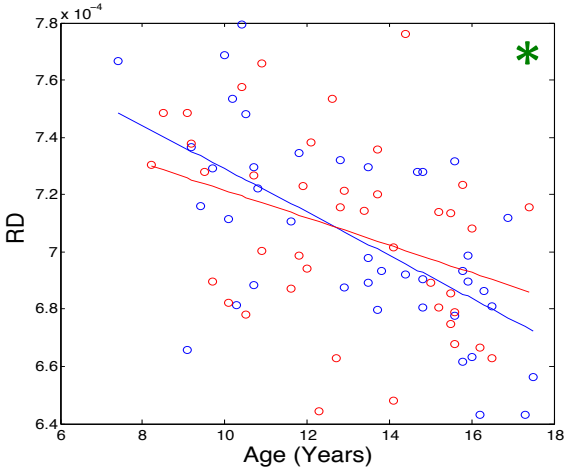
M47

ASD : CONTROL

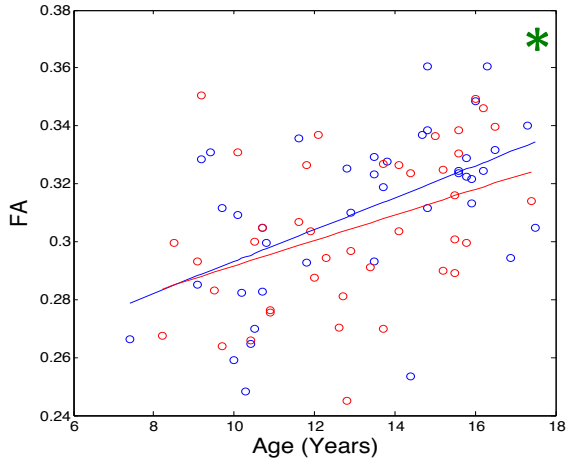
Cingulum (right)

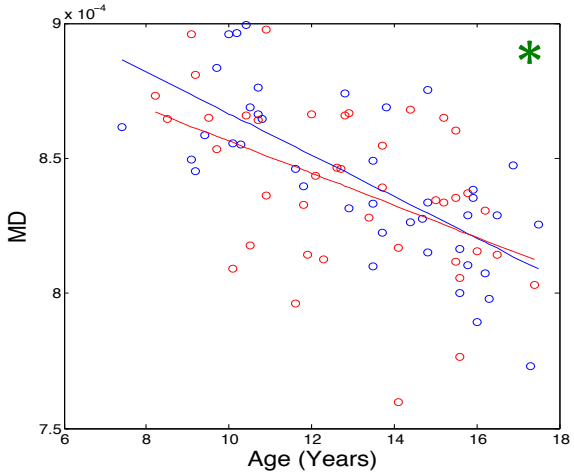


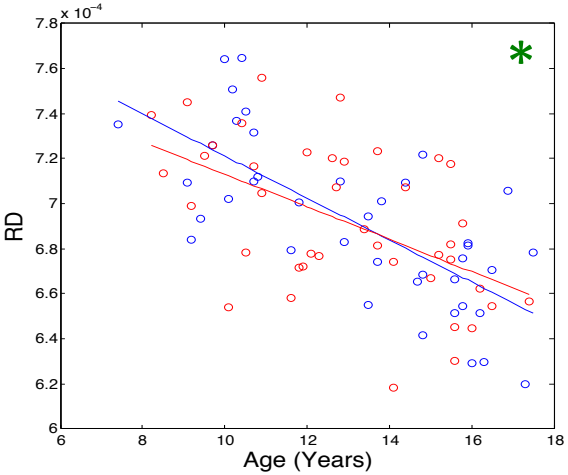




Cingulum (left)



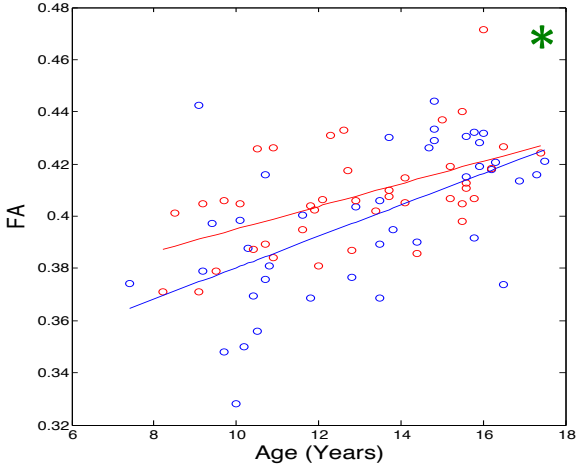


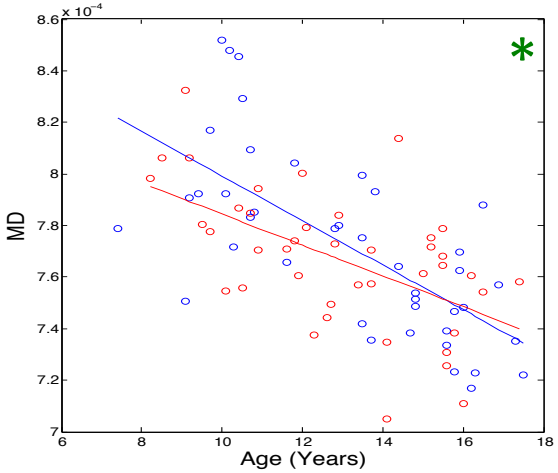


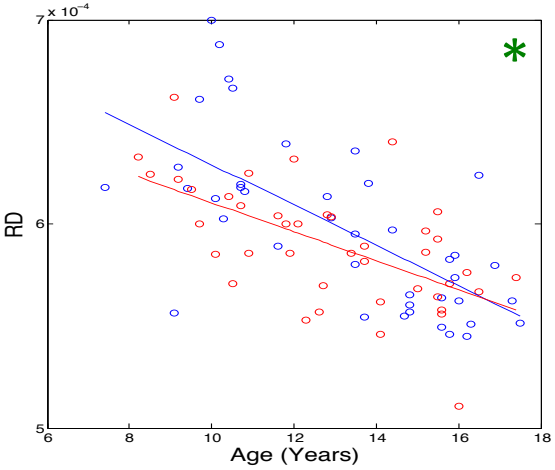
M48

ASD : CONTROL

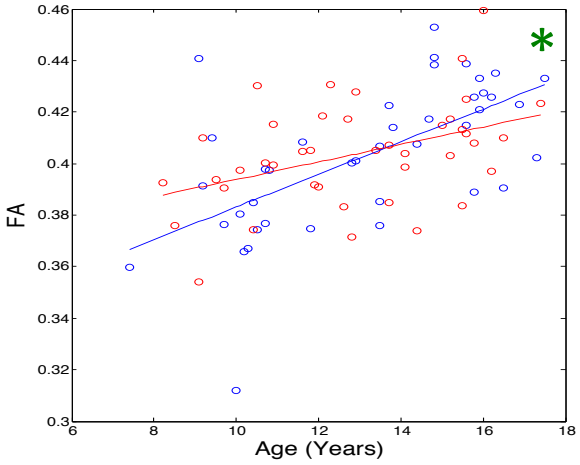
Superior Longitudinal Fasciculus (right)

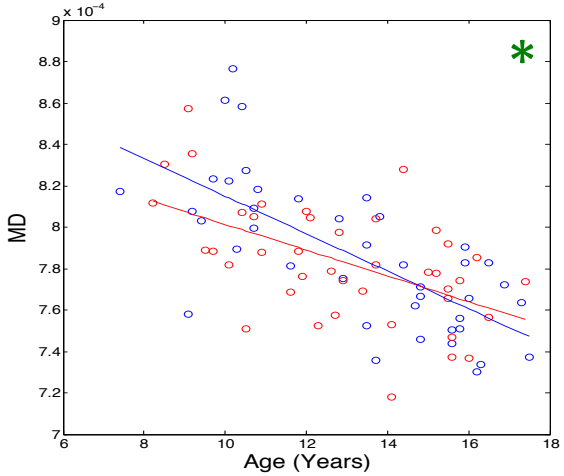


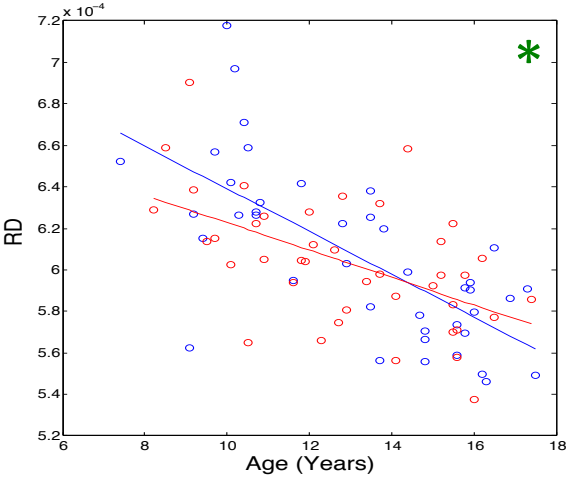




Superior Longitudinal Fasciculus (left)



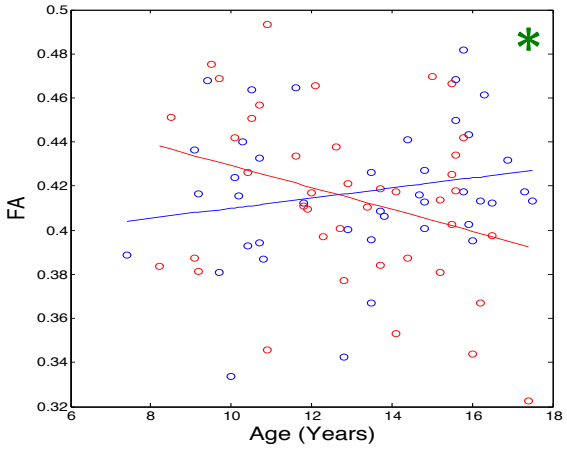


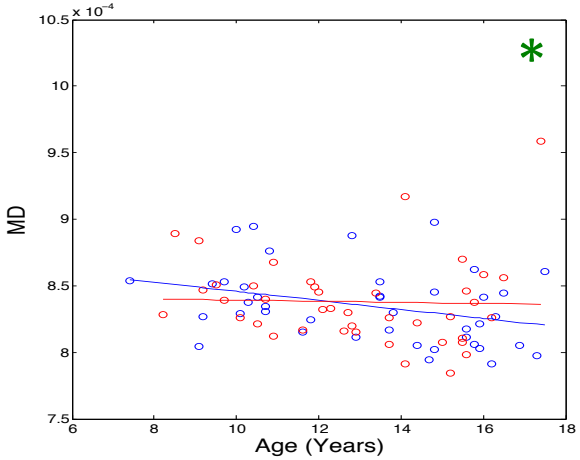


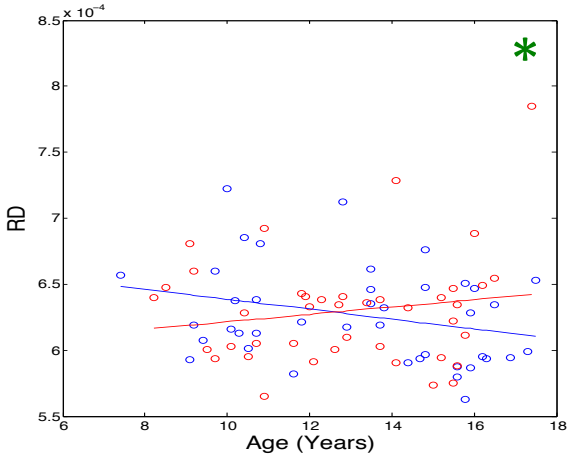
M49

ASD : CONTROL

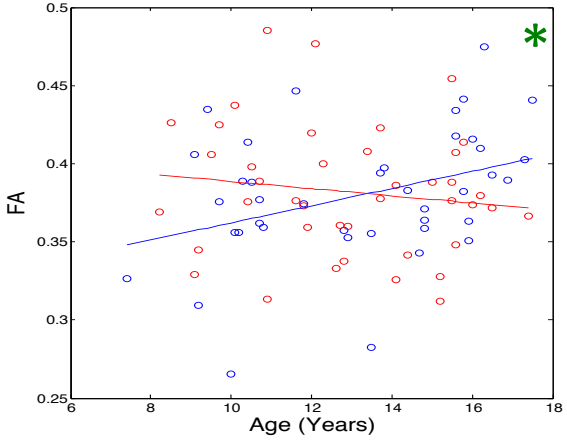
Uncinate (right)

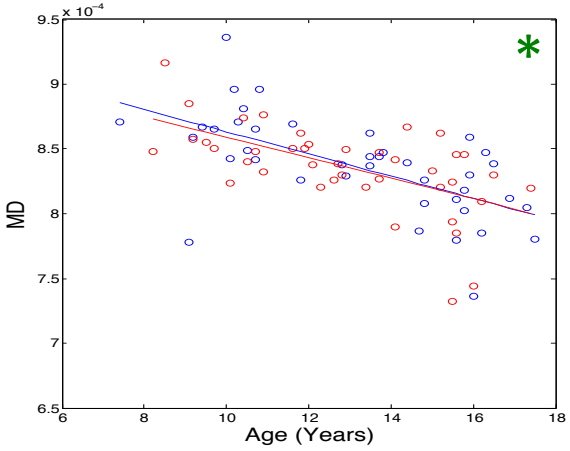


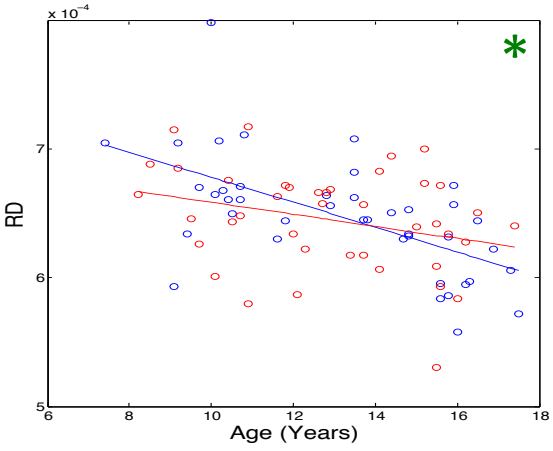




Uncinate (left)







M50

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CHAPTER. **7**

GYRIFICATION AND ITS RELATIONSHIP WITH STRUCTURAL CONNECTIVITY IN AUTISM SPECTRUM DISORDER

ABSTRACT

Autism spectrum disorder is a lifelong neurodevelopmental condition accompanied by differences in cortical grey-matter morphometry and white-matter connectivity. However, the relationship between these grey- and white-matter differences remains to be elucidated. Therefore, we examined age-related differences in gyrification alongside DTI diffusion measures in a cross sectional sample including 23 male children and adolescents with ASD, and 27 neurotypical controls matched for age and IQ. Initially we examined linear, quadratic, and cubic effects of age, and discovered that the quadratic model best predicted the developmental trajectory of gyrification. Subsequently, gyrification (lGI) was analysed using a general linear model including both linear and quadratic age terms, as well as their interactions with the main effect of group. When controlling for the effects of age, we found significant increases in lGI measures in ASD relative to controls in two significant clusters in right temporo-parietal and occipital regions. When we examined white-matter structural connectivity in the tracts that originated or terminated within these significant lGI clusters, no corresponding between-group differences or age x group interactions for measures of diffusion were found. However, despite the lack spatial correspondence for between-group differences in DTI measures, we did observe a significant correlation between lGI measures and tract-specific measures of diffusion in the right temporo-parietal cortex cluster. Our results indicate that the degree of cortical gyrification is correlated with the underlying white-matter architecture. Therefore, measures of grey- and white-matter connectivity should not be interpreted independently, but jointly during development as they elicit the atypical patterns of cortico-cortical connectivity typically observed in ASD.

INTRODUCTION

Autism Spectrum Disorder is a life-long condition defined by early-onset impairments in social communication and reciprocity, and a propensity

for repetitive and stereotyped behaviour [1]. It is accepted that these primary symptoms are accompanied by differences in cortical grey-matter morphometry and white-matter connectivity [2-4]. However, despite abundant research into the condition, our understanding of the neuropathological underpinnings of ASD remains incomplete, particularly with regards to the relationship between these grey- and white-matter differences.

Evidence suggests that ASD is a disorder of early developmental origin, marked by a period of accelerated brain growth during early postnatal life [5-7], which drives the brain to be comparatively larger in toddlers and young children with ASD than typically developing controls [8-11]. This accelerated increase in total brain volume is then followed by a period of atypically slow or hindered growth throughout the remainder of childhood, such that no global differences are generally observed by adulthood [12-14]. Nevertheless, it is clear that this altered trajectory has expansive consequences on brain morphology and neural circuitry, demonstrated by residual abnormalities in multiple, spatially-distributed neurocognitive systems.

It has been proposed that functional connectivity, and 'brain coherence' is an emergent property of collaboration among brain areas [15]. Consequently, the expansion of early-developing lower-order brain regions becomes a limiting factor for the elaboration of more complex higher-order association cortices, which integrate higher cognitive functions [16]. This is particularly important in relation to social behaviour, language and communication, as these core domains demand the time-sensitive integration of information from spatially discrete brain regions [17], and so the development of each depends greatly on the capacity of earlier developing components [4, 18]. Concurrently, there is a large body of evidence for the disrupted organization of these cerebral networks in ASD [19, 20]. Patterns of disrupted connectivity have been documented in childhood, adolescence and adulthood [15, 21-25], and a number of studies also suggest a pattern of local over-connectivity coupled with long distance under-connectivity in ASD [26, 27]. For example, reductions in long-range functional connectivity have been identified mostly in frontal regions using several functional MRI (fMRI) models of

cognition, including executive functioning [28], working memory for faces [29] and facial-affect processing [30].

Evidence for divergent patterns of over- and under- connectivity also comes from structural MRI findings, based on the examination of white matter tracts using techniques such as diffusion tensor imaging. For example, studies using DTI have reported lower FA in the corpus callosum of children, adolescence and adults with ASD [31-35], thus providing evidence for abnormalities in inter-hemispheric white-matter connectivity in ASD. Local over-connectivity, on the other hand, has been a more difficult concept to prove, as it is difficult to measure using conventional neuroimaging approaches due to limitations with regards to the spatial resolution of MRI techniques [36]. However increased volume and microstructural integrity in radial white matter structures has been interpreted as evidence for local over-connectivity [37, 38].

Another aspect of brain structure that may be related to the concept of local connectivity is the degree of cortical gyrification, which is thought to be reflective of brain development during prenatal and early post-natal life [39, 40]. Although the determinants of specific gyral arrangements are poorly understood, it has been theorized that cortical shape is driven by patterns of neural connectivity. For example, the tension-based theory of convolutional morphogenesis (van Essen) proposes that strongly interconnected cortical regions are drawn towards one another during embryological development as a result of the cumulative tension exerted by the axons that connect them [41]. This theory closes the link between brain surface morphology and the underlying regional (i.e. local) micro-circuitry of the brain, within a developmental framework. Concepts such as the axonal-tension theory are thus of direct relevance to a neurodevelopmental disorder such as ASD, which is also accompanied by differences in white matter.

Indeed, there is evidence to suggest that the brains of children and adolescents with ASD are atypically shaped, owing in part to gyral abnormalities. More precisely, increased gyrification in ASD has been noted in the bilateral posterior cortices [42], right parietal cortex [43], and left inferior frontal language regions [44], whilst other studies have reported increased

overall gyral complexity [45] relative to typically developing controls. In addition, Hardan et al., reported findings of greater prefrontal gyrification in children and adolescents, but not adults with ASD [46], indicating a possible interaction between age and diagnosis on measures of gyrification. These observations also highlight parallels with recurrent findings of white matter alterations in ASD, particularly in the long association pathways that connect intra-hemispheric cortical regions (e.g. the inferior and superior longitudinal fasciculi, uncinate, and arcuate fasciculus [34, 47-49]).

Despite the abundance of evidence for disordered connectivity and atypical surface morphometry in ASD, few studies have combined findings from different MR modalities to correlate coexisting differences. There are a few integrated studies that examine how differences in cerebral morphology and connectivity fit together (reviewed in, [50]) but multi-modal approaches to examining brain structure and connectivity are rare. Recently, a study by Schaer et al. (2013), examined cerebral morphometry and structural connectivity in a small sample of children and adolescents (11 ASD : 11 controls), using an approach that combined DTI measures and vertex-wise measures of cortical thickness and local the Gyrification Index (lGI). Reduced gyrification was noted for the ASD group compared to controls in four clusters across the right inferior frontal, and medial parieto-occipital regions of the cortex, and tractography analysis revealed coincident reductions in (anterior) callosal volume, and inter-hemispheric frontal streamlines. Interestingly, no correlation was found between the observed differences in gyrification and long-range connectivity, but short-range fibres in the frontal lobe and inferior parietal cortex correlated positively with the observed lGI changes [35]. Schaer et al's novel findings demonstrate the feasibility of multimodal investigations for integrating findings of altered cortical morphometry and connectivity across imaging modalities. However, these preliminary findings are limited by a small sample size and inclusion of both male and female participants (adding the complication of sexual dimorphism), and thus warrant replication in a large and well-characterized sample of individuals with ASD and matched neurotypical controls.

Thus, in the present study, we examined differences in brain morphometry and white-matter connectivity in ASD, utilizing a multi-modal approach to examine age-related differences in DTI diffusion measures alongside vertex-based measures of gyrification. In addition, we examine these differences within a developmental framework as studies of typical development have shown that white matter pathways continue to mature linearly [51, 52], and the cerebral cortex continues to become more complex [53, 54] through childhood and adolescence (despite a pronounced decrease in GM occurring during adolescence [16, 55]). At present, the developmental trajectory for *l*GI measures remains unknown. Therefore, we examine linear, quadratic, and cubic age effects in order to find the most 'parsimonious' model (i.e., model with the smallest number of parameters) that allows the examination of between-group differences in *l*GI measures in the presence of significant age effects, as well as age x group (age 'by' group) interactions.

Materials And Methods

Participants

23 males with ASD (aged 9–17years) and 27 typically developing male controls (aged 9–17years) were recruited by advertisement and assessed at the Institute of Psychiatry, London. All participants were right handed (measured using The Edinburgh Handedness inventory [56]), and native English speakers. Exclusion criteria included; pre-existing medical conditions or complications (e.g. head trauma, epilepsy); use of medication affecting brain function; mental retardation; a history of major psychiatric disorder (e.g. psychosis); chromosomal abnormality (e.g. fragile X, Tuberous Sclerosis, VCFS); and any MRI contraindications. Intellectual ability was assessed using the WASI [57]. All participants had an IQ greater than 70 (within the high-functioning range of the autistic spectrum). For the autistic group, inclusion was based on a confirmation of autism diagnosis as required by the International Statistical Classification of Diseases, 10th Revision (ICD-10) research criteria and

confirmed using the ADI-R [58]. Current symptoms were assessed using the ADOS [59], but not used as an inclusion criterion. All participants and their parents or guardians gave informed written consent in accordance with ethics approval by the National Research Ethics Committee, Suffolk, UK.

Structural MRI and DTI Data Acquisition

All imaging for this study was acquired at the Centre for Neuroimaging Sciences, Institute of Psychiatry, London, UK, using a General Electric 3T MR system. High-resolution structural T1-weighted volumetric images were acquired through a high-resolution sagittally-oriented IR-SPGR sequence with full-head coverage. Nominal acquisition parameters: Field of View (FOV): 24cm2 x 16cm, matrix: 240 x 240 x 160, echo time (TE) / repetition time (TR) / inversion time (TI): 2.1ms/6ms/450ms and flip angle: 35°.

Diffusion tensor MRI scans were acquired with a spin-echo echo-planar imaging (SE-EPI) double refocused sequence providing whole head coverage with isotropic image resolution (2.4 x 2.4 x 2.4mm), 32mm diffusion-weighted volumes with different non-collinear diffusion directions with b-factor 1300 sec/mm^2 and 6 non-diffusion-weighted volumes; 60 slices; no slice gap; TE 104.5 msec; TR 20 R-R intervals; 128 x 128 acquisition matrix; FOV = 30.7 cm^2; peripherally gated (parameters compatible with [60]).

Total acquisition time for DTI and IR-SPGR and was approximately 19 minutes. Consistent image quality was ensured by a semi-automated quality control procedure. If participants were unable to tolerate scanning or obvious head movement was detected during the acquisition (due to anxiety or hyperactivity for example), they were invited to return for a second time - at which time scan quality was usually significantly improved.

CORTICAL RECONSTRUCTION USING FREESURFER

The FreeSurfer analysis suite (*vFS5.3.0 release,* http://surfer.nmr.mgh.harvard.edu/) was used to derive models of the cortical surface in each T1-weighted image. These well-validated and fully automated procedures have been extensively described elsewhere (e.g. [61-64]). In brief, a single filled white matter volume was generated for each hemisphere after intensity normalization, skull stripping, and image segmentation using a connected components algorithm. Then, a surface tessellation was generated for each white matter volume by fitting a deformable template. This resulted in a triangular cortical mesh for grey and white matter surfaces consisting of approximately 150,000 vertices (i.e. points) per hemisphere. Following standard FreeSurfer pre-processing, each reconstructed surface was then visually inspected for reconstruction errors, and images that did not reconstruct correctly (i.e. with visible anatomical abnormalities) were further excluded from the statistical analysis (dropout 4%).

Vertex-level measures of local gyrification were then derived as described by; [65]. The local gyrification index (lGI) is a local variant of the classical 2-dimensional (2D) gyrification index (GI) originally proposed by Zilles et al. (1988), which is defined as the ratio of the total pial surface area over the perimeter of the brain delineated on 2D coronal sections [66]. The lGI utilizes the high-resolution surface reconstructions provided by FreeSurfer to measure the degree of gyrification at each cerebral vertex, thus providing 3-dimensional (3D) measures of local gyrification at each spatial location on the entire cortical surface. The lGI at a given vertex v_i is computed as the ratio between the surface of a circular patch (i.e. geodesic circle with radius r centred at v_i) on the outer surface of the brain, and the surface of the corresponding patch at v_i on the pial surface (vertex positions are preserved across surfaces). Thus, the lGI at each point v_i reflects the amount of cortex buried within the sulcal folds in the surrounding area [65]. Clusters of significant between-group differences in lGI were utilized as regions-of-interest (ROIs) for subsequent DTI analysis.

DTI PRE-PROCESSING AND ROI-BASED AUTOMATED TRACTOGRAPHY

Diffusion data was pre-processed and analysed using TORTOISE software (https://science.nichd.nih.gov/confluence/display/nihpd/TORTOISE) [67], to reduce the effects of motion, and eddy current based deformations. Corrections were performed in the native space of each subject, and appropriate rotations were applied to the b-matrix [68, 69]. All deformations in TORTOISE were computed and applied in a single step to avoid multiple interpolations of the data. After correction, data was visually inspected and any volumes with residual artefacts were removed (accounting for <0.3% of the total number of volumes acquired across the cohort). Differences in subject motion have been shown to be an important consideration for group comparisons of DTI data [70, 71]. Therefore, to rule out the effect of age and between-group differences in head motion, a univariate analysis of variance was carried out to identify the main effect of age and group, as well as their interaction on the number of volumes removed and the amount of distortion and motion-correction applied during processing.

Fibre tracking was done using the standard FACT (Fibre Assignment by Continuous Tracking) propagation algorithm [72] implemented in DiffusionToolkit. The threshold angle for tract termination was set at 35°. FACT uses variable step sizes, depending on the length of the trajectory needed to pass through a voxel and tracts terminate if the selected threshold angle is exceeded between two consecutive tractography steps. Finally, diffusion tensor maps and whole-brain tractography were exported to TrackVis [73] for ROI-based tract dissection and visualization.

To examine the relationship between differences in local gyrification and white-matter structural connectivity, the individual's structural MRI data (T_1-weighted volumes and brain surfaces) were firstly co-registered with the DTI data using FreeSurfer tools and FSL FLIRT linear registration (fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT), which allowed us to create volumetric ROIs based on surface-based clusters (or labels). To create ROIs, cluster(s) with a significant between-group difference in lGI were mapped from the average

cortical surface in standard space to the individual's reconstructed white-matter surface in native space, thus preserving the individuals' cortical geometry within the cluster. The individual's surface-based clusters in native space were then converted to 3D volumes, and dilated spatially to cover both grey- and white-matter tissue within the ROI. This approach generated volumetric ROIs large enough to select tracts based on clusters defined on the cortical grey-matter surface, and to dissect white-matter fibre tracts within these ROIs in an automatic fashion using TrackVis (Figure 7.1). We included all tracts that originated and terminated in the surface-based ROI.

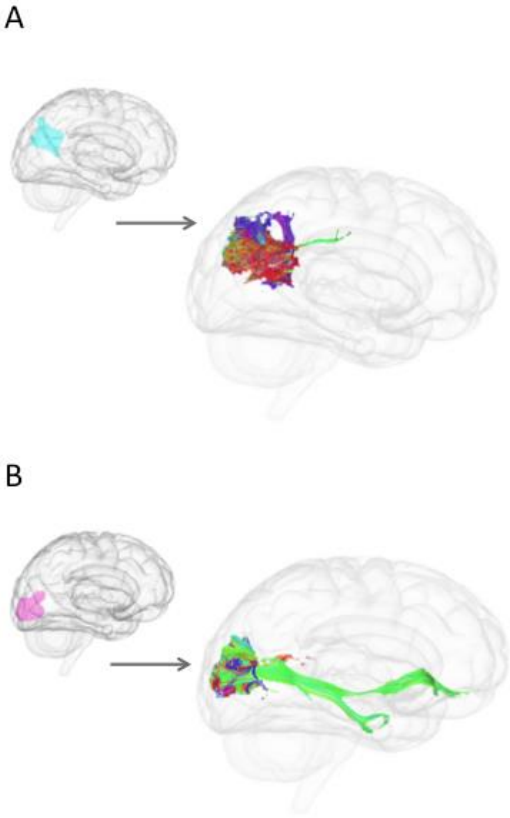

FIGURE 7.1.

Representation of automated fibre-tracking from surface based ROIs in (A) the temporo -parietal junction (B), the lateral occipital lobe.

Fractional anisotropy (FA), mean diffusivity (MD), axial or perpendicular diffusivity (AD), radial diffusivity (RD), and the number of streamlines for the dissected tracts were calculated using the Along-tract Statistics Toolbox for MATLAB [74].

Statistical Comparisons

Between-group differences in lGI.

Exploratory vertex-based statistical analysis of lGI measures was conducted using the SurfStat Toolbox (www.math.mcgill.ca/keith/surfstat/) for Matlab (R2010b, The Mathworks, Massachusetts). As the developmental trajectory for lGI measures currently remains unknown, we initially tested for linear, cubic and quadratic age effects at each cerebral cortex, in addition to the main effect of group. The procedure for model fitting and model comparison was the same as described in detail in; [75]. Briefly, the linear model was initially compared to the quadratic model. If the quadratic model performed significantly better, it was then compared to the cubic model, which contained a linear, quadratic and cubic age term. This allowed us to identify the most parsimonious model at each vertex, i.e. most simple plausible model that explains variations in measures of brain morphology with the smallest set of predictors. Parameter estimates for measures of lGI (Y_i) were estimated separately by regression of a general linear model (GLM) at each vertex *i* and subject *j*, with (1) group (G) as categorical fixed-effects factor; (2) linear, quadratic and cubic terms for age as well as their interactions with group, and (3) FSIQ as continuous covariate. Between-group differences in lGI were subsequently examined based on the main effect of group, while controlling for the effects of age and age x group interactions. Corrections for multiple comparisons across the whole brain were performed using random-field theory (RFT) based cluster-corrected analysis for non-isotropic images using a $p<.05$ (2-tailed) cluster-significance threshold [76].

Between-group differences in DTI measures within cluster of significant differences in LGI.

The statistical analysis of tract-specific DTI measures was based on tracts that originated or terminated within the clusters of significant differences in lGI measures to (1) examine between-group differences in

white-matter structural connectivity as measured by DTI within these clusters, and (2) to establish the relationship between differences in lGI and white-matter connectivity.

Prior to the statistical analysis of tract-specific DTI measures, the statistical distribution of tract lengths of tracts dissected using the surface-based ROIs across subjects was examined in order to further subdivide the automatically generated tracts into more homogeneous tract classes based on their lengths, and to reduce inter-individual variability in tract-specific measures examined across individuals. Here, we utilized Hartigan's dip statistic (HDS) to test for multimodality in the distribution of tract lengths (i.e. existence of multiple tract classes), as well as Gaussian Mixture Models (EM algorithm in R for statistical computing) to model the distribution (and cut-off) for individual tract classes. The number of components were derived by cross-validation, which estimates the log-likelihood (i.e. model fit) for different component solutions by performing a simple data-set splitting, where a randomly-selected half of the data is use to fit the mode, and half to test. The aim of this approach was to test for the existence of different tract classes within the automatically generated streamlines based on their respective distribution of tract lengths (e.g. short U-shaped fibres vs. long-range associative tracts).

To assess between-group differences in tract-specific DTI measures, a multivariate General Linear Model (SPSS software) was subsequently used, which included a main effect of group, as well as a linear effect of age, and an age x group interaction using a test-wise error rate of $p<0.05$ (two-tailed). Last, Pearson correlation coefficients were examined to investigate significant associations between lGI and DTI measures.

RESULTS

SUBJECT DEMOGRAPHICS

There were no significant differences between individuals with ASD and controls in age ($t(48) = 0.43$, $p = 0.67$), or IQ ($t(48) = 0.94$, $p = 0.35$). There were also no significant between-group differences in total area of the grey- and white-matter surface area ($t(48) = -0.47$, $p = 0.96$); ($t(48) = -0.15$, $p = 0.98$), respectively). We therefore did not covary for total brain measures in the statistical analysis of *l*GI measures. Demographic data is presented in Table 7.1.

TABLE 7.1. SUBJECTS DEMOGRAPHICS

	ASD (n = 23)	**CONTROL (n = 27)**
Age (years)	13.6 ± 2.6 (9 - 17)	13.3 ± 2.3 (9 - 17)
IQ (WASI)	113.5 ± 15.6 (70 - 140)	109.8 ± 12.3 (79 - 132)
ADI-R Reciprocal Social Interaction	17.3 ± 4.4	-
ADI-R Communication	15.4 ± 3.8	-
ADI-R Restricted, Repetitive, Stereotyped Behaviour	5.1 ± 2.3	-
ADOS Communication	3.7 ± 1.5	-
ADOS Reciprocal Social Interaction	7.7 ± 3.1	-
ADOS Imagination/Creativity	0.7 ± 0.6	-
ADOS Stereotyped Behaviours, Restricted Interests	1.7 ± 1.6	-
Total Surface Area of Grey Matter [cm2]	216115 ± 15089	216351 ± 19374
Total Surface Area of White Matter [cm2]	169095 ± 12021	169719 ± 16484

Note. Data expressed as mean ± standard deviation (range)

Nested Model Comparisons

For *l*GI measures, we found that the quadratic model provided a significantly better good-ness-of-fit than the linear model in one significant cluster located in the left lateral occipital lobe (see Figure 7.2.a). However, there was no significant improvement in fit when comparing the quadratic with the more complex cubic model, and no clusters survived correction for multiple comparisons (RFT-based, cluster-corrected, $p<0.05$) (Figure 7.2.b). Thus, we selected the quadratic model as the most parsimonious model for examining between-group differences in *l*GI (i.e. model with the smallest number of predictors), which also allowed us to investigate age x group interactions for the linear and quadratic age term.

Between-group Differences LGI and Age x Group Interactions

Based on the outcome of the nested model comparison, a quadratic model was used to examine the effects of group, age, and their interactions *l*GI measures. We found significant increases in *l*GI measures in ASD relative to controls in two significant clusters located in the right hemisphere when controlling for the effects of age (linear and quadratic terms), as well as for age x group interactions (RFT-based cluster corrected, cluster threshold $p<0.05$). Relative to controls, individuals with ASD had significantly increased *l*GI (1) at the right temporo-parietal junction ($t_{max}=2.79$, $N_{vertices}=3649$, $p_{cluster}=0.011$, Brodman Area (BA) 39), and (2) in the right lateral occipital lobe ($t_{max}=3.08$, $N_{vertices}=1833$, $p_{cluster}=0.005$, BA19) (see Figure 7.3.a). In these regions, we also observed a significant interaction between age x group (i.e. linear effect of age by group) (Figure 7.3.b). A significant interaction between age^2 x group (i.e. quadratic effect of age by group) was, however, only observed in the right lateral occipital cluster (Figure 7.3.c).

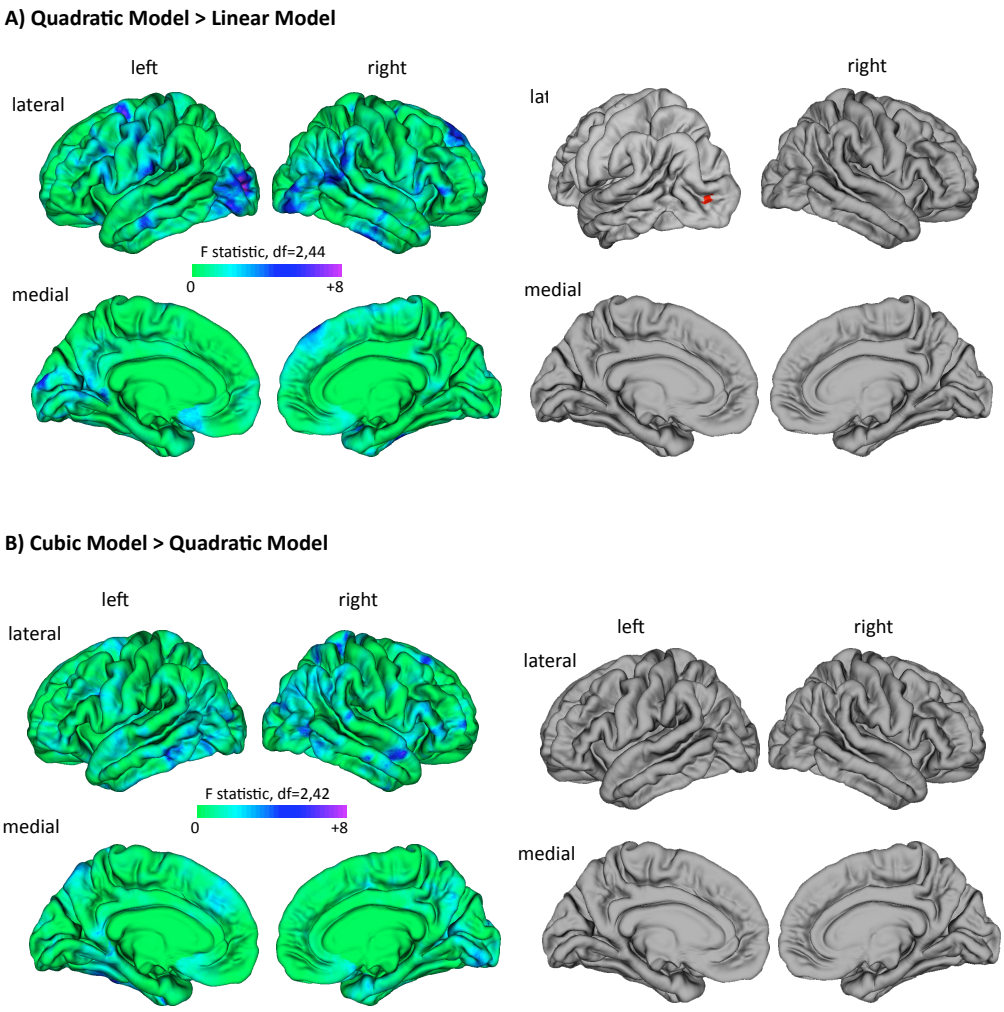

FIGURE 7.2. NESTED MODEL COMPARISONS FOR GYRIFICATION.

A) Linear vs. quadratic model. B) Quadratic model vs. cubic model. Left panel shows the difference map resulting from the model comparison (F statistic, unthresholded). F values (green to blue) indicate voxels where the more complex model fits better than the more reduced model. Right panel indicates random-field theory (RFT)-based, cluster-corrected ($p < 0.05$) difference maps indicating regions where the more complex model provides a significant better goodness of fit than the simpler model

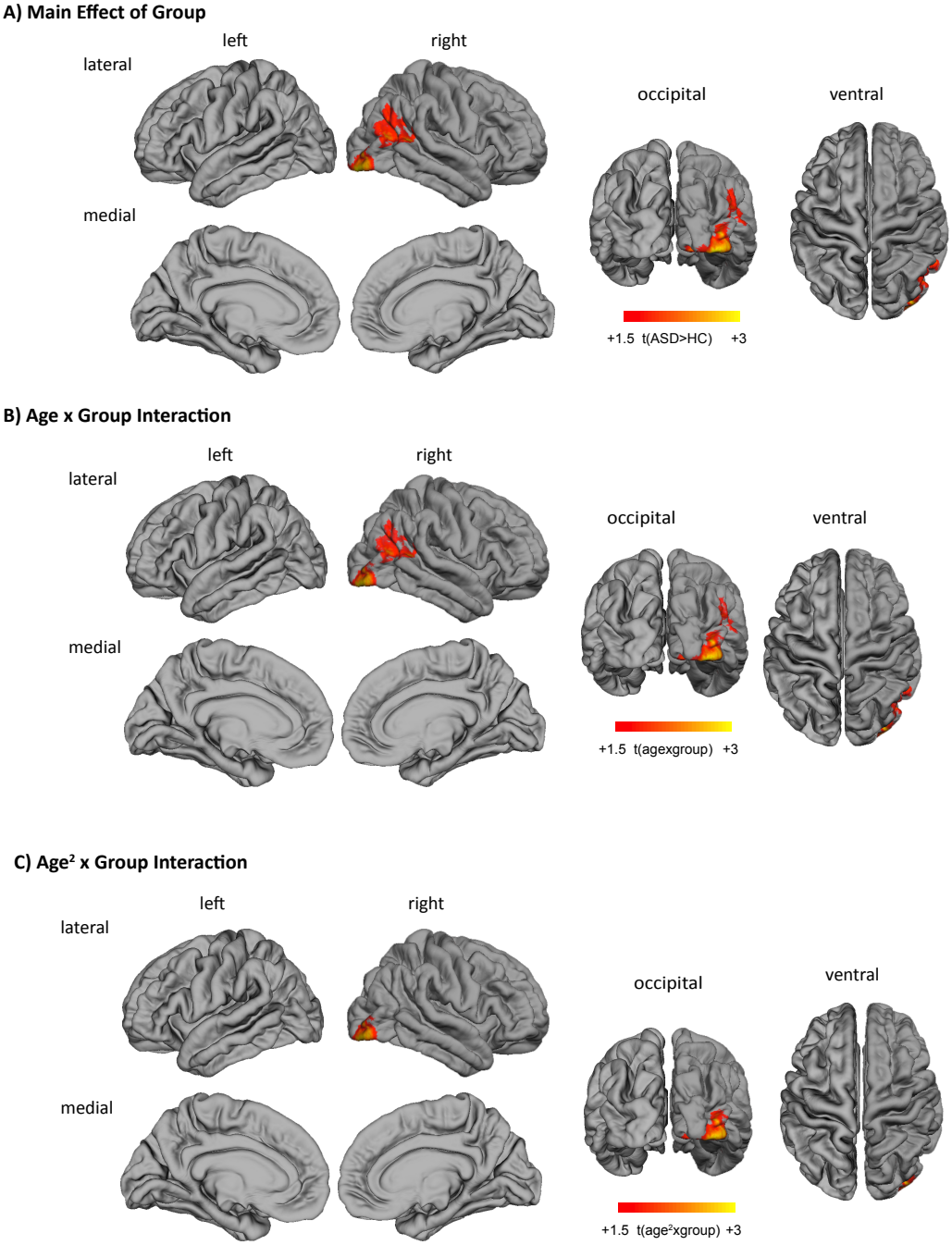

FIGURE 7.3. BETWEEN-GROUP DIFFERENCES AND AGE-BY-GROUP INTERACTIONS FOR MEASURES OF GYRIFICATION.

A) Clusters with significantly reduced cortical thickness measures (RFT-based, cluster-corrected, $p<0.05$) in ASD compared to controls while controlling for the effects of age and age-related interactions (i.e., main effect of group). B) Clusters with significant interactions between age x group (RFT-based, cluster-corrected, $p<0.05$). C) Clusters with significant interactions between age2 x group (RFT-based, cluster-corrected, $p<0.05$)

Distribution Of Tract Lengths Of Tracts Dissected Using Surface-Based ROIs

The distribution of track lengths for tracts originating or terminating in Cluster 1 (right temporo-parietal junction) significantly deviates from a unimodal distribution (Hardigan's Dip = 0.0045, $p < 0.001$) (see Figure 7.4), thus indicating the existence of one (or more) tract classes. More specifically, the density distribution of tract lengths was modelled with a four-component solution, above which no significant improvement in fit was observed (Figure 7.4.b). The bulk of the distribution of streamlines contains tracts up to a length of approximately 35mm, while longer tracts (> 35 mm) seem to be individually rare. The density distribution of tract lengths originating or terminating in Cluster 2 (right lateral occipital lobe) was also modelled with a four-component solution, above which no significant improvement in fit was observed (Figure 7.4.d). We therefore subsequently investigated short tracts (tract lengths: 0 – 35 mm), which mainly contained U-shaped fibres within both surface-based ROIs.

Between-Group Differences In Tract-Specific DTI Measures And Their Relationship To Variations In lGI

There were no significant between-group differences or age x group interactions in any of the examined DTI measures based on the fibre tracts originating or terminating in the surface-based ROI at the right temporo-parietal junction (Cluster 1) or right lateral occipital lobe (Cluster 2)(see Table 7.2). However, for both clusters we found a significant effect of age on tract-specific measures of diffusion, which were confirmed using a bivariate correlation analysis across and within groups (results are displayed in Table 7.3).

In addition, we also found a significant correlation between lGI measures in Cluster 1 and tract-specific diffusion measures (see table 3).

Thus suggesting that variations in lGI are associated with variations in DTI measures in U-shaped fibres in the right temporo-parietal cortex.

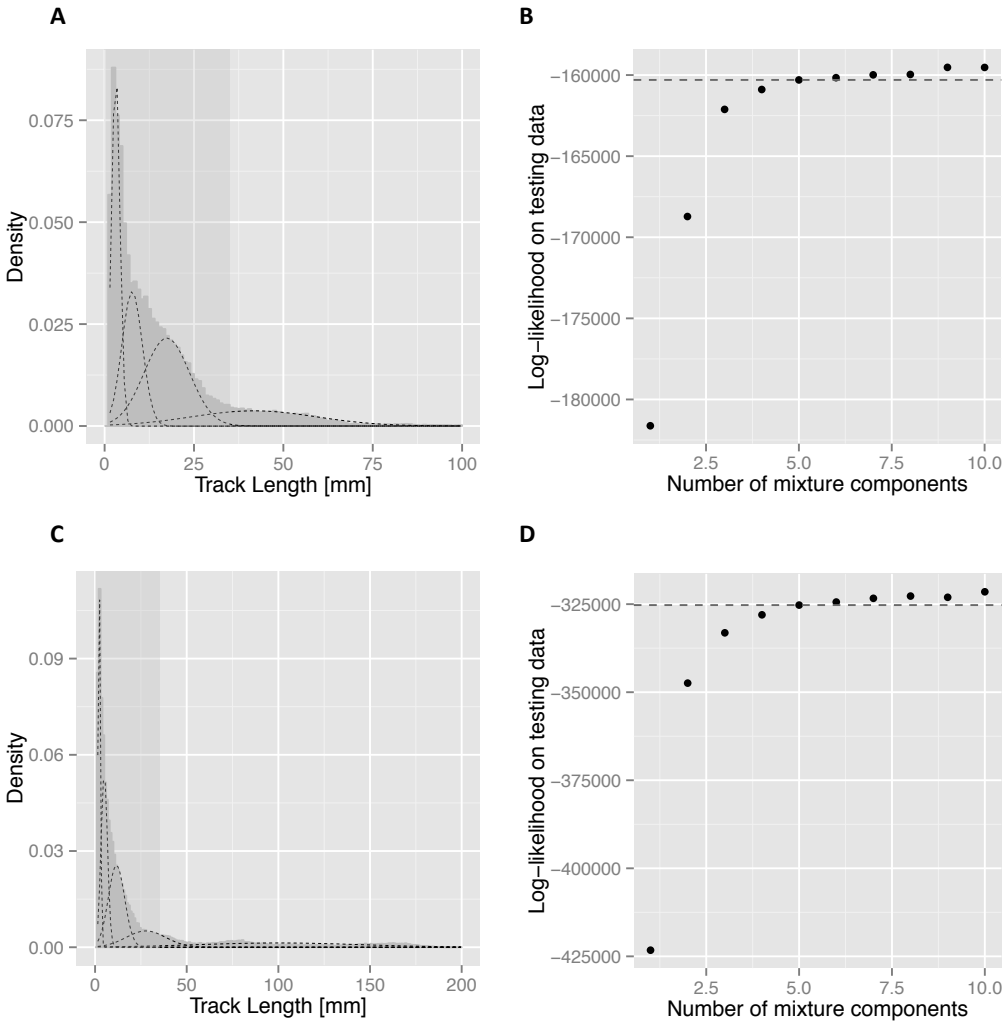

FIGURE 7.4. DISTRIBUTION OF TRACT LENGTHS OF TRACTS DISSECTED USING SURFACE-BASED ROIS

A) The distribution of track lengths for tracts originating or terminating in Cluster 1 (right temporo-parietal junction). B) Four-component solution for cluster 1, above which no significant improvement in fit was observed. C) The distribution of track lengths for tracts originating or terminating in cluster 2 (right lateral occipital lobe). D) Four-component solution for cluster 2, above which no significant improvement in fit was observed.

TABLE 7.2. BETWEEN-GROUP DIFFERENCES IN TRACT-SPECIFIC DTI MEASURES

Cluster 1. Right temporal-parietal junction (t_{max} = 2.79, $N_{vertices}$ = 3649, $P_{cluster}$ = 0.011, BA39)

	Subject groups						Effect of Group		Effect of Age		Age x Group Interaction	
	ASD (*n*=23)			HC (*n*=27)			F (*df*=1)	*p* value	F (*df* =1)	*p* value	F (*df* =1)	*p* value
Fractional Anisotropy	2.43E-01	±	1.68E-02	2.39E-01	±	1.79E-02	0.193	0.662	13.419	0.001*	0.123	0.727
Mean Diffusivity	8.51E-04	±	2.05E-05	8.50E-04	±	2.95E-05	0.000	1.000	7.374	0.009*	0.000	1.000
Radial Diffusivity	7.44E-04	±	2.28E-05	7.45E-04	±	3.16E-05	0.026	0.873	8.297	0.006*	0.043	0.837
Axial Diffusivity	1.07E-03	±	2.31E-05	1.06E-03	±	3.04E-05	0.158	0.693	3.101	0.085	0.040	0.843
Number of Tracts	4739.87	±	691.91	4671.81	±	620.93	1.007	0.321	0.362	0.550	0.970	0.330

Cluster 2. Right lateral occipital lobe (t_{max} = 3.08, $N_{vertices}$ = 1833, $P_{cluster}$ = 0.005, BA19)

	Subject groups						Effect of Group		Effect of Age		Age x Group Interaction	
	ASD (*n*=23)			HC (*n*=27)			F (*df* =1)	*p* value	F (*df* =1)	*p* value	F (*df* =1)	*p* value
Fractional Anisotropy	2.11E-01	±	2.10E-02	2.14E-01	±	1.23E-02	0.646	0.426	4.814	0.033*	0.475	0.494
Mean Diffusivity	8.47E-04	±	3.56E-05	8.37E-04	±	2.21E-05	0.534	0.469	9.181	0.004*	0.242	0.625
Radial Diffusivity	7.56E-04	±	3.93E-05	7.46E-04	±	2.29E-05	0.614	0.438	7.091	0.011*	0.312	0.579
Axial Diffusivity	1.03E-03	±	3.25E-05	1.02E-03	±	2.37E-05	0.232	0.632	11.811	0.001*	0.060	0.808
Number of Tracts	2655.78	±	589.17	2872.56	±	392.32	0.466	0.498	0.218	0.643	0.211	0.648

Note. Data expressed as mean ± standard deviation. F values resulting from the multivariate GLM including group as categorical fixed effect, and age and IQ as continuous covariates; a star (*) denotes statistical significance (p<0.05, two-tailed)

TABLE 7.3. TRACT-SPECIFIC DTI MEASURES AND THEIR RELATIONSHIP TO VARIATIONS IN AGE AND LGI

Subject Group		Cluster 1				Cluster 2			
		Effect of Age		Correlation with lGI		Effect of Age		Correlation with lGI	
		Pearson Correlation	*p*-value	Pearson Correlation	*p*-value	Pearson Correlation	*p*-value	Pearson Correlation	*p*-value
Fractional Anisotropy	Across Groups (n=50)	0.473	0.001*	-0.06	0.681	0.318	0.025*	-0.179	0.214
	ASD (n=23)	0.466	0.025*	-0.205	0.348	0.354	0.097	-0.345	0.107
	Control (n=27)	0.477	0.012*	0.053	0.793	0.301	0.127	0.022	0.911
Mean Diffusivity	Across Groups (n=50)	-0.354	0.012*	0.354	0.012*	-0.396	0.004*	0.187	0.195
	ASD (n=23)	-0.459	0.027*	0.494	0.017*	-0.411	0.051	0.358	0.094
	Control (n=27)	-0.305	0.122	0.29	0.142	-0.431	0.025*	-0.001	0.994
Radial Diffusivity	Across Groups (n=50)	-0.379	0.007*	0.286	0.044*	-0.359	0.011*	0.179	0.213
	ASD (n=23)	-0.545	0.029*	0.426	0.043*	-0.38	0.074	0.354	0.098
	Control (n=27)	-0.342	0.081	0.212	0.288	-0.388	0.046*	-0.023	0.909
Axial Diffusivity	Across Groups (n=50)	-0.225	0.117	0.414	0.003*	-0.428	0.002*	0.18	0.211
	ASD (n=23)	-0.328	0.127	0.475	0.022*	-0.431	0.04*	0.32	0.136
	Control (n=27)	-0.175	0.382	0.401	0.038*	-0.46	0.016*	0.045	0.823
Tract Count	Across Groups (n=50)	-0.111	0.444	0.254	0.075	0.089	0.54	0.195	0.175
	ASD (n=23)	-0.251	0.248	0.011	0.959	0.147	0.503	0.061	0.782
	Control (n=27)	0.467	0.014*	0.028	0.889	0.049	0.808	0.357	0.067

Note. Values resulting from the bivariate correlation analysis across and within groups.

A star (*) denotes statistical significance ($p<0.05$, two-tailed).

DISCUSSION

Here we present the results of a multi-modal neuroimaging study examining age-related differences in gyrification and structural connectivity in a large and well-characterized sample of male children and adolescents with ASD, and matched typically developing controls. Based on previous observations demonstrating that the complexity of grey matter development is regionally variable [77], we examined linear, quadratic, and cubic effects of age. We found that the quadratic model best predicted the developmental trajectory of gyrification. Using this model, we examined between-group differences in gyrification in the presence of significant age effects, and found that individuals with ASD showed significantly increased gyrification in two clusters in right temporo-parietal and occipital regions when controlling for the effect of age. In these regions, we also observed a significant interaction between age and diagnostic category (i.e. ASD vs. controls). These findings suggest that gyrification increases more rapidly between childhood and adolescence in ASD, relative to typically developing controls. However, when we examined white-matter structural connectivity in tracts that originated or terminated within the clusters of significant between-group differences in lGI, no corresponding between-group differences or age x group interactions for measures of diffusion were found. Interestingly, despite the lack of between-group differences in DTI measures, we observed a significant correlation between lGI measures and tract-specific measures of diffusion in the right temporo-parietal cortex. Thus, our results indicate that the degree of cortical gyrification is correlated with the underlying white-matter architecture. Measures of grey- and white-matter connectivity should therefore not be examined in isolation, for they interact during development to elicit the atypical patterns of cortico-cortical connectivity typically observed in ASD.

Our findings agree with earlier studies showing increased gyrification in ASD [44, 46], particularly in the parietal and occipital cortices [42, 43], which may mediate some of the autistic symptoms in the social domain of ASD. For example, the right temporo-parietal junction has been implicated in mentalizing and theory of mind impairments in ASD [78], and other authors have suggested that this region is activated during tasks involving attention [79]. Furthermore, the lateral occipital cortex has been associated with face processing, affect processing, and performance in language-related tasks [80, 81]. Therefore it is coherent that we observed group-differences in gyrification in these regions.

However, there are also some discrepancies between our findings and earlier literature. For example, Schaer et al., (2013) noted four frontal clusters of reduced gyrification in individuals with ASD compared to controls, using the same high-resolution technique as employed in the current study. This difference in the location and direction of gyrification changes may relate to disparities in subject demographics (e.g. the previous study investigated a small sample of both males and females). The clinical heterogeneity observed between males and females with ASD might also be accompanied by differential neurodevelopmental trajectories. Indeed, there is evidence for sexual dimorphism in both grey- and white matter volume [82-84], and connectivity [85-87]. Thus highlighting the importance of modelling gender as a contributing factor in the analysis of neuroanatomical and data.

In a second example, Wallace et al., (2013) noted a bilateral increase in gyrification in the lateral occipital lobe, in partial agreement with our right lateralised findings for the same region. However, Wallace et al. observed no significant interactions between age and diagnostic group [42]. Again, these differences may relate to differences in subject demographics (e.g. the Wallace et al., sample was older and more than half of the subjects with ASD were taking psychotropic medications, whereas our sample was entirely medication naïve). Additionally, and more specifically with regards to aging,

only linear age effects were examined. While typical neurodevelopmental trajectories are well established for measures of cortical thickness, there is currently no comparable data for vertex-based measures of gyrification. The more complex (cubic and quadratic) developmental trajectories of grey matter make cross-sectional comparisons challenging as accelerated or delayed development in a particular subject group could lead to a significantly positive difference at one age and a negative difference at a different age [88]. It is thus important that studies examining age-related differences in the development of grey matter examine a variety of statistical models in order to ascertain which model is best suited to a particular measure or region, which is the approach we employed in the present study.

To tackle our findings of cortical folding within a developmental framework it is also important to acknowledge that adolescence is a pivotal stage in neurodevelopment in which the trajectory of brain development changes from growth to decline in certain brain regions (after peak maturation has been reached) – for example, there is a considerable amount of synaptic pruning that occurs during adolescence [89, 90] and this is thought to underlie a decrease in GM volume [16, 54, 55, 91]. Not surprisingly, there is also evidence for a gradual decrease in brain surface complexity throughout childhood and adolescence [92] and early adulthood [93]. Hence, our finding of increased gyrification in ASD may reflect aberrant cortical pruning. In line with the observation of increased cortical neurons in ASD [94], and the suggestion that atypical synaptic pruning may drive the atypical brain growth trajectory of the disorder [95].

Furthermore, our right-lateralised findings may reflect differences in the time-course of right and left hemisphere development that has been described previously [96, 97]. Notably, there is evidence for right hemisphere predominance in the development of typical brain asymmetry between 1 and 3 years [98]. Thus, our results could be related to different right and left hemisphere vulnerabilities that result from atypical early development in ASD.

We observed a significant correlation between lGI and variations in MD, RD and AD, in the short U-shaped fibres of the right temporo-parietal cortex, indicating a link between surface morphology and underlying white-matter connectivity. These findings converge with those of Kates et al (2009) who showed that right parietal white matter volumes contributed significantly to variance in right parietal lobe gyrification. Correlations between diffusion measures and lGI were positive across groups – but it appears that differences in MD and RD are driven predominantly by the ASD group. MD refers to directionally-independent average diffusivity, and has been shown to decrease over the course of white matter maturation, in line with increasing microstructural integrity [99]. AD represents the diffusivity of water in the direction parallel to the fibre bundles, and RD measures diffusion perpendicular to the axonal wall. RD is often interpreted as a reflection of myelination, whilst AD may be explained by changes in axonal packing [100, 101]. As such, an increase in radial and axial diffusivities as seen in our results could potentially reflect either loss myelin or an increase in axonal diameter, which may accompany increased gyrification [102].

However, it has also been demonstrated that a change in radial diffusivity can cause an unreliable change in axial diffusivity, and vice versa in voxels characterized by crossing fibres [103]. Moreover, both measures of AD and RD may vary as a consequence of crossing fibres and partial volume effects rather than on the basis of the underlying tissue structure [104]. It is important to note that the WM directly beneath the cortical mantle is highly variable across subjects, and contains more complex crossing fibre trajectories than deeper white matter [105]. Additionally, in vivo and post-mortem research shows that U-shaped fibres constitute the terminal zone of myelination and remain weakly myelinated until adulthood [105-107], making diffusion measures for superficial WM inherently difficult to interpret.

Nonetheless, our focus on U-shaped fibres in the current study may explain why our grey and white matter findings of between-group differences

were discordant within the two clusters of increased gyrification. There is strong evidence for impaired long-range connectivity in ASD [26, 27], and numerous authors have suggested that a period of rapid enlargement might tend towards altered optimal connectivity patterns [108-111], which may suggest a possible reliance on shorter tracts in ASD [112]. In the present study, we only investigated short fibres as the bulk of the distribution of streamlines contained tracts up to a length of approximately 35mm, while longer tracts (> 35 mm) were negligible. By focussing on the short subgroup of streamlines from the ROI we were also able to reduce variability across individuals and examine more homogeneous tracts within a cluster. As such, our findings for these two clusters are not affected by long-range under connectivity. In the future it may however be beneficial to examine correlations between WM and GM measures across the whole brain (in both typical and disordered development) and across a variety of tract lengths, in order to examine correlations between gyrification and connectivity in cortical regions where the distribution of tract lengths tends towards longer connections.

Placed in the context of the broader literature, our findings are in line with Van Essen's theory proposing that tension along the lengths of axons induces compacting folds in the cortical surface [41]. It is possible that the summation of tension along the lengths of numerous short connections generate a tangential force comparable to longer connections, thus compensating for long-range under-connectivity in ASD. Our cluster based ROIs may represent an exception to this compensatory effect in ASD, as neurotypical brains are also characterised by short fibres in these particular regions.

LIMITATIONS

Our findings are limited to the short-range connections exclusively within two clusters of abnormality. Although informative, it would be of further merit to investigate correlations between WM and GM measures inclusively across the whole brain. In addition, an important consideration in

the interpretation of these findings is that although WM microstructure is commonly seen as a macro-structural correlate of connectivity, structural and functional connectivity are not truly co-referential [113-115]. For example, tonic neuro-modulatory changes have been shown to affect the functional activation of neurons without alteration to their structural connections [116]. Thus, future studies may benefit from combining the same high-resolution gyrification metrics with functional connectivity.

CONCLUSIONS

The present study extends previous findings by demonstrating age-related differences in cortical gyrification in children and adolescents with ASD. In addition, we show that gyrification measures correlate with metrics of structural connectivity within and across groups. Thus, atypical white-matter connectivity in the brain of individuals with ASD should not be examined in isolation, but should be interpreted in the light of atypical brain morphometry.

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CHAPTER. **8**

MAPPING WHITE MATTER DEVELOPMENT IN CHILDREN AND ADOLESCENTS WITH AUTISM SPECTRUM DISORDER

CHAPTER PREFACE

Prior to mcDESPOT, a group of myelin mapping methods were developed for quantitative, in vivo imaging of fundamental parameters determining the magnetization transfer effect in tissues, labeled broadly as quantitative magnetization transfer (qMT) [1-3]. It has been suggested that magnetization transfer imaging gives a more direct estimate of myelination than DTI [4]. It relies on the transfer of magnetization from protons that are bound to macromolecular structures (for example, myelin and cell membranes) to protons in free water [5]. The quantity of magnetic transfer differs between tissue types and is larger where more macromolecular structure is present [6]. This exchange of magnetization between the pools produces an observable change in the magnetization of the liquid spins. This property is exploited in qMT experiments, to yield a magnetization transfer ratio (MTR). The magnetization transfer ratio describes the proportion of MR signal reduction that is attributed to the presence of macromolecules [1] (the principles of qMT are detailed further in Chapter 2). Thus, within white matter, myelin-bound water disproportionally contributes to this measure, rendering the MTR an index of myelination [7].

Changes in the MTR in central nervous system tissue have often been attributed to changes in myelin content [8, 9] and previous studies have shown histopathological and *post mortem* evidence of a significant correlation between myelin and MTR [10]. This is reasonable because myelin does contribute to the motionally restricted (macromolecular) proton pool and consequently, loss of myelin leads to a reduction of MTR. In addition, MTR measurements have been used extensively to track the progression of demyelinating lesions as well as changes in normal-appearing white matter in multiple sclerosis [8, 9, 11, 12], and more recently to examine fronto-striatal myelination in ADHD [6].

MTR values are quantitative in the sense that they are reproducible and comparable among subjects and repeated scans. However, the resulting images reflect a complex combination of sequence and relaxation parameters

in addition to magnetization transfer [13, 14]. Furthermore, changes in water content due to edema and inflammation may also cause changes in MTR, which are completely unrelated to myelin [15-17]. Studies have shown the MTR to be significantly correlated with water content but only weakly correlated with myelin water content [18]. Thus, caution should be used when associating magnetization findings exclusively with myelin, although sensitive to myelin, it is not myelin specific.

In the past studies have also relied on rather lengthy T_1 or T_2-weighted signal contrast measurements to suggest differences in myelin [19-21]. Unfortunately, differences in these signals illustrate a broad range of microstructural and biochemical alterations, including lipid and cholesterol content, macromolecule and protein content, iron and oligodendrocyte content, free water content, and water mobility [22]. Thus, it is not feasible to interpret observed T_1 or T_2 contrast changes into meaningful estimates of myelin content. Further, conventional MR imaging metrics, including T_1, T_2 and magnetization transfer, often suggest the presence of myelin (in infants) before it can be observed histologically [23-25]; or show little difference in the myelination trajectory between regions [25]. This limits the efficacy of these techniques in examining myelin through development. In contrast, studies using multi-component relaxometry techniques, such as mcDESPOT suggest a spatial and temporal myelination pattern that faithfully reproduces histological findings [26-30].

Multi-component relaxometry is a more specific method of investigating tissue microstructure. MCR is described in more detail later in this chapter, but in brief, the aim of MCR is to decompose the measured MR signal into contributions from two distinct water environments that exist in the CNS [26, 29, 31]. The first is characterized by slow signal relaxation attributed to the less-restricted intra and extra-cellular water and the second, is characterized by more rapid relaxation, which is broadly attributed to water trapped between the myelin lipid bilayers (there is also a third non-exchanging water pool) [32, 33]. This yields the myelin water fraction (MWF), which relates

to the relative fraction of the myelin-associated signal, i.e. the signal that is measured from the rapidly relaxing water molecules trapped within myelin.

The MWF has previously been used to investigate demyelination in disorders, such as multiple sclerosis [34-36], as well as to study normal neurodevelopment in infants and young children [30, 37, 38]. Thus, the addition of MCR imaging may aid the interpretation of prior reports from DTI and qMT in ASD, and provide further evidence that altered myelin is an important etiological characteristic of the disorder.

mcDESPOT, which is described herein, is a relatively new multicomponent relaxometry MR-imaging technique that provides whole-brain, high resolution quantitative T1, quantitative T2, and myelin water fraction maps in a clinically feasible time.

ABSTRACT

Magnetic resonance imaging studies of children and adolescents with autism spectrum disorder have consistently revealed differences in regional white-matter microstructure, and development. There is a growing body of evidence that these differences may be related to myelin, yet few imaging studies have specifically examined the maturation of this crucial white matter component in typical versus autistic neurodevelopment. Therefore, we performed a cross-sectional MRI study of eighty-five males (45 with autism spectrum disorder, and 40 age and IQ matched controls) aged from 6-17 years. Utilizing a multicomponent-relaxometry technique entitled mcDESPOT, we examined age related differences in white matter myelin water fraction and correlated myelin content with clinical measures. We report significant age-related differences in myelination between autistic and typically developing groups in several brain regions, including; the body, splenium and genu of the corpus callosum, fornix, anterior thalamic radiation, right inferior-longitudinal-fasciculus and the bilateral uncinate. In the autistic group these regions were characterised by reduced myelin water fraction in early childhood, and steeper developmental trajectories, such that myelin water fraction was increased relative to controls in late adolescence. Additionally, reduced myelin content was associated with higher sub-domain scores in the Autism Diagnostic Interview (i.e. more marked symptomatology). Our results show, for the first time, that the neurodevelopmental trajectory of myelin is altered in autism spectrum disorder and that myelin content is negatively correlated with symptom severity. More powerful, longitudinal investigations are required to elucidate the precise extent, direction, and time course of these differences.

Autism Spectrum Disorder is a neurodevelopmental condition characterised by impairments in communication and social interaction, and restricted, repetitive and stereotyped behaviours [39, 40]. These 'core' symptoms of ASD typically manifest before two years of age and are accompanied by developmental differences in brain anatomy and connectivity [41-43]. However, defining the anatomy of ASD has been problematic, the neural systems underlying ASD are complex and involve abnormalities in multiple, spatially distributed neurocognitive systems [44]. Therefore, the neural substrates of ASD remain the subject of on-going investigation.

Despite this, some consistent neurobiological trends have emerged. The most consistent MRI findings in ASD are those that are prominent on the global level, such as differences in overall brain growth – including total grey and white matter volume [45-51]. Research suggests that the ASD brain undergoes a period of accelerated growth during early postnatal life (up to age 2) [52-55], followed by atypically slow or arrested growth throughout the remainder of childhood, such that no global differences are generally observed in adulthood [46, 56]. It is increasingly evident that this growth trajectory steers the ASD brain to be larger in early childhood, and this may be disproportionately accounted for by increased white matter [45, 57-59]. A second, well-replicated trend is that of altered connectivity. ASD is increasingly viewed as a 'developmental disconnection syndrome' [43], associated with differences in anatomical [60-62], functional [63-66], and electrophysiological connectivity [67-69]. Patterns of disrupted connectivity have been documented in adults [62, 70] and children [62, 63, 67, 68, 71], and investigations have implicated specific neural networks that mediate particular symptoms [57, 72, 73]. The myelinated axons of white matter play a vital role in establishing and maintaining brain connectivity and consequently numerous investigations have set out to examine WM differences in ASD.

Investigations of WM microstructure using quantitative T_1 and T_2 relaxation time measures have revealed regional and global increases in WM T_2

in children with ASD [74, 75]. During typical development, T_2 shortens, owing to decreasing intra- and extracellular fluid, secondary to increased microfilament, microtubule and myelin production [76]. Thus the observed increase in T_2 relaxation time could, in part, reflect WM myelin abnormalities [74]. However, despite demonstrated value, T_1 and T_2 relaxation times have seldom been investigated in autism (to our knowledge only three prior studies exist; [74, 75, 77]), likely owing to the exhaustive scan times required. More often, investigators have employed diffusion tensor imaging to quantitatively gauge the micro-structural properties of WM, based on measures of fractional anisotropy (FA), which describes the degree of directionality of intra-voxel diffusivity within a tissue [78].

An array of studies examining FA values in ASD have reported altered WM microstructure, during infancy [79], early childhood [60, 80, 81], late childhood and adolescence [82-87], and adulthood [88-93]. However, the pattern of abnormality is highly variable across the age range and affects widespread neural systems. For example, increased mean FA has been documented in ASD infants and toddlers [79, 80] but the dominant pattern in childhood and adolescence is lower FA [81-87, 89, 92, 94]. The FA literature describing later adolescence and adulthood is less consistent and it has been hypothesised that WM microstructural differences begin to normalise with age [83]. For example, WM tracts involved in complex socio-emotional processing have been implicated in children, but not in adolescents with ASD [95]. Thus, the effect of age is very relevant to understanding ASD. Indeed, it has been proposed that the time course of brain development rather than the final product may be most disturbed in ASD [42]. However, few studies of WM integrity have examined a broad enough sample to inform on the effect of age spanning childhood and adolescence.

In addition to the need for a better understanding of age-related change, there is a need for imaging measures with improved biological specificity. Although informative, the neuroimaging techniques used thus far cannot inform us about the underlying cellular events that mediate the observed effects. Phenomena visible with conventional MRI are unlikely to be

the result of single, independent processes, but probably depict a number of changes, involving various cell types. For example, FA is influenced by a range of factors, including the underlying fibre architecture and geometry of WM, inflammation, gliosis and axonal loss as well as myelination [26, 30, 96-101]. Thus, it is desirable to untangle the mechanisms underlying WM matter change in ASD, and draw more specific conclusions about the changes we observe *in vivo*.

Myelin constitutes a substantial portion of WM and is a likely candidate underlying the WM differences observed in ASD. It is essential for the rapid transmission of electrical impulses and its damage can impair conduction and consequently, sensory, motor, and cognitive functions [102, 103]; all of which are relevant to ASD. The human brain continues to undergo myelination into adulthood [104] and the frontal regions of the cerebral cortex, responsible for higher-level cognitive functions, are the last to be myelinated [105]. In addition, many studies of experience-dependent WM plasticity in healthy development propose changes in myelin as a potential mechanism [106-109], supporting that myelination is a dynamic process through development and into adulthood. Lately, beyond extensively studied demyelinating pathologies, such as multiple sclerosis, myelin is drawing new interest as a potential contributor to a collection of psychiatric and neurodevelopmental disorders, including depression, schizophrenia and ASD [110-112]. Support for myelin involvement in these disorders arises from gene expression studies [110, 111, 113, 114], histological analyses of *post mortem* tissue [115], and numerous brain imaging methods that indirectly illustrate an association with myelin (for example; [46, 49, 60, 61, 82, 112, 116, 117]).

Improved sensitivity and specificity to myelin content may be obtained through multi-component relaxometry (MCR) [26, 29, 31]. MCR decomposes the measured MRI signal into contributions from micro-anatomically distinct water compartments (Figure 8.1). In CNS tissue, at least two water environments have been consistently observed, 1.) the slow-relaxing “free” water, in intra- and extra-axonal space; and 2.) the rapidly-relaxing “trapped” water between the lipid bilayers of myelin [32, 33]. These water compartments produce distinct MR signals, or “signatures”, based on their respective relaxation

characteristics. Based on these signatures the relative fraction of myelin associated signal can be calculated, and is termed the myelin water fraction (MWF). Unlike DTI measures, the MWF is not influenced by fibre architecture [29, 118], and robustly correlates with histological estimates of myelin content [26-29]. MWF measures have previously been used to investigate demyelination in human and animal models of multiple sclerosis [34-36], as well as to study normal neurodevelopment and myelination in infants and young children [30, 37, 38, 99]. Encouragingly, the most rapid period of myelination described by these studies is temporally coincident with the early period of brain overgrowth described in ASD [30, 37, 38, 99].

Thus, we present a study using the mcDESPOT MCR technique (multicomponent driven equilibrium single pulse observation of T_1 and T_2) [33] to investigate differences in MWF between individuals with ASD and typically developing controls, through childhood and adolescence.

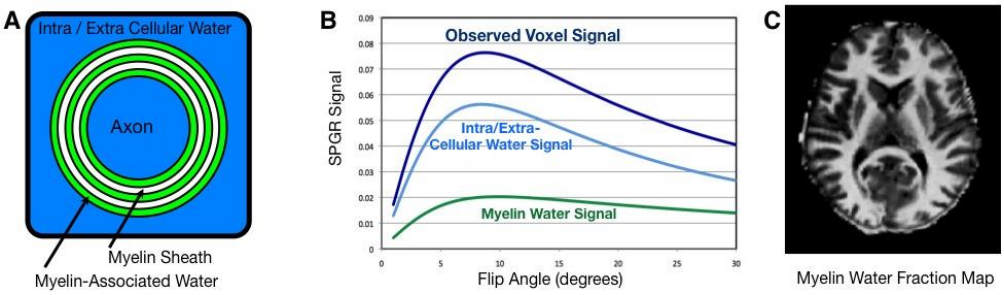

FIGURE 8.1. MULTI-COMPONENT RELAXOMETRY

Protons in the intra/extra-cellular water and myelin-associated water pools (A) (Figure from Davison AN, Peters A. Myelination [119]) have different relaxation properties and, therefore, contribute unique MRI signatures (B). The observed signal is the summation of these signatures. By resolving the signature from the myelin water pool, a quantitative map of its volume fraction can be produced (C) (Deoni et al. 2008. Magn. Reson. Med. 60, 1372-1387 [33]).

PARTICIPANTS

Forty-five ASD males (5.9-17.6 years of age, mean = 11.8 years) and forty TD males (7.5-17.4 years of age, mean = 12.3 years) were recruited into the study. All were right-handed (measured using The Edinburgh Handedness inventory [120]), native English speakers, and had an IQ ≥ 70 as measured by the WASI. Full demographics are provided in Table 8.1. ASD participants were diagnosed according to ICD-10 research criteria [121]. Clinical diagnosis was confirmed using the Autism Diagnostic Interview-Revised (ADI-R) [122]. All cases of ASD reached ADI-R algorithm cut-off values in all three domains. Current symptoms were then assessed using the Autism Diagnostic Observation Schedule (ADOS) [123], however, participants were allowed to fall below ADOS algorithm cut-offs by a single point. Exclusion criteria included, intellectual disability (i.e. IQ<70); major psychiatric disorder (e.g., psychosis); use of medication affecting brain function; any pre-existing medical conditions or complications affecting brain function (e.g. head injury, epilepsy); chromosomal abnormality associated with ASD (e.g., fragile X syndrome, tuberous sclerosis); and any MRI contraindications. The study was approved by the National Research Ethics Committee, Suffolk, UK. Informed written consent was obtained from all study participants and their parents.

IMAGE ACQUISITION

To investigate tissue MWF, the mcDESPOT MCR technique was used [33]. This involved the combined acquisition of 8 SPoiled GRadient recalled echo (SPGR, spoiled FLASH, or Fast Field Echo) and 8 fully-balanced steady-state free-precession (bSSFP, FIESTA or TrueFISP) images over a range of flip angles with constant acquisition timings. In addition, an inversion-prepared IR-SPGR image was acquired in order to allow correction of flip angle (B1 field) inhomogeneities [30]. Two sets of bSSFP data were acquired with different

radio-frequency (RF) phase-cycling patterns in order to correct for main and transmit magnetic field (B0) inhomogeneities [30].

Whole-brain, sagittally-oriented data were acquired with a common 22cmx22cmx16cm field of view (FOV) and 128x128x92 imaging matrix. To minimise acoustic noise and increase our paediatric population's comfort and compliance, maximum gradient amplitudes and slew rates were decreased by 75% and 55% respectively. To reduce acquisition time, data were acquired with 3/4 partial Fourier acquisition and parallel imaging with an acceleration factor of 1.75. Sequence-specific acquisition parameters were as follows:

SPGR: Echo time (TE)/Repetition Time (TR) =4.9ms/11ms, flip angles (α) = {3, 4, 5, 6, 7, 9, 12 and 18}°, receiver bandwidth (BW) =217Hz/pixel. IR-SPGR: TE/TR =4.9ms/11ms, inversion time (TI) =450ms, α =5°, BW =217Hz/pixel. bSSFP: TE/TR =3.8ms/7.6ms, α ={12, 16, 21, 27, 33, 40, 51 and 70}°, RF phase-cycling patterns ={0 and 180} °, BW =217Hz/pixel. All imaging was performed on a 3 Tesla GE Signa HDx clinical scanner equipped with an 8-channel head RF coil array. Total acquisition time for the protocol was less than 15 minutes.

MWF Calculation and Image Alignment

Following data acquisition, the SPGR, IR-SPGR and bSSFP data from each participant were linearly co-registered to correct for subtle intra-session motion [124]. Non-parenchymal signal was removed [125], and voxel-wise MWF estimates were calculated using a 3-pool tissue model (as described in [32, 37], the 3-pool model includes the volume fractions and relaxation times for intra/extra-axonal water, myelin-associated water, and non-exchanging free water).

A common T_1-weighted MRI template in approximate MNI space was constructed using the high flip-angle SPGR images from each of the 85 participants using symmetric diffeomorphic normalization (SyN; [126]) as implemented in the ANTs package and a cross-correlation similarity measure (http://picsl.upenn.edu/ANTS). This was performed using the buildtemplateparallel.sh script distributed with the ANTs package [126]. A rigid

affine transformation was calculated from this template to the MNI T_1 template. Following MWF map calculation, each participant's high-angle SPGR image was non-linearly aligned to the common template. The quantitative MWF map was then transformed into the 1.5mm^3 space of the approximate MNI template using this non-linear transformation and the rigid affine transformation to MNI space.

Group Comparisons of MWF

Initially a group-wise comparison of MWF between the ASD and typically developing controls was performed via voxel-wise unpaired two-tailed t-tests. A 2.5 mm full-width-at-half-maximum Gaussian kernel was used to smooth the MWF data, and non-parametric permutation testing used to perform the group comparison (using the randomize tool included in the FMRIB Software Library; http://www.fmrib.ox.ac.uk/fsl/). Significance was defined as $p<0.05$, corrected for multiple comparisons using a cluster-based technique, a commonly used multiple testing method for determining corrected significances while accounting for the high level of spatial dependencies between adjacent voxels [127].

Comparisons of MWF vs. Age Trajectories

Group comparisons of the MWF vs. age trajectories were performed on predetermined WM regions that were highlighted to be of relevance to ASD, based on evidence from previous neuroimaging studies. For region-wise analysis, masks for 12 (*a priori* chosen) WM regions/pathways were derived from the John Hopkins University WM atlas [128]. Regions included; the body, genu and splenium of the CC, the fornix, and right and left anterior thalamic radiations, uncinate fasciculus (UF), inferior longitudinal fasciculus (ILF), and posterior limb of the internal capsule (PLIC). These masks were superimposed on the MWF data and mean MWF values were obtained for each masked region for each participant.

Initially exploratory tests were carried out in order to establish the most parsimonious model with regards to the effect of age. Applying a quadratic age term did not significantly increase the goodness-of-fit, thus, the linear model was favoured for examining myelin. Next, a line, described by MWF (age) =α×age+β, was fitted to each group's MWF vs. age data. To perform a group comparison of the slope of these trajectories, a wild bootstrap with residual re-sampling approach [129] with 5,000 re-samples was used to estimate the distribution of slope (α) and y-intercept (β) for the ASD and typical control groups. An unpaired two-tailed *t*-test was then performed to compare the slope distributions between the two groups. A *p*-value less than 0.004 ($p < 0.05$ corrected for 12 comparisons) was used to define statistical significance.

A voxel-wise comparison of WM MWF slope was also performed. Each participant's spatially aligned MWF data was first smoothed with a modest 4mm full-width-at-half-maximum Gaussian kernel, and the same slope distribution comparison detailed above was applied at each image voxel within a white and grey matter mask derived from the mean template using the FAST package (part of the FMRIB FSL Tool Library [130]). A non-parametric unpaired two-tailed t-test was performed to compare the slope distributions calculated for the ASD and typical control groups (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise). Significance was defined as $p<0.05$, cluster corrected.

Correlations with Symptom Measures

To investigate associations between MWF values and symptom measures in participants with ASD, correlation analysis was performed on a voxel-wise level between the MWF values and ADOS and ADI-R sub-domain measures of reciprocal social interaction, communication, and restricted, repetitive and stereotyped behaviour. Analysis was performed using non-parametric testing (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise), with participant age included as a covariate. The spatially aligned MWF data was

smoothed with a 4mm full-width-at-half-maximum Gaussian kernel prior to analysis. Areas of significant correlations were identified as regions with p<0.05 cluster corrected for multiple comparisons as above.

RESULTS

PARTICIPANT DEMOGRAPHICS

Between-group differences in age and IQ, were calculated using unpaired two-tailed t-tests. There were no significant between-group differences in either age (t(83) = -1.37, p = 0.18) or IQ (t(83) = -1.16, p = 0.25). A summary of demographic data, including mean age, IQ, ADOS and ADI-R sub-domain measures is provided in Table 8.1.

TABLE 8.1. SUMMARY OF SUBJECT DEMOGRAPHICS

	ASD (n = 45)	CONTROL (n = 40)
Age (Years) ± SD (range)	11.8 ± 3.1 (6 - 17)	12.3 ± 2.8 (7 - 17)
IQ (WASI score) ± SD (range)	108 ± 16.4 (70 - 140)	110.7 ± 13.3 (79 - 148)
ADI-R Reciprocal Social Interaction	18.92 ± 4.7	-
ADI-R Communication	16.6 ± 3.7	-
ADI-R Restricted, Repetitive, Stereotyped Behaviour	5.7 ± 2.4	-
ADOS Communication	3.75 ± 0.62	-
ADOS Reciprocal Social Interaction	9.1 ± 1.78	-
ADOS Imagination/Creativity	1.7 ± 0.58	-
ADOS Stereotyped Behaviours, Restricted Interests	1.9 ± 1.82	-

Note. Data is expressed as; mean ± standard deviation (range).

There were no significant between group differences in MWF. However, 11 out of the 12 regions examined were found to display significant differences in developmental trajectory between the ASD and typical control groups, with only the left ILF not reaching significance. Scatter plots with superimposed mean linear development trajectories, as well as the ASD and typical control slope distributions (histograms) for each of the 12 WM pathways examined are displayed in **Figure 8.2.**

Tract specific differences were confirmed in a voxel-wise analysis of MWF trajectories (**Figure 8.3**), which show significant differences (with ASD greater than typical controls) in the bilateral cortico-spinal tracts, anterior thalamic radiations, forceps, cingulum, inferior froto-occipital fasciculus (IFOF), portions of the ILF and superior longitudinal fasciculus (SLF), and cerebellum.

FIGURE 8.2. (DISPLAYED OVERLEAF)

Region-Wise Comparison Of MWF Development. For each WM pathway examined, a plot of the raw data and mean linear trend-line, as well as the bootstrap-derived development slope distributions are shown. Red corresponds to participants with ASD, and blue with TD controls. A star denotes pathways with a significant ($p < 0.05$ corrected) difference in developmental trajectory.

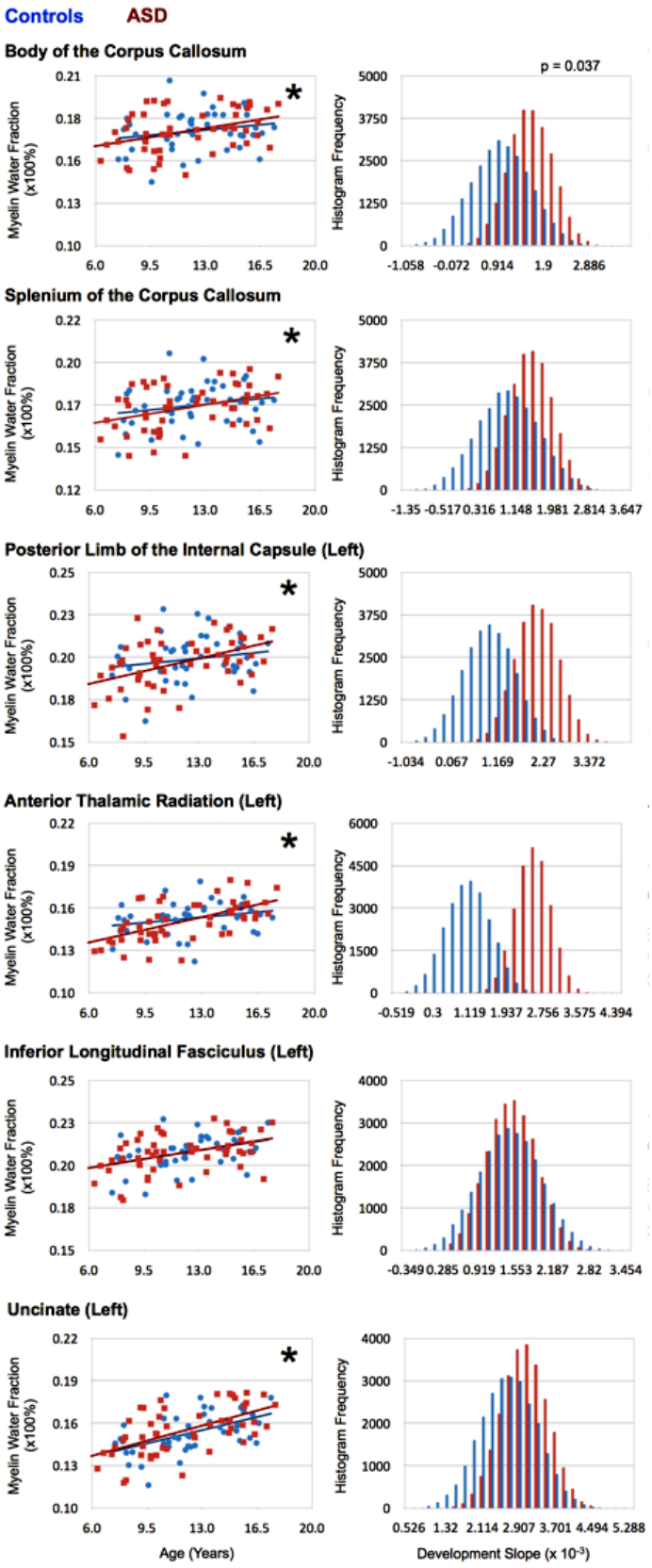

FIGURE 8.2. (PAGE 1 OF 2)

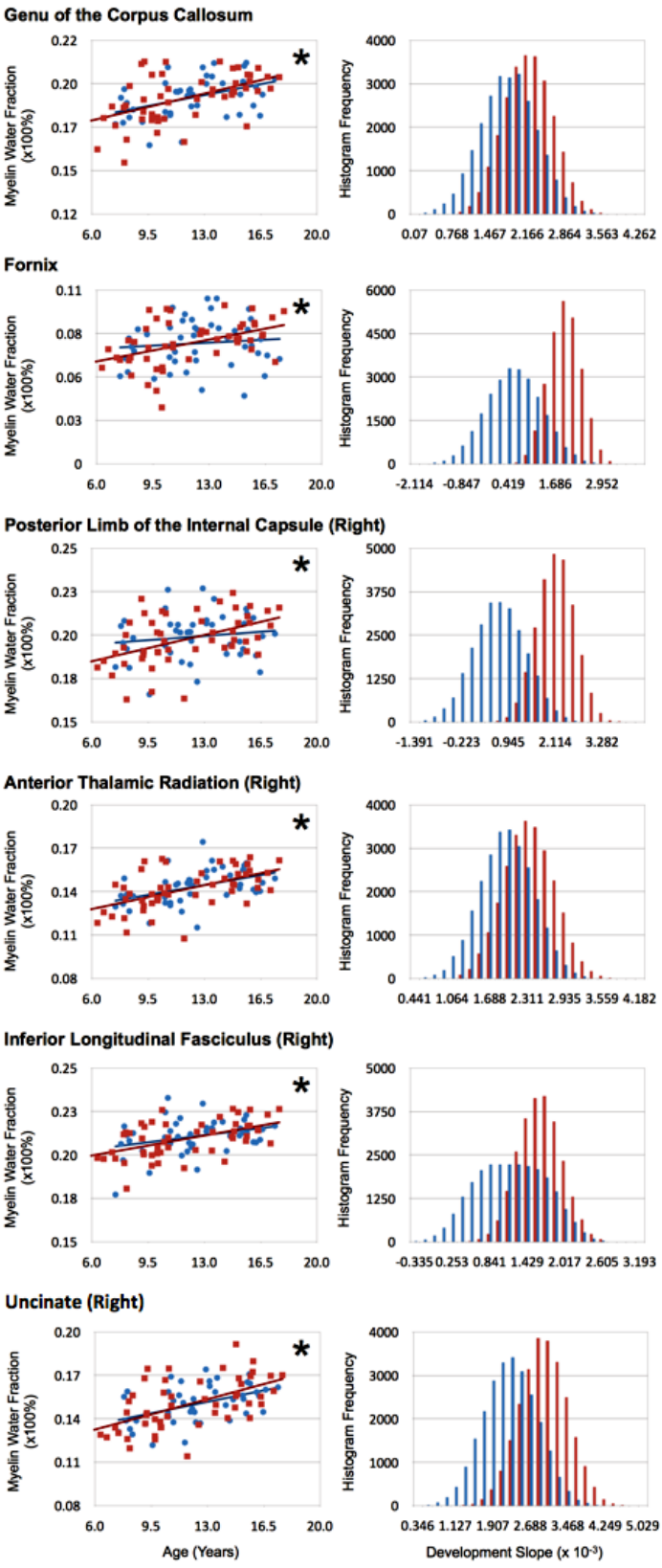

FIGURE 8.2 (PAGE 2 OF 2)

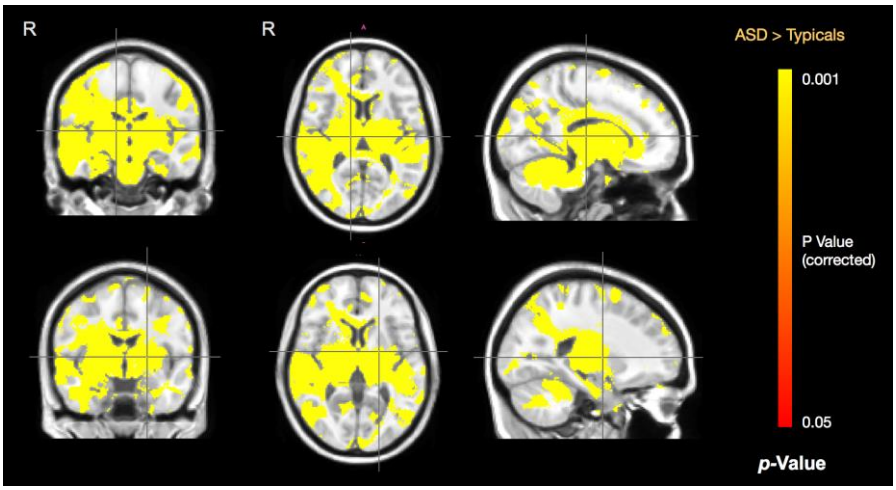

FIGURE 8.3. VOXEL-WISE COMPARISON OF MWF DEVELOPMENT

Thresholded p-value map overlaid on the common T1-weighted template. Areas correspond to regions where subjects with ASD show significantly ($p<0.05$ corrected) increased MWF development rate compared to controls.

CORRELATIONS WITH SYMPTOM MEASURES

Figure 8.4 displays results of our analysis investigating correlations between MWF and ADI-R sub-scores within the ASD group. Corrected for multiple comparisons, significant ($p<0.05$) *negative* correlations (reduced MWF predicted worse performance) were identified between MWF and *reciprocal social interaction (ADI-A)* in bilateral cerebellum, CC, internal capsule, striatum, IFOF cingulum, and the ILF and SLF (mean effect size, r^2, for the cluster was 0.175). Significant *negative* correlations were identified between MWF and *communication (ADI-B)* in left cingulum, SLF, IFOF, and premotor cortex (mean effect size, $r^2 = 0.143$). Finally, significant *negative* correlations were identified between MWF and *restricted repetitive and stereotyped behaviour (ADI-C)* in right cortico-spinal tract, bilateral forceps, and other portions of the frontal

lobe (mean effect size, r^2 = 0.124). No positive correlations were identified. No significant correlations were identified between MWF and ADOS measures.

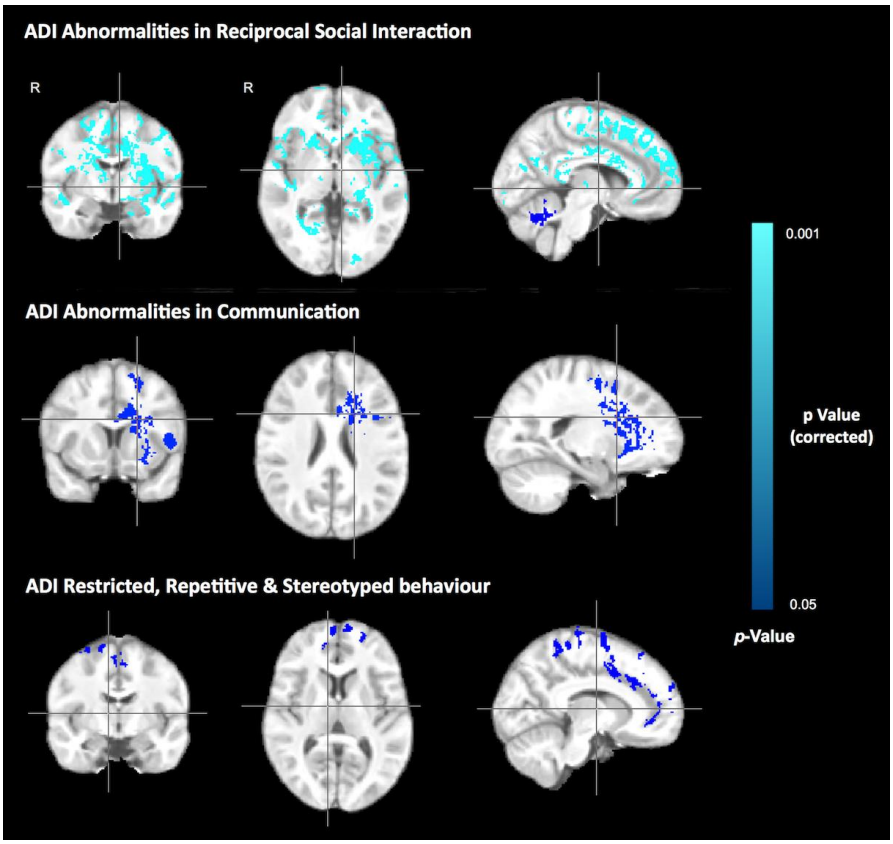

FIGURE 8.4. CORRELATION WITH SYMPTOM MEASURES

Correlation analysis examining relationships between ADI sub-domain scores and MWF in the ASD group. Identified regions correspond to areas where there was a significant ($p<0.05$ corrected) negative correlation with each ADI score. No areas with positive correlation were found.

DISCUSSION

Our results suggest a distinct neurodevelopmental trajectory for myelin in ASD. In this first cross-sectional analysis of MWF in children and adolescents, we found that our ASD group had a significantly steeper developmental trajectory of myelin in brain regions and pathways previously implicated in the disorder. The fact that we observed no between-group differences but documented extensive age-related between-group differences reinforces that the effect of age is particularly relevant to understanding ASD aetiology.

Although significant individual variability is seen in our results, there was a consistent pattern of increasing MWF from childhood through adolescence across groups. The slope of MWF vs. age differed significantly between the two groups in 11 out of the 12 tracts examined (with the exception of the left Inferior-Longitudinal Fasciculus). Fibre tracts for the ASD group were characterized by lower MWF values at 6 years of age, but increased MWF relative to controls by the age of 17. The 'cross-over' point, where the ASD group went from reduced to increased MWF relative to healthy controls appeared consistently between 12 and 13 years of age for most tracts. An exception to this time-course was the UF, in which the slopes were seen to intersect much earlier, between approximately 6 and 9 years. We also observed a substantial hemispheric difference in timing for this tract, with the left UF preceding the right by several years. This hemispheric asymmetry has been noted in previous DTI studies [85] and may be attributed to hemispheric specialization, as this fibre bundle connects cortical regions involved in speech and language [131].

The age-dependent differences that we have observed may help explain some of the variability that can be seen in existing ASD WM literature. Weak concordance among previous investigations can be attributed, in part, to the heterogeneity of the ASD, but also rests strongly on the methods of sampling and analysis used. As we have shown, the age of the subject pool will significantly influence results. Based on our results it is easy to see how

findings of significantly reduced (children sampled under 12 years), no change (uniformly sampled around 12 years of age), or significantly increased white matter integrity (older adolescents) could be obtained through cross-sectional investigations. This highlights the need for more statistically powerful longitudinal investigations that extend downwards to infancy and upwards through early adulthood.

In correlation with symptom measures we found that MWF was reduced in connection with increased impairment, as measured by the ADI-R. Impairments in social and reciprocal interaction (measured in ADI-R domain A), were correlated with reduced MWF in several regions, including, the CC; internal capsule; ILF; SLF; IFOF; cingulum; fronto-striatal pathways; and the cerebellum. All of these areas have previously been associated with autism [46, 85, 86, 89, 90, 132-139] and reduced FA in both the SLF and cerebellum have specifically been correlated with impairments in this particular ADI-R domain [89]. We identified similar negative correlations with communication (measured in ADI-R domain B), for the SLF, IFOF and cingulum. The cingulum is the most prominent tract connecting the limbic system and its deterioration has been linked to social attention impairments [140] and abnormalities in expressive language [133]. Likewise the SLF, which connects fronto-temporal networks (Broca's and Wernicke's areas), has been robustly associated with language production [141]. Interestingly reduced MWF in the right CST was associated with restricted repetitive and stereotyped behaviours (measured in ADI-R domain C). Reduced FA has previously been noted in the CST of individuals with ASD [86, 90, 142] and hypoplasia of the CST has been correlated with stereotyped motor behaviour in mice [143], however, its relationship with restricted and stereotyped behaviour in ASD has not previously been explored.

Interestingly, no significant correlation was made between ADOS scores and MWF. The ADI-R and ADOS are the 'gold standard' diagnostic measures for ASD, based on eliciting a developmental history from informants or assessing current symptoms, respectively. In older individuals, current symptoms are often modulated by coping strategies developed over the life span [144], which would undoubtedly impact what is seen during the ADOS and

possibly impact our findings. Additionally, from a diagnostic perspective ADI-R scores hold more bearing than ADOS as information collected about developmental history and age of onset is vital for diagnosis and cannot be seen during the ADOS for older children or adolescents.

It is interesting that our ASD group is characterised by an accelerated change in myelin relative to controls, yet within the ASD group increased myelin seems to be an adaptive change associated with fewer symptoms. This might suggest that after an early lag in micro-structural organization or myelination, the ASD brain attempts to "catch up" (and perhaps then "over-compensates") throughout later childhood and adolescence. We know that in typical development, neural connections are continuously refined through subtractive processes such as *axonal pruning* [145] and productive processes such as *myelination* [146] and *myelin remodeling* [147]. Adolescence is thought to be a critical time for cortical pruning [148] and myelination is a process that continues in an active manner through childhood adolescence [147, 149]. Thus, these processes are all possible candidates for the dissimilarities we have observed.

Our findings, although seemingly paradoxical, do conform with accepted clinical definitions of ASD, which recognize a 'most abnormal' period between 4 and 5 years of age. We have observed that MWF is most reduced at the bottom of our study age range (closest to the 'most abnormal' period) and that reduced MWF is associated with more pronounced symptomatology. MRI literature at this age range is lacking and since our data is cross-sectional we cannot derive predictive assumptions, but only hypothesise that the 'most abnormal' period in ASD is temporally coincident with a period of significantly low myelination.

To conclude, MWF imaging has a long history in the field of known demyelinating disorders [36], however, its use in examining development is novel. The verification of mcDESPOT MWF measures has been limited to qualitative histological comparisons in the Shaking Pup model of demyelination [150], and through comparison with the known histological time-course of myelination in healthy human infants [30, 37] and demyelination in multiple

sclerosis [34, 35]. As such, the specificity of mcDESPOT MWF measures as a reflection solely of myelin may be questioned - it has been suggested that additional effects, such as magnetization transfer, may also influence mcDESPOT [37, 151]. In any case, phenomena visible in MRI are unlikely to be the result of single, independent processes, but probably reflect a number of coordinated changes, involving various cell types. The best way to explore and interpret these ideas would be with more statistically powerful longitudinal studies of MWF, taken hand-in-hand with studies of myelin gene expression, brain chemistry and histology, to observe changes to the WM matrix that may be below the resolution of magnetic resonance imaging.

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CHAPTER. **9**

LONGITUDINAL DIFFERENCES IN WHITE MATTER MATURATION IN CHILDREN AND ADOLESCENTS WITH AUTISM SPECTRUM DISORDER

ABSTRACT

Numerous neuroimaging studies have investigated the neural correlates of Autism Spectrum Disorder revealing differences in regional white-matter microstructure and development, with considerable heterogeneity in their results. Most of these prior studies have been cross-sectional, making it difficult to accurately model the developmental trajectories of specific brain regions and determine the spatial and temporal emergence of structural abnormality in ASD. Therefore, we performed a longitudinal MRI study examining white matter maturation in a sample of 95 male children and adolescents aged between 6 and 20 years. 51 subjects with ASD and 44 controls (matched for age and IQ) were scanned up to 3 times (the average time between scans 1 and 2 was 1.13±0.21 years; and between scans 2 and 3 was 1.02±0.06 years). The main outcome measures were the longitudinal trajectories of myelin water fraction, quantitative T_1 (qT_1), and quantitative T_2 (qT_2). Statistical comparisons of ASD and typical trajectories revealed altered MWF trajectories in the splenium of the corpus callosum, left anterior thalamic radiation, bilateral posterior limb of the internal capsule, and the right inferior longitudinal fasciculus. Overlapping results were found with qT_1, with the addition of left ILF. Differences in qT_2 were identified in the fornix, left ATR, right uncinate fasciculus, bilateral PLIC, and right ILF. For the first time, we show differences in myelin development in children and adolescents with ASD compared to typical development. These results complement prior reports of altered white matter development in ASD, and further support ASD being associated with distinct patterns of brain development.

INTRODUCTION

Autism spectrum disorder is characterized by an early childhood onset of impairments in communication and social interaction, and restricted and repetitive behaviours [1, 2]. The neurological foundation of these altered behaviours has been the focus of numerous studies using advanced imaging

techniques [3-5] to look at differences in brain structure and function spanning infancy through adulthood. The results from these studies are heterogeneous and in some cases contradictory. The heterogeneity evident in magnetic resonance imaging literature is heavily influenced by study design (e.g. cross-sectional or longitudinal, retrospective or prospective), study cohort (e.g. differences in age, number of subjects, diagnostic criteria, and choice of control population) and analysis methodology (e.g. voxel-wise, tract-specific, regional, or global). In addition to specific study parameters, confounds including head motion, physiological noise and a small effect size also contribute to inconsistencies, particularly within white-matter literature [6-8].

Despite this heterogeneity it is established that ASD is accompanied by developmental differences in brain anatomy and connectivity [9-11]. One of the most reproduced MRI findings is that of an altered trajectory of brain growth in ASD [12-15]. More specifically, research suggests that the ASD brain undergoes a period of accelerated growth during early postnatal life (up until the age of 2) [12, 16-18], followed by atypically slow or arrested growth throughout the remainder of childhood [14]. A second, well-replicated finding is that of altered connectivity in ASD, more specifically, a number of studies indicate increased short-range connectivity alongside reduced long-range connectivity [19-24]. In addition, it has been suggested that functional connectivity and 'brain coherence' is an emergent property of collaboration among brain areas through development [25]. As such, structural development and functional connectivity are inextricably linked and an improved understanding of white matter maturation may provide important insight into the neural correlates of dis-connectivity in ASD.

Currently, the most common approach to studying WM is diffusion tensor imaging, which provides quantitative metrics of the microstructural architecture of brain tissue [26, 27]. The large body of DTI ASD literature lends support to the hypothesis of altered WM development in ASD [8, 19, 21, 28-32]. In a longitudinal study of high-risk ASD siblings 6-24 months of age, Wolff et al., showed that fractional anisotropy was greater in ASD infants at 6 months compared to typical subjects, but by 24 months, typical subjects had not only

caught up, but had higher FA values than ASD subjects [21]. Walker et al., noted an age by group interaction in a cross-sectional study of 2-9 year olds with ASD, showing similar FA values in ASD and typical children at around 2 years of age, with diverging developmental trajectories with higher FA in typical children by 9 years of age [8]. Cross-sectional DTI studies of very young subjects show higher FA in ASD compared to typical subjects [19, 32], while studies of older subjects show lower FA in ASD [20, 28, 29]. In addition, cross-sectional studies of adolescents and adults with ASD tend to show fewer regions with differences in FA [20, 28, 33-35] suggesting that brain differences between ASD and typical subjects may normalize in magnitude and extent into adolescence and adulthood.

While these and other results suggest ASD is associated with abnormal brain development, the majority of brain studies have been cross-sectional in nature, most commonly examining young children or adults. Therefore, it is largely unknown how the brain develops during adolescence in ASD. Studies of typical development suggest that adolescence is a pivotal stage of elaboration, during which the trajectory of brain development changes from growth to decline in certain brain regions - grey matter volume plateaus (after reaching its peak) [36-38] whilst the developmental trajectory of WM remains dynamic and protracted [39-41] with white-matter tracts responsible for higher-order, multimodal cognitive functions reaching their peak at around 30 years of age [39]. In addition the modulation of WM may persist in an activity-dependent fashion through adulthood [42-44]. Thus, longitudinal studies are required to develop accurate models of brain development spanning childhood and adolescence. From these models, more sensitive and specific analyses of developmental trajectories may be performed to determine whether ASD is associated with altered brain growth, and how differential development may contribute to the spectrum of behaviours characteristic of the disorder.

In addition to the need for longitudinal studies, there is also a need for imaging measures with improved biological specificity. Changes in myelin are often suggested as a potential mechanism for observed changes in WM diffusivity, however, FA and other DTI-derived measures are sensitive to a

number of other changes, in addition to myelination [45]. Therefore, the suggestion of myelin involvement is purely speculative. Nonetheless, myelin is a plausible candidate - it is essential for the rapid transmission of electrical impulses, and its damage can impair conduction and consequently, sensory, motor, and cognitive functions [46, 47]; all of which are related ASD. Support for myelin involvement in ASD also comes from gene expression studies [48, 49]. Thus, MR techniques offering improved sensitivity and specificity to myelin content may provide new insight into the underpinnings WM maturation in ASD.

Henceforth, we present a longitudinal study of myelin development in children and adolescents with ASD and typically developing controls from 6 to 20 years of age. We examine the developmental trajectories of myelin water fraction as well as quantitative longitudinal and transverse relaxation times (qT1 and qT2), using the multi-component relaxometry technique, mcDESPOT [50] (described in Chapter 8). We perform longitudinal, nonlinear, mixed-effects modelling of the developmental trajectories of myelin water fraction, qT1 and qT2, and examined regional differences in the development of these measures in ASD and typical control groups.

PARTICIPANTS

51 male participants with ASD and 44 typically developing male participants between 6 and 17 years of age were recruited into the study (demographics are provided in Table 9.1). Subjects returned annually for up to 3 scans, with a total of 220 scans included in this analysis (119 ASD, 101 controls). The distribution of scans by age and group are shown in Figure 9.1. ASD participants were diagnosed according to the ICD-10 research criteria [51]. Clinical diagnosis was confirmed using the Autism Diagnostic Interview-Revised (ADI-R) and current symptoms assessed using the Autism Diagnostic Observation Schedule (ADOS).

Exclusion criteria included; intellectual disability (i.e. IQ<70), major psychiatric disorder (e.g., psychosis), use of medication affecting brain function, any pre-existing medical conditions or complications affecting brain function (e.g. head injury, epilepsy), chromosomal abnormality associated with ASD (e.g., fragile X syndrome, tuberous sclerosis), and any MRI contraindications. The study was approved by the National Research Ethics Committee, Suffolk, UK. Informed written consent was obtained from all study participants and their parents.

MR IMAGING

To investigate tissue MWF, the mcDESPOT MCR technique was used [50]. This involved the combined acquisition of 8 SPoiled GRadient recalled echo (SPGR, spoiled FLASH, or Fast Field Echo) and 8 fully-balanced steady-state free-precession (bSSFP, FIESTA or TrueFISP) images over a range of flip angles with constant acquisition timings. In addition, an inversion-prepared IR-SPGR image was acquired in order to allow correction of flip angle (B_1 field) inhomogeneities [52]. Two sets of bSSFP data were acquired with different

radio-frequency (RF) phase-cycling patterns in order to correct for main and transmit magnetic field (B_0) inhomogeneities [52].

Whole-brain, sagittally-oriented data were acquired with a common 22cmx22cmx16cm field of view (FOV) and 128x128x92 imaging matrix. To minimise acoustic noise and increase our paediatric population's comfort and compliance, maximum gradient amplitudes and slew rates were decreased by 75% and 55% respectively. To reduce acquisition time, data were acquired with 3/4 partial Fourier acquisition and parallel imaging with an acceleration factor of 1.75. Sequence-specific acquisition parameters were as follows: SPGR: Echo time (TE)/Repetition Time (TR) =4.9ms/11ms, flip angles (α) = {3, 4, 5, 6, 7, 9, 12 and 18}°, receiver bandwidth (BW) =217Hz/pixel. IR-SPGR: TE/TR =4.9ms/11ms, inversion time (TI) =450ms, α =5°, BW =217Hz/pixel. bSSFP: TE/TR =3.8ms/7.6ms, α ={12, 16, 21, 27, 33, 40, 51 and 70}°, RF phase-cycling patterns ={0 and 180} °, BW =217Hz/pixel.

All imaging was performed on a 3 Tesla GE Signa HDx clinical scanner equipped with an 8-channel head RF coil array. Total acquisition time for the protocol was less than 15 minutes.

MRI data were quality assessed visually by an experienced researcher. Data were rejected if incomplete or contaminated by motion artefacts. Consequently, five year-1 scans, six year-2 scans, and one year-3 scan were rejected (totalling 12 out of 232 total scans).

Image Processing and Longitudinal Registration

Quantitative T_1, T_2, and MWF quantification was performed using an established analysis pipeline. First, the SPGR, IR-SPGR and bSSFP images for each participant were linearly co-registered to account for subtle inter-scan head motion during acquisition [53]. Non-brain signal was then removed from each dataset [54]. Quantitative T_1 and T_2 were calculated using an established single-pool tissue model [55], and MWF was quantified at each voxel using a 3-pool tissue model (as in Chapter 8, described in [56, 57]).

It has been shown previously that registering individual longitudinal data points to a single template may introduce additional unwanted variability [58]. To minimize this affect, we used a modified longitudinal registration approach that has been used previously [59]. Briefly, a T_1-weighted template was created for each subject using the high flip angle SPGR images from the longitudinally acquired time-points, using symmetric diffeomorphic normalization and a mutual information cost function. First, rigid registration was performed to align the high flip angle SPGRs into rough alignment. The single subject template was then created from the aligned SPGRs using buildtemplateparallel.sh included in the ANTs package [60]. Finally, the subject template was nonlinearly registered to MNI space using symmetric diffeomorphic normalization. The transformations from this process were then applied to the individual time-point MWF, qT_1 and qT_2 maps with a single interpolation step. Registered MWF maps were then smoothed with a modest 3mm Gaussian kernel to account for residual mis-registration errors while preserving anatomical fidelity.

Statistical Analysis

Non-linear mixed effects modelling was used to characterize trajectories of MWF, qT_1 and qT_2 development. Over the age range of this study (6-20 years), previous imaging studies have shown a linear developmental trajectory for white matter. To confirm that this was the most appropriate model for our data, we fit each brain region (cross-sectionally) to a linear and logarithmic function. No significant difference in R-squared values was noted, thus we opted for the simpler linear model, of the form:

$$MWF(t) = m \times t + b$$

Where m = slope of the trajectory (growth rate), t = age in days, and b = intercept.

Modelling was performed in the following 12 (*a priori* chosen) brain regions, as defined by the John Hopkins University WM atlas [61]; the genu, body and splenium of the corpus callosum (CC), fornix, and bilaterally in the anterior thalamic radiation (ATR), inferior longitudinal fasciculus (ILF), posterior limb of the internal capsule (PLIC), and uncinate fasciculus (UF). Region masks were overlaid on the registered MWF, qT_1, and qT_2 data, and mean values were obtained for each individual at each time-point. To ensure the data support separate fits for ASD and typically developing subjects, we first fit all subjects to a single linear model, and then fit linear models to the ASD and to the typically developing subjects separately. An *F*-Test was used to determine if the data justified separate fits for the two groups. In regions where two models were supported (*F*-stat > 1.43 corresponding to $p<0.05$ corrected for the 12 comparisons), pair-wise t-tests were used to determine if the slope (development rate) and intercept of the ASD and typical trajectories differed significantly ($p < 0.05$ corrected for the 12 comparisons).

RESULTS

There were no significant differences between individuals with ASD and controls in either age or IQ; for time-point 1, age (t(91)=-0.38, p=0.71), IQ (t(91)=-0.71, p=0.48); time-point 2, age (t(66)=0.38, p=0.70); or time-point 3, age (t(57)=0.24, p=0.81), IQ (t(57)=-0.95, p=0.34). Demographic data is presented in Table 9.1.

Differences in developmental trajectories between Typical and ASD subjects were found for MWF, qT_1 and qT_2 (Table 9.2 and Figure 9.2). Trajectories of MWF development were found to differ significantly ($p < 0.05$) in the splenium CC, left ATR, right ILF, and bilateral PLIC. Trajectories of qT_1 were significantly different ($p < 0.05$) in the splenium of the CC, left ATR, bilateral ILF, and bilateral PLIC. Finally, trajectories of qT_2 were significantly different ($p < 0.05$) in the fornix, left ATR, right ILF, bilateral PLIC, and right UF. All regions remained significant after correction for multiple comparisons (Bonferonni correction).

Overall, the mean MWF values for ASD subjects were lower than the typically developing subjects. Developmental trajectories were positively correlated with age for both ASD and typical children, and followed two patterns: (1) diverging trajectories in the splenium of the corpus callosum (and approaching significance in the fornix, UF and left ILF), with a faster growth rate in typical subjects; (2) converging trajectories in the right ILF, ATR, and PLIC, with a faster growth rate in ASD subjects. In all cases, both the slope and intercept of the trajectories were significantly different for at least one measure (MWF, qT_1 or qT_2). Results are displayed in Figure 9.2.

Mean qT_1 times of ASD subjects were longer (i.e. larger qT_1 values) than typically developing subjects, and decreased with age for both groups, although with more variation than MWF. Trajectories in the splenium of the CC and right ILF did not differ in growth rate, but had statistically significant differences in intercept. In the left ATR and bilateral PLIC, ASD subjects had a steeper growth rate (stronger negative correlation with age), while in the left ILF typical subjects had a steeper growth rate. All trajectories differed statistically for intercept.

qT_2 slopes were very modest for most regions with some increasing and some decreasing with age. Overall, qT_2 values were higher in ASD subjects compared to typical subjects in early childhood, with trajectories converging in late adolescence (Table 9.2), such that qT_2 times were shorter (lower values) in ASD subjects compared to typically developing subjects at the top of our age range (Figure 9.2).

TABLE 9.1. SUBJECTS DEMOGRAPHICS.

YEAR 1	ASD (n = 50)	Control (n = 43)
Age (years)	11.9 ± 3.2 (6.3 - 17.6)	12.1 ± 2.8 (7.5 - 17.4)
IQ	108.2 ± 16.8 (70 - 140)	110.3 ± 13.1 (79 - 148)
ADI-R Reciprocal Social Interaction	18.8 ± 4.9	-
ADI-R Communication	16.0 ± 3.9	-
ADI-R Restricted, Repetitive, Stereotyped Behaviour	5.8 ± 2.4	-
ADOS Communication	4.1 ± 1.7	-
ADOS Reciprocal Social Interaction	9.0 ± 3.2	-
ADOS Stereotyped Behaviours, Restricted Interests	2.0 ± 1.8	-

Year 2	ASD (n = 36)	Control (n = 32)
Age (years)	13.6 ± 3.0 8.4 - 18.5	13.3 ± 2.8 8.4 - 18.4

Year 3	ASD (n = 33)	Control (n = 26)
Age (years)	14.1 ± 3.0 (9.4 - 19.2)	13.9 ± 3.0 (9.5 - 19.4)
IQ	108.8 ± 15.1 (79 - 139)	112.4 ± 13.7 (84 - 148)
ADOS Communication	3.6 ± 1.6	-
ADOS Reciprocal Social Interaction	6.6 ± 3.0	-
ADOS Stereotyped Behaviours, Restricted Interests	1.4 ± 1.5	-

Data expressed as: mean ± standard deviation (range). IQ was measured using the Wechsler Abbreviated Scale of Intelligence [62]

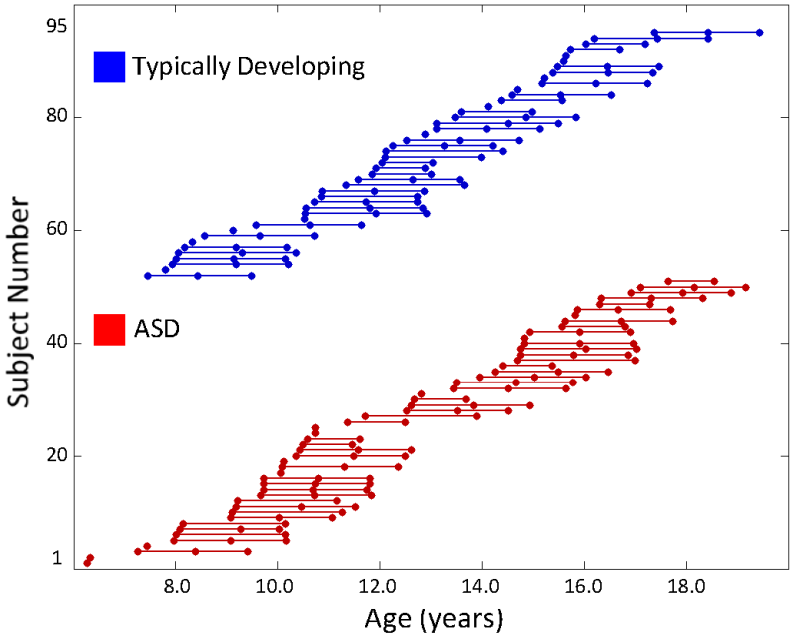
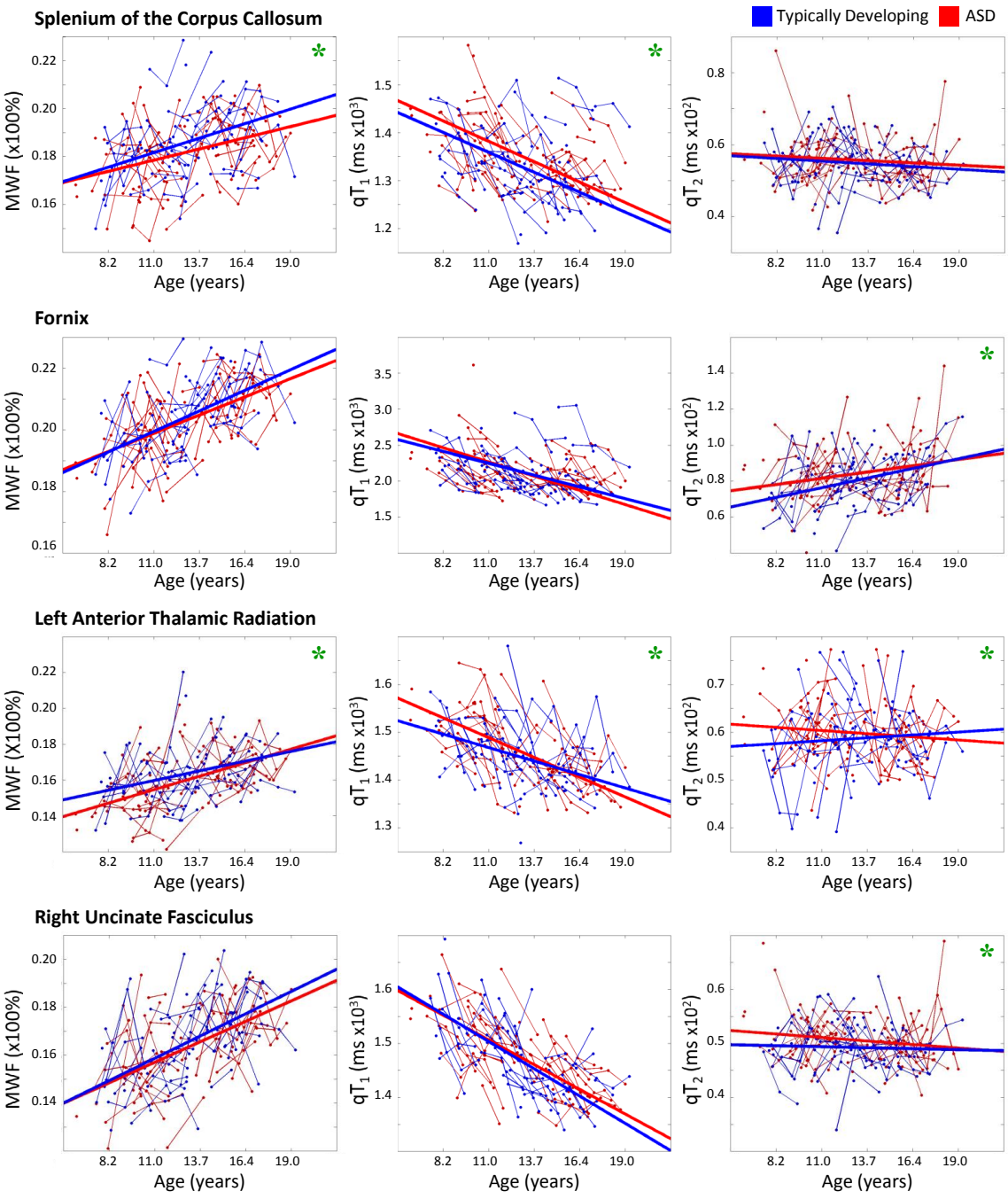


FIGURE 9.1

Ages of the 44 typically developing subjects and 51 subjects with ASD. Each row represents a single subject, with individual scan time-points indicated by a filled circle and repeated measurements connected by a solid line. Blue = typically developing, Red = ASD.

FIGURE 9.2

Developmental trajectories and individual data-points of MWF, qT1 and qT2 of typically developing (blue) and ASD (red) subjects. Statistical significance indicated with (*). Only regions with a statistically significant difference in the trajectories of at least one measure are shown.



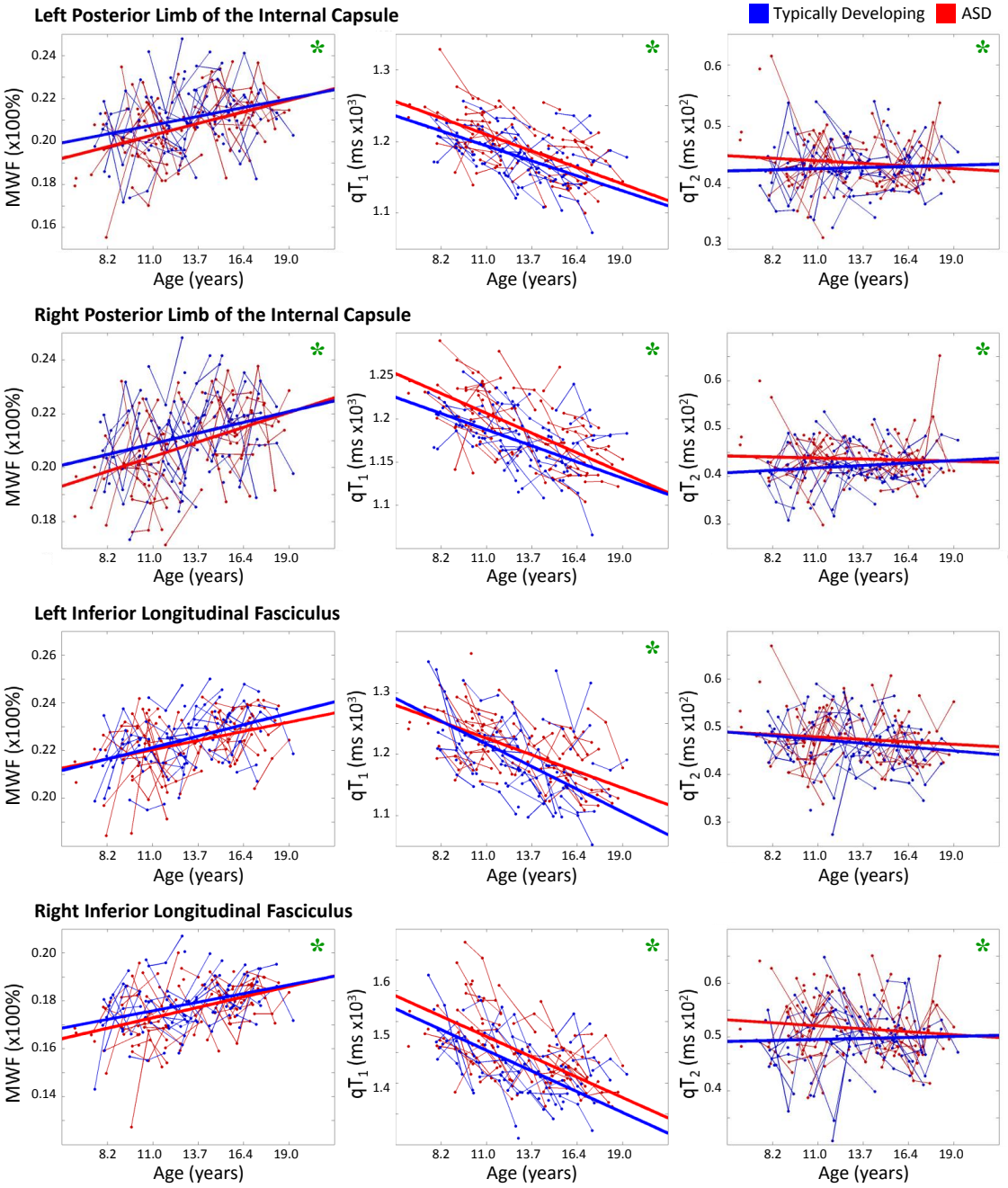


Figure 9.2 (Page 2 of 2)

TABLE 9.2. (A) RESULTS FOR MWF

Myelin Water Fraction (MWF)	F-test P Value	Slope (SE[a]), x10^{-6} Typical	Slope (SE[a]), x10^{-6} ASD	Intercept (SE) P Value	Intercept (SE) Typical	Intercept (SE) ASD	Intercept (SE) P Value	Age of convergence (Years)
Genu Corpus Callosum	0.59							
Body Corpus Callosum	1							
Splenium Corpus Callosum	0.003[b]	6.05 (1.64)	4.66 (1.20)	<0.001	0.157 (0.007)	0.160 (0.006)	0.008	4.8
Fornix	0.96							
Anterior Thalamic Radiation (Left)	<0.001	5.40 (1.61)	7.51 (1.26)	<0.001	0.138 (0.007)	0.125 (0.006)	<0.001	17.5
Anterior Thalamic Radiation (Right)	0.99							
Inferior Longitudinal Fasciculus (Left)	0.99							
Inferior Longitudinal Fasciculus (Right)	<0.001	3.61 (1.09)	4.37 (0.91)	<0.001	0.211 (0.005)	0.205 (0.004)	<0.001	21.5
Posterior Limb Internal Capsule (Left)	<0.001	4.12 (1.64)	5.42 (1.10)	<0.001	0.191 (0.008)	0.181 (0.005)	<0.001	20.7
Posterior Limb Internal Capsule (Right)	<0.001	3.99 (1.59)	5.48 (1.11)	<0.001	0.192 (0.007)	0.182 (0.005)	<0.001	19.8
Uncinate Fasciculus (Left)	1							
Uncinate Fasciculus (Right)	1							

Note. SE = standard error

Table continued overleaf…

TABLE 9.2. (B) RESULTS FOR QUANTITATIVE T1

	F-test	Slope (SE[a]), $x10^{-2}$		Intercept (SE)				Age of convergence
Quantitative T1 (qT_1)	P Value	Typical	ASD	P Value	Typical	ASD	P Value	(years)
Genu Corpus Callosum	1							
Body Corpus Callosum	1							
Splenium Corpus Callosum	<0.001	-4.16 (0.83)	-4.26 (0.61)	0.32	1525 (37)	1552 (30)	<0.001	n/a
Fornix	0.14							
Anterior Thalamic Radiation (Left)	<0.001	-2.82 (0.67)	-4.14 (0.54)	<0.001	1581 (32)	1654 (26)	<0.001	15.3
Anterior Thalamic Radiation (Right)	0.97							
Inferior Longitudinal Fasciculus (Left)	<0.001	-3.71 (0.57)	-2.70 (0.39)	<0.001	1365 (26)	1334 (19)	<0.001	8.5
Inferior Longitudinal Fasciculus (Right)	<0.001	-3.37 (0.52)	-3.31 (0.43)	0.36	1337 (24)	1357 (21)	<0.001	n/a
Posterior Limb Internal Capsule (Left)	<0.001	-2.10 (0.33)	-2.30 (0.28)	<0.001	1278 (15)	1302 (14)	<0.001	31.8
Posterior Limb Internal Capsule (Right)	<0.001	-1.88 (0.33)	-2.31 (0.28)	<0.001	1263 (15)	1299 (14)	<0.001	23.2
Uncinate Fasciculus (Left)	1							
Uncinate Fasciculus (Right)	1							

Note. SE = standard error

Table continued overleaf…

TABLE 9.2. (C) RESULTS FOR QUANTITATIVE T2

Quantitative T2 (qT_2)	F-test P Value	Slope (SE[a]), $x10^{-4}$ Typical	Slope (SE[a]), $x10^{-4}$ ASD	Intercept (SE) P Value	Intercept (SE) Typical	Intercept (SE) ASD	Intercept (SE) P Value	Age of convergence (years)
Genu Corpus Callosum	1							
Body Corpus Callosum	0.99							
Splenium Corpus Callosum	1							
Fornix	<0.001	53.6 (1.4)	34.7 (1.2)	<0.001	54.8 (6.8)	67.7 (5.9)	<0.001	18.7
Anterior Thalamic Radiation (Left)	<0.001	6.05 (7.9)	-6.62 (5.5)	<0.001	55.8 (3.8)	63.0 (2.7)	<0.001	15.6
Anterior Thalamic Radiation (Right)	1							
Inferior Longitudinal Fasciculus (Left)	1							
Inferior Longitudinal Fasciculus (Right)	<0.001	1.89 (6.0)	-5.5 (4.6)	<0.001	43.8 (2.9)	49.2 (2.3)	<0.001	20.3
Posterior Limb Internal Capsule (Left)	0.001	1.84 (4.4)	-4.15 (3.6)	<0.001	42.3 (2.1)	46.0 (1.8)	<0.001	16.9
Posterior Limb Internal Capsule (Right)	<0.001	5.09 (4.4)	-2.18 (3.7)	<0.001	39.8 (2.1)	44.7 (1.8)	<0.001	18.6
Uncinate Fasciculus (Left)	1							
Uncinate Fasciculus (Right)	<0.001	-1.83 (4.7)	-6.48 (3.7)	<0.001	50.1 (2.3)	53.7 (1.8)	<0.001	21

Note. SE = standard error

DISCUSSION

In this first longitudinal study of MWF development through childhood and adolescence, we have shown that the trajectory of myelin development varies significantly between typically developing individuals and those with ASD. Whilst we observed a high degree of individual variability, developmental trajectories for MWF indicate a pattern of increasing myelin from childhood through adolescence, with region specific differences in developmental trajectories between groups. The observed differences varied by region and followed one of two patterns; (1) diverging trajectories with a faster growth rate in typical subjects and (2) converging trajectories with a faster growth rate in ASD subjects.

AGREEMENT WITH DTI FINDINGS

These findings complement and extend the existing white matter imaging literature on ASD. We show that ASD subjects have lower overall myelin content in childhood compared to typically developing subjects (with the exception of the splenium of the CC), in agreement with cross-sectional findings of lower FA in children with ASD [63-66]. On the other hand, studies examining older children and adolescents show more heterogeneous results, including both region-specific increases, decreases, or no difference in FA [64, 67-71]. The region- and age-dependent differences that we have observed may help to explain some of the heterogeneity of findings across the existing literature - which is characterized by widely varying results, but also widely varying subject pools for age, gender, diagnosis, IQ, and severity of symptoms. In particular our findings show that the age of a particular cohort could significantly influence results, skewing them towards differences that are seen only at a specific age.

Nevertheless, there are regions that have been frequently identified as altered in ASD regardless of age, such as the splenium of the CC [19, 28, 66, 70, 72].

In our work, we have found that myelin development of this area diverges across the age-span of our study. These results, coupled with others showing differences in splenium development [21], suggest that this region begins to develop differently in ASD at a very early age and this persists at least up to 20 years of age. We also identified this trend of continuing divergence in other commonly identified brain regions, including the body and genu of the CC, fornix, bilateral UF, and left ILF, however these regions failed to reach significance. The remaining regions investigated, (ATR, PLIC and right ILF) showed a converging pattern, suggesting that the myelin development of ASD subjects may be normalizing with respect to typical development. Whilst it is unclear what drives this trend toward normalization, it may represent a later, compensatory myelin growth.

For the regions in which we observed a converging trend, the age of cross-over was close to 20 years for most regions (Table 9.2), with the exception of the left ATR, which crossed at 17.5 years. This is in line with the only other longitudinal study of white matter development in ASD [21], which examined diffusion differences at 6, 12 and 24 months of age in a set of typically-developing infants/toddlers, and a matched set of infants with increased familial risk of ASD who went on to be diagnosed with ASD at 2 years of age. Wolff et. al. noted that the ATR was the only structure where group differences were observed at 12-months of age, while the rest of the brain structures were only different at 24 months of age [21]. This suggests that differences in the ATR may occur earlier and subsequently normalize earlier, as we have observed here. For tracts showing a diverging trend, the cross-over age was near the youngest of our age range. It is possible that this age is biased by the start- and end- ages of our participants, and that the cross-over is actually much younger and more in-line with the observations of Wolff, et. al. In addition, at younger ages developmental trajectories have been shown to be non-linear [59, 73] and so these ages may be over- or underestimated.

Specificity for Myelin

The application of mcDESPOT to disorders of neurodevelopment is novel. To date, mcDESPOT derived MWF measures have shown a strong correlation with the known time-course of myelination in typical development [59, 74, 75]. In addition, MWF has shown strong correlation with clinical disability in multiple sclerosis [76, 77] and amyotrophic lateral sclerosis [78]. While quantitative histological comparison studies of mcDESPOT derived measures have yet to be performed, these findings provide the first evidence that MWF is extremely sensitive to myelin content. The increased specificity to myelin may be an advantage over other WM imaging methods, such as diffusion tensor and magnetization transfer imaging.

Atypical myelin development is further supported by our findings of qT_1 and qT_2. While more difficult to interpret in terms of a specific biological process, T_1 and T_2 are known to be sensitive to water and lipid content [79], with increasing lipid content resulting in shorter T_1 and T_2 times (hence the contrast between grey and white matter). We found significant trajectory differences between typically developing and ASD subjects, with longer T_1 and T_2 times in ASD subjects. This result is consistent with reduced lipid (myelin) content in subjects with ASD relative to controls [80].

Conclusion

Our results, suggest that the altered time-course of brain maturation in ASD is not restricted to early development. Whilst there is a trend towards normalization in some brain regions, there are persistent differences in others. This highlights the need for further longitudinal studies, which follow children at-risk of ASD from birth, through childhood and into young adulthood, in order to establish an appropriate model covering the lifespan, and to investigate whether the severity of later symptoms correlate with the degree of deviation from what is considered the typical developmental trajectory.

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DISCUSSION

CHAPTER. **10**

10.1. THESIS SYNOPSIS

The overarching theme of this thesis was the examination of differences in brain connectivity and brain structure in ASD throughout childhood and adolescence. Adolescence is a crucial neurodevelopmental stage, where peak maturation in some brain regions has been reached, while other regions continue to mature. Yet, atypical neurodevelopment in childhood/adolescence remains currently underexplored in ASD.

An important question at the outset of this work was: where to focus?

ASD is a disorder that is known to affect many different brain systems. However, the results of brain imaging studies to date are highly heterogeneous, and also point to differences in various image modalities and measures (e.g. differences in grey-matter morphology and differences in white-matter connectivity). The overall aim of this PhD thesis was therefore (1) to apply well-established neuroimaging techniques, to further examine brain differences in ASD during late brain maturation (i.e. during late childhood/adolescence and early adulthood), and (2) to utilize novel analytical techniques and imaging measures to further dissect the neuropathological underpinning of ASD and to combine (i.e. unite) findings from different imaging

modalities into a coherent picture of neurodevelopment in ASD. Below, I summarise the empirical findings of my work. Subsequently, I will tease apart/integrate differences and similarities between projects.

STUDY 1

In **Chapter 5**, we examined neurodevelopmental differences in cortical volume as a product of two biologically distinct neuroanatomical features, namely cortical thickness and surface area. Utilizing high-resolution structural T_1-weighted volumetric MR imaging, we examined age-related differences in cortical thickness and surface area in a broad cross-sectional cohort of individuals with ASD and matched controls, spanning from childhood into adulthood.

In this sample, we examined linear, quadratic, and cubic age effects on measures of cortical thickness and surface area in order to identify the most parsimonious model that allowed us to examine between-group differences in the presence of significant age effects, as well as age x group interactions. When controlling for the effects of age, we found that individuals with ASD had spatially distributed reductions in cortical thickness relative to controls, particularly in fronto-temporal regions. We also noted significantly reduced surface area in the prefrontal cortex and the anterior temporal lobe, relative to controls. Significant group x age interactions were also noted for both measures. However, while cortical thickness was best fit to a quadratic developmental trajectory, the neurodevelopmental trajectory for measures of surface area was mostly linear. Our findings indicate that ASD is accompanied by age-related and region-specific reductions in cortical thickness and surface area between childhood and early adulthood and, that differences in the neurodevelopmental trajectory for both these measures need to be taken into account in interpreting volumetric findings.

STUDY 2

In **Chapter 6,** we examined age-related differences in white matter diffusion measures in ASD. Utilizing diffusion tensor MR imaging to examine differences in fractional anisotropy, radial diffusivity and mean diffusivity at both voxel-wise and region-wise levels, in a broad, cross-sectional cohort of children and adolescents with ASD versus matched typically developing controls.

Here we found that individuals with ASD have a distinct neurodevelopmental trajectory of white matter relative to controls. This was characterised by a stronger positive correlation between FA and age in the ASD group, and a stronger negative correlation between age and RD, across multiple white matter pathways. In addition, we examined the relationship between DTI measures and the severity of current symptoms, and found a significant negative correlation between FA and restricted and repetitive behaviour as measured by the ADOS. Regions of significant between-group differences in FA included the uncinate, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, corticospinal tract, and the anterior thalamic radiation. These results suggest that individuals with ASD have a distinct neurodevelopmental trajectory of white matter relative to typically developing controls, and that certain symptoms may be accompanied by reductions in fractional anisotropy. Last, we investigated group differences in hemispheric asymmetry for FA measures, and found a cluster in the post-central gyrus where FA values were significantly more left lateralized in the ASD group relative to controls.

STUDY 3

In **Chapter 7**, we presented the results of a multi-modal neuroimaging study. Here we combined morphological measures of cortical grey-matter with diffusion-weighted measures of cortical white-matter, to examine age-related differences in local cortical gyrification and white-matter structural

connectivity, in a cross-sectional cohort of children and adolescents with ASD and matched typically developing controls.

Here we found that a quadratic model best predicted the developmental trajectory of local cortical gyrification (lGI). Subsequently, we examined between-group differences in lGI and noted significantly increased gyrification in the ASD group, localised in two clusters in the right temporo-parietal and occipital cortices. In these regions, we also observed a significant age x group interaction. We then went on to examine white-matter structural connectivity in tracts that originated or terminated within these significant lGI clusters. We did not find corresponding between-group differences or age x group interactions for measures of diffusion. However, we did observe a significant correlation between l GI measures and tract-specific diffusion measures in the right temporo-parietal cortex, indicating an association between surface morphology and underlying white-matter architecture.

STUDY 4

In **Chapter 8**, we looked more closely at white matter differences in ASD using a multicomponent-relaxometry technique called mcDESPOT. Here, we examined age related differences in white matter myelin water fraction at both the voxel- and region-wise level in a broad cross-sectional cohort of children and adolescents with ASD and matched typically developing controls.

Here we reported significant age-related differences in myelination between autistic and typical groups in several brain regions, including; the body, splenium and genu of the corpus callosum; fornix; anterior thalamic radiations; right inferior-longitudinal-fasciculus; and the bilateral uncinate. In ASD these regions were characterised by reduced myelin water fraction in early childhood, and steeper developmental trajectories, such that myelin water fraction was increased relative to controls in late adolescence. Additionally, reduced myelin content was associated with more marked symptomatology, denoted by higher sub-domain scores in the ADI. These

results show that the trajectory of myelination is altered in ASD and that myelin content is negatively correlated with symptom severity.

STUDY 5

In **Chapter 9** we utilised mcDESPOT to longitudinally compare white matter maturation in children and adolescents with ASD to typically developing children. We examined the developmental trajectories of quantitative T_1, quantitative T_2 and the myelin water fraction for 12 white matter regions.

MWF trajectories were found to differ significantly between autistic and control participants, and followed one of two patterns: diverging trajectories in the corpus callosum, fornix, UF and left ILF, with a faster growth rate (higher slope) in typical subjects; converging trajectories in the right ILF, ATR, and PLIC, with a faster growth rate in ASD subjects. Overlapping results were found for quantitative T_1, with the addition of left inferior longitudinal fasciculus. Differences in quantitative T_2 were identified in the fornix, left anterior thalamic radiation, right uncinate fasciculus, bilateral posterior limb of the internal capsule, and the right inferior longitudinal fasciculus. These results showed for the first time, that myelin development is altered in children and adolescents with ASD compared to typically developing controls. These results complement prior reports of altered white matter development, and further support ASD being associated with distinct patterns of brain development.

INTEGRATION OF FINDINGS ACROSS STUDIES

The studies conducted as part of this PhD thesis have utilized a series of distinct imaging techniques, which exploit differing physiological properties of biological tissues. For example, in volumetric T_1-weighted imaging, the measurement of interest is the time taken for magnetization to recover following excitation. In DTI, we measure the directionality of water diffusion whilst in MWF imaging, we exploit the differing properties of trapped versus

free water. Despite these methodological differences, however, important parallels can be drawn between each of these studies.

AGE RELATED DIFFERENCES

Across studies, the most consistent finding is that there are age-related differences in development of grey- and white-matter from childhood into adolescence in ASD. We observed significant age x group interactions in all of the studies, even in the absence of significant between-group group differences in the different measures – as was true for both our studies of myelin water fraction and diffusion measures. While MWF and measures of diffusion reflect different aspects of brain structure, both are sensitive to changes in myelin content, thus, offering some continuity between these findings. We showed (in **Chapters 6** and **8**) that both FA and MWF are positively correlated with age, and that this positive correlation is stronger in individuals with ASD than in controls. Thus the ASD group is characterised by an "accelerated" change in white matter relative to controls – although the term "accelerated" should be used with caution, as these two studies were not longitudinal, and so cannot not inform about changes within individuals.

Interestingly, increases in both of these measures (MWF and FA) were also associated with reductions in symptom severity, which may suggest that the accelerated change in diffusion and myelin measures in the ASD group could in fact be adaptive. For example, after an early delay in micro-structural organization or myelination in early childhood, the ASD brain attempts to "catch up" throughout later childhood and adolescence. Encouragingly, these findings correspond with accepted clinical definitions of ASD, which identify a 'most abnormal' period between 4 and 5 years of age. In addition, we note that micro-structural organization and myelination is most reduced at the lower end of our age distribution (closest to this 'most abnormal' period), and this is associated with more pronounced symptomatology. In line with this, a number of studies have suggested that abnormalities in the ASD brain become less pronounced with age [1, 2].

MODELS OF AGING

For both our measures of white-matter connectivity (described in Chapters, 6, 8 and 9), the most parsimonious statistical model of aging was found to be linear. This finding agrees with earlier observations demonstrating that white matter undergoes linear increases in volume and density with age [3-6]. Grey matter development, on the other hand, follows a non-linear trajectory [7-11]. As such, we examined cortical thickness, surface area and gyrification using more complex age terms, and found differences in the neurodevelopmental trajectories of all three features.

As expected, based on previous research (Shaw et al. 2008), the neurodevelopmental trajectories of cortical thickness and gyrification were best predicted by a more complex quadratic age term. The neurodevelopmental trajectory for measures of surface area however, was linear, which is more in line with our white matter findings. To explain this, it has been suggested that reduced cortical surface area may correspond to underlying reductions in the area of WM underlying the cortical mantle [12]. Additionally, reconstructing a true representation of the cortical surface area in FreeSurfer involves surface tessellation or 'tracing' of the underlying white matter volume to produce a cortical mesh representing grey and white matter surfaces. As such, measures of cortical surface area may be more closely related to the underlying white matter, than those of cortical thickness. This would also connect with our finding of altered neurodevelopmental trajectories in white matter tracts that connect the cortical regions where we have seen abnormalities in surface area (discussed below).

REGION SPECIFIC DIFFERENCES

In addition to our common observations of age related change we also observed some common region-specific differences. One example of this is the uncinate fasciculus - in both our cross-sectional studies of diffusion and myelination (Chapters 6 and 8, respectively), the developmental slope that we

observed for the UF stood out from the trends that we recorded for other regions. Therefore, both these studies point to a pathway of development that is unique to the uncinate both in terms of timing and hemispheric asymmetry. In addition, the uncinate fasciculus connects social and emotional grey matter regions, such as the anterior temporal lobe, orbitofrontal, and prefrontal cortex, thus, presenting a link between our studies of white–matter connectivity and morphological measures of cortical grey-matter. More specifically, in **chapter 5,** we observed significant reductions in vertex-based measures of surface area in the medial orbitofrontal and anterior temporal lobe in our ASD group. Additionally, complex group by age interactions were observed in the anterior temporal and prefrontal lobe where individuals with ASD tend to have reductions in cortical thickness during childhood, but increased cortical thickness during adulthood. These areas are thought to play crucial roles in a number of socio-cognitive domains which are impaired in ASD, including theory of mind, empathy and self-referential cognition [13-17].

Another example of a region-specific finding is the cortico-spinal tract, for which both FA and MWF were negatively correlated with restricted, repetitive and stereotyped behaviour in ASD. Whilst reduced FA has previously been noted in the cortico-spinal tract of individuals with ASD [18-20], its relationship with restricted and stereotyped behaviour in ASD has not previously been explored. However, hypoplasia of the CST, which is involved with voluntary movement, has been correlated with stereotyped motor behaviour in mice [21], which may indicate the possibility of a relationship between this region and repetitive and stereotyped body movements seen in ASD.

10.2. Methodological Considerations

One of the main strengths of this study is the use of a large, well-characterised and homogenous sample of right-handed males. For example, it has been reported that handedness effects brain laterality [22] and therefore - by including only right-handed subjects - we could attribute any significant laterality effects to differences related to ASD, rather than differences in handedness. There is also evidence for sexual dimorphism in the brain [23], and it is known that ASD is more prevalent in males than females [24-27]. Thus, including only males in our sample enhanced its phenotypic homogeneity. Another strength is the medication naivety of our sample. Individuals with ASD are often prescribed medications such as Risperidone and SSRIs [28], and these can have long term effects on structural brain development [29]. Therefore, by recruiting a medication naïve sample, we removed this potential confound.

However, these same factors that infer statistical strength and precision also limit the generalizability of our findings. Our sample inevitably represents only a subpopulation of the characteristically heterogeneous autistic spectrum. As a result, our findings may not be replicated in other groups on the autism spectrum (e.g. females or individuals with intellectual disability). Future research is thus needed to better characterise the diversity of individuals on the autism spectrum, and to examine alternative subgroups of individuals with ASD (e.g. individuals in the low-functioning range as well as the family members of affected individuals, who exhibit the broader autism phenotype but do meet the clinical requirements for diagnosis).

Finally, an important limitation of this work is that in four out of the five conducted studies, we have employed a cross-sectional design to investigate age-related differences in brain anatomy between groups. The trajectories discussed in these studies are not co-referential with longitudinal developmental trajectories that describe change within individuals. This will be discussed further in the following section.

CROSS-SECTIONAL VERSUS LONGITUDINAL FINDINGS

In **Chapters 8** and **9**, I presented the findings of respective cross-sectional and longitudinal examinations of the same sample (at time-point 1). Below I discuss the methodological considerations of both.

The study of development consists of observing samples at different age levels in order to establish age-dependent relationships. In both cross-sectional and longitudinal designs this rests on the assumption that the differences between different age groups can be can be attributed solely to the effect of age (or age x group interactions). Thus, both should generate comparable age-dependent relationships. However, in the present work and previous studies, discrepancies have been observed between cross-sectional and longitudinal study designs. For example, cross-sectional investigations of IQ produce an aging curve depicting a plateau followed by gradual decline of intellectual ability from the age of thirty, whereas longitudinal studies suggest that intellectual ability continues to increase until mid-life [30, 31]. Similarly, cross-sectional studies of personality differences have been shown to suggest more dramatic developmental trends than longitudinal studies [32]. These discrepancies may be attributed to the methodological differences between both designs.

For example, the primary difference between cross-sectional and longitudinal designs is the respective use of independent (single observations of n individuals), versus dependent sampling (n observations of a single individual). The independent sampling in the cross-sectional designs enables us to investigate neuroanatomy across a relatively large age-range, in a large number of subjects. However, it has not allowed us to determine neurodevelopmental trajectories within individuals. On the other hand, the dependent sampling of the longitudinal study permits the analysis of individual trends through repeated observations of individuals. However, repeated observations require repeated participation and this may lead to participant drop-out, which - if systematic - may lead to selection bias [33].

In our longitudinal study, participants were required to return on three occasions, and a number of measures were taken in order to minimise attrition (see Chapter 4). Nonetheless, our study was affected by participant drop-out.

Attrition occurred due to a number of reasons, such as:

1. Participants who were eligible at the start of the study became ineligible during the time-course of the study. This included individuals who were newly medicated and individuals who no longer passed MRI safety screening, for example, due to operations or orthodontic fixtures.
2. Participant withdrawal. This occurred infrequently but common reasons included parental ill health or divorce.
3. Poor scan quality. Of those participants who returned for repeated participation, a number of scans had to be excluded from the study, as participants were unable to lie still for the duration of the scan.

Therefore, participant drop-out may have increased the homogeneity of our longitudinal sample in a positive direction (reducing those participants with social, psychiatric and medical complications). This could account for the stronger group by age interactions that we observed in our cross-sectional sample.

10.3. Potential Directions For Future Research

Women With ASD

In the present work, we chose to study males exclusively, as the disorder is more prevalent in males rather than in females [24-27]. However, the study of females with ASD is of great importance in advancing our understanding of ASD aetiology as a whole. For example, it is known that there is significant sexual dimorphism in the brain [23, 34], particularly during development [35, 36]. However, recent work has shown that typical sexual dimorphism found in controls is attenuated in participants with ASD [37], thus, implicating factors underlying gender-specific brain differentiation in the aetiology of ASD. Additionally, it is known that the clinical manifestation and cognitive profile of individuals with ASD differs by gender [38-40], as does neuroanatomy [41] and brain activation [40]. Furthermore, recent research has suggested the existence of female protective effects against the disorder [42]. Therefore, it is essential to explore these gender differences further in future research.

Experience-Dependent Plasticity In ASD

Many recent studies have applied MRI techniques in the investigation of group differences that reflect a certain skill or expertise. One of the first and most well known studies was the demonstration of greater posterior hippocampal volume in London's taxi drivers [43]. This study illustrated the plasticity of this brain structure involved with spatial navigation. In addition, the measured effects increased as a function of experience and practice, suggesting an experience-dependent plasticity of its structure [44].

However, we do not know whether such correlational relationships relate to different environmental factors like persistent training, or certain predispositions, such as anatomical variations that exist prior to skill acquisition. Moreover, anatomical variation is likely to have many precursors,

including environmental, genetic and epigenetic ones. Therefore, it would be of great interest to test these hypotheses in individuals with ASD, in order to establish whether ASD is accompanied by differences in activity-dependent white- or grey matter plasticity. Particularly, for the following reasons:

1. We know that the clinical profile of ASD changes with age, and this also applies to brain anatomy.
2. The clinical phenotype of ASD may also change as a result of intervention and behaviour-based therapeutic approaches [45-47].

GENETICS

An important issue that was not addressed in this thesis was the influence of genes on brain development. Throughout this work we have observed a considerable amount of individual variation across both typically developing and ASD groups. Inter-individual differences in brain structure are unquestionably influenced by genetic variation, which has previously been investigated in twin studies examining how genetic variations explain differences in grey and white matter findings. For example, it has been shown that genetic influences on grey matter are most prominent in the frontal and temporal lobes, incorporating language areas [48]. Whilst in white matter, genetic factors have been shown to explain almost 80% of the variance in fractional anisotropy, most notably in parietal and frontal lobes [49]. As such, the application of the imaging methods described in this thesis to monozygotic and dizygotic twin pairs who are affected, unaffected, or discordant for ASD would be of great relevance.

GENES THAT CODE FOR MYELIN

There are a host of myelin related genes, and the roles these genes play on myelination are diverse. A number of genes have been linked to WM microstructure and volume [50-53]. However, these genes largely control poorly understood and highly complex molecular pathways, which complicate the

direct investigation of the relationship between genes and myelination. This particularly applies to mechanisms of axon ensheathment and myelin growth, which involve several stages [54]. The genetic regulation of these processes is of critical importance in neurodevelopment.

Below, I have listed some genes that are thought to be important in myelin sheath development and maintenance, and could provide potential targets for future investigations in ASD.

1. Myelin basic protein (MBP) - The protein encoded by the classic MBP gene is a major constituent of the myelin sheath of oligodendrocytes in the central nervous system [55]. Previous studies have reported higher levels of anti-MBP auto-antibodies in the blood serum of autistic children than typically developing children [56, 57].
2. Myelin associated glycoprotein (MAG) - The protein encoded by this gene is a type I membrane protein and a member of the immunoglobulin superfamily. MAG mediates certain myelin-neuron interactions and is believed to be involved in the process of myelination. Three alternatively spliced transcripts encoding different isoforms have been described for MAG [55]. Increased serum anti-MAG antibodies have also been reported in autistic children [58].

So far, few studies have examined the effects of specific polymorphisms of these genes on early myelination, so future work would require tagging single nucleotide polymorphisms across the whole gene to fully characterise it. This could be combined with neuroimaging findings with the end goal of determining whether differences in certain alleles within these genes influence myelin development at critical windows in development.

Another interesting genetic target is the FADS gene cluster, which is responsible for the biosynthesis of long chain polyunsaturated fatty acids (LC-PUFAs) [59], which have been implicated in myelination [60, 61]. A recent study combing genetics with DTI showed that the FADS genes are associated with age-related WM differences from childhood to early adulthood [62]. In future

this gene cluster could be studied alongside more myelin specific imaging methods like mcDESPOT to more specifically elucidate age x genotype interaction with myelin.

10.4. Conclusion

Collectively this work supports the notion that ASD affects the brain at a systems level. ASD is accompanied by multiple, spatially-distributed differences in the brain, and these differences can be observed at different levels of magnification. For example, our DTI findings have shown age-related WM differences in ASD, and we have been able to correlate WM diffusion measures in short U-shaped fibres with surface topology. However, interpretation of these findings is difficult as white matter is a complex biological milieu with numerous components to untangle. Thus, our effort of fractionating out the contribution of myelin facilitates a more precise interpretation. Similarly, we have shown that findings from volume-based approaches that focus on differences in global or regional brain volume can be fractionated into contributions from distinct morphometric sub-components to provide more precise candidates for the observed differences. In the same vein, there are microstructural and molecular mechanisms that fall below the resolution of structural MR-imaging techniques - such as changes in chemistry and cell metabolism. Techniques allied to MR-imaging, such as positron emission tomography and MR-spectroscopy hold great potential for tapping into the molecular level *in vivo,* and advances in these areas will greatly enhance our understanding and interpretation of MRI based discoveries.

To conclude, our findings consistently point to the importance of developmental trajectories. It is possible that the altered course of maturation seen in ASD influences the functional characteristics of the condition more than brain structure at any fixed time-point. Therefore, the aetiology of ASD may in fact be as dynamic as the process of development itself.

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Perhaps the most significant and translational facet of this research is

its predictive quality. The work, which culminates in longitudinal findings of altered neurodevelopment along with changing symptom profiles has likely value in helping those with autism, for example through bringing new understanding to clinicians of developmental trajectories or stimulating new lines of research that may ultimately enhance biological psychological or social interventions. In the simplest sense, knowing and understanding where an individual falls on the ***neurodevelopmental continuum*** of autism is the first step in predicting and potentially steering, with intervention, where they may fall in 6 months, 1 year or 5 years time.

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APPENDIX

CONTENTS

ASD PARTICIPANTS

"The Institute of Psychiatry is starting a new and exciting project investigating brain development in young people with ASD. We believe that by improving our understanding of brain development, it may help us to explain better the behavioural difficulties that some young people with autism have.
The project involves three visits to the Institute of psychiatry over a 3-year period. During each visit, you will be asked to complete some questionnaires, have a brain scan and give a DNA sample. The brain scan is a painless procedure, with no known side effects.
We are looking for right-handed males with ASD, aged 6-16 yrs. All participants will be reimbursed for their time and travel expenses will be fully refunded.
If you would like to hear more about this project, or to take part, please contact: <name> at the Institute of Psychiatry, King's College London <telephone, email>.

REC Reference Number: 09/H0720/115"

CONTROL PARTICIPANTS

"The Institute of Psychiatry is starting a new and exciting project investigating the developing brain.
Healthy, right-handed boys aged 6-16years are sought to take part in this research. The project involves three visits to the Institute of psychiatry over 3 years. You will be asked to complete some questionnaires, have a brain scan and give a DNA sample. The brain scan is a painless procedure, with no known side effects.
All participants will be paid for their time and travel expenses will be refunded. If you would like more information please contact <name> at the Institute of Psychiatry, King's College London <telephone, email>.

REC Reference Number: 09/H0720/115"

***Personal Details*:**

	Parent(s)		Son
Title	Mr / Mrs / Miss / Ms		Master
Forename			
Surname			
Age		Date of Birth	
Contact Phone No	Home	Mobile	
Address			
Email address			
Year at school		Type of School	
What is your first language English/Other			
Are you right handed?	Yes / No		
Where did you hear about this project?			

Medical Information

How is your son's physical health?	Good
Does your son have any medical diagnosis (past or present), including any psychiatric disorders? Please specify:	Yes / No
Is your son on any medication?	Yes / No
Does your son have a history of head trauma?	Yes / No
Does your son suffer from epilepsy? Has your son ever used anti-epileptic medication?	Yes / No
Does your son suffer from chromosomal abnormalities associated with ASD, such as fragile X, Tuberous Sclerosis or VCFS?	Yes / No
Does your son have a learning disability?	Yes / No

MRI Scanning Information:

Has your son ever had an MRI scan before?	Yes / No
Does your son have a pacemaker?	Yes / No
Has your son ever had any cardiac or neurological surgery/Any surgery?	Yes / No
Does your son have any metal implants anywhere in his body? (i.e. metal pins/plates, clips)	Yes / No
Has your son ever sustained an injury involving metal, where the metal has not been successfully removed?	Yes / No
Has your son ever had a fit or blackout or suffer from epilepsy or diabetes?	Yes / No
Does your son have any of the following (Please specify): ▪ Metal dental work ▪ A hearing aid ▪ An artificial limb ▪ Tattoos ▪ Body piercings that cannot be removed	 Yes /No Yes / No Yes / No Yes / No Yes / No

B. INITIAL SCREENING FORM (ASD)

Does your son suffer from regular dizziness, extreme claustrophobia, or frequent migraines?	Yes / No
How tall is your son? (approximate height)	
How much does he weigh? (approximate weight)	
Can you think of anything else that may make your son unsuitable for an MRI scan?	Yes / No

ASD Diagnosis:

What is your son's diagnosis?	• Asperger's Syndrome • High-Functioning Autism • Autism Spectrum Disorder • Autism • Pervasive Developmental Disorder
At what age was your son given this diagnosis?	
Who gave your son this diagnosis?	
Is your son currently under the care of a psychologist or psychiatrist?	

Parental Involvement

In order for your son to be involved in this study, we would need to conduct an interview with one (or both) of you about your son's development. However, if you have already completed this interview in the past we will not need to repeat it.

Have you ever been interviewed regarding your son's diagnosis?	Yes /No
Have you ever done an Autism Diagnostic Interview (ADI)? ▪ When? ▪ Where? ▪ By who?	Yes / No
If this interview hasn't been done, we would need to meet or telephone you to complete this. Would one of you be available to be interviewed for this purpose?	

Would you be happy for researchers from the Institute of Psychiatry to contact you regarding future research?	Yes / No

Conversation with parent:

Date of conversation:.....

Agreed to ☐ ADI

Interview ☐ scheduled

Would you be happy for researchers from the Institute of Psychiatry to contact you regarding future research? Yes / No

***Personal Details*:**

	Parent(s)		Son
Title	Mr / Mrs / Miss / Ms		Master
Forename			
Surname			
Age		Date of Birth	
Contact Phone No	Home	Mobile	
Address			
Email address			
Year at school		Type of School	
What is your first language English/Other			
Are you right handed?	Yes / No		
Where did you hear about this project?			

Medical Information

How is your son's physical health?	Good
Does your son have any medical diagnosis (past or present), including any psychiatric disorders? Please specify:	Yes / No
Is your son on any medication?	Yes / No
Does your son have a history of head trauma?	Yes / No
Does your son suffer from epilepsy? Has your son ever used anti-epileptic medication?	Yes / No
Does your son suffer from chromosomal abnormalities associated with ASD, such as fragile X, Tuberous Sclerosis or VCFS?	Yes / No
Does your son have a learning disability?	Yes / No

MRI Scanning Information:

Has your son ever had an MRI scan before?	Yes / No
Does your son have a pacemaker?	Yes / No
Has your son ever had any cardiac or neurological surgery/Any surgery?	Yes / No
Does your son have any metal implants anywhere in his body? (i.e. metal pins/plates, clips)	Yes / No
Has your son ever sustained an injury involving metal, where the metal has not been successfully removed?	Yes / No
Has your son ever had a fit or blackout or suffer from epilepsy or diabetes?	Yes / No
Does your son have any of the following (Please specify): ▪ Metal dental work ▪ A hearing aid ▪ An artificial limb ▪ Tattoos ▪ Body piercings that cannot be removed	 Yes /No Yes / No Yes / No Yes / No Yes / No

Does your son suffer from regular dizziness, extreme claustrophobia, or frequent migraines?	Yes / No
How tall is your son? (approximate height)	
How much does he weigh? (approximate weight)	
Can you think of anything else that may make your son unsuitable for an MRI scan?	Yes / No

Conversation with parent:

Date of conversation:.....

Would you be happy for researchers from the Institute of Psychiatry to contact you regarding future research? Yes / No

MRI SAFETY SCREENING QUESTIONNAIRE

SURNAME..... **FIRST NAMES**.....
D.O.B......**HOME TEL:**.....
ADDRESS:.....
.....

(Please circle correct response)

1. Have you had any Scans or X-rays here before? **MRI / CT / X-rays / None**

2. Do you have a pacemaker or artificial heart valve fitted? **Y/N**

Any other heart or chest operations? **Y/N**

3. Have you had any operations on your head, ears or spine? **Y/N**

4. Have you had any operations where metal might have been inserted into your body? **Y/N**

If 'Y', please give details

.....

Have you had any other operations? **Y/N**

If 'Y', please give details

.....

5. Do you have any foreign metallic bodies in your eyes? **Y/N**

Have you done any welding or metalwork? **Y/N**

Do you have any shrapnel in your body? **Y/N**

6. Do you have any of the following:

Dentures, dental plates or bridges **Y/N** False limb, calliper or brace **Y/N**

Tattoos / metallic make-up **Y/N** Hearing aid or ear implant **Y/N**

Body Piercings **Y/N**

Any implanted device that is electrically, magnetically or mechanically activated? **Y/N**

7. Do you have a history of (a) Seizures **Y/N**

(b) Diabetes **Y/N**

(c) Allergic reaction to drugs? **Y/N**

please state which drugs .....

8. Is there any chance that you may be pregnant? **Y/N**

9. Do you have a history of any problems with your heart or arteries? **Y/N**

10. Do you have any history of kidney problems **Y/N**

11. Are you able to lie flat without becoming breathless? **Y/N**

12. How much do you weigh? ....

The reverse of this questionnaire gives some background information and reasons why your brain scan may help further our understanding of the human brain. Please read this carefully.

Signature.....**Date……**

Authorised Signatory.....

FOR RESEARCH SUBJECTS

ESTABLISHING AN MRI LIBRARY OF NORMAL AND DISEASED BRAIN SCANS OF ADULTS AND CHILDREN - Ethics Approval number 033/03

The KCL and SLaM Centre for Neuroimaging Sciences (CNS) and Wellcome Trust NIHR KCH Foundation Trust Clinical Research Facility (WT NIHR KINGS CRF) are building a picture library of the brain in health and illness. These pictures are very valuable to us, because we have very special machines that can take pictures which other hospitals may not be able to take. We hope to be able to understand more about the structure and the function of the brain, and by looking at many of these pictures, and linking them with different things, such as your age, we will get a better understanding of how the brain develops from childhood to old age.
The pictures we collect today will be kept on file and added to the picture library mentioned above, and may be used in another study at a later date. Rest assured that these pictures will have your name and other personal details removed before they are used for any further investigation. In addition, for each project that uses pictures from this library, permission from the Local Research Ethics Committee will be asked for, and we will only release these pictures when the Local Research Ethics Committee approves of the project.
Although it may be unlikely, it is possible that at some point in the future you might become unable to give consent for research such as this, so in order to be able to comply with your wishes, should this happen, we need to ask you now for your permission to continue to treat the pictures we collect from you in exactly the same way, even is you are no longer able to tell us that you are happy for us to do this.
If you have any particular questions, please feel free to ask any of the Radiographers or Radiologists.

I have read and understood this information sheet, and agree to my pictures being stored in the picture library, and also to them remaining in the library even if, at some time in the future, I lose should the capacity to give such consent.

Name of subject / guardian

_____

Signature of subject / guardian

_____

Date _____

The pictures taken today will be reviewed by a Radiologist, provided relevant medical history is available. If anything abnormal is found, the Radiologist will contact your GP, who will then contact you for further consultation if appropriate
(Note only examinations of the head are reviewed)

I understand and accept this and my GP Details are as follows
GP details:
Name _____
Address_____

____
Postcode_____

Name of subject / guardian

_____

Signature of subject / guardian

_____

Date _____

Version 4.3 25/10/2012

Imaging myelination during typical development and in autism
REC reference nr: 09/H0720/115
Participant Information Sheet 12/01/2010, version 2.0

Institute of Psychiatry

at The Maudsley

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Division of Psychological Medicine

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KING'S College LONDON
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University of London

Information sheet for young children

Imaging myelination in typical development and in individuals with autism

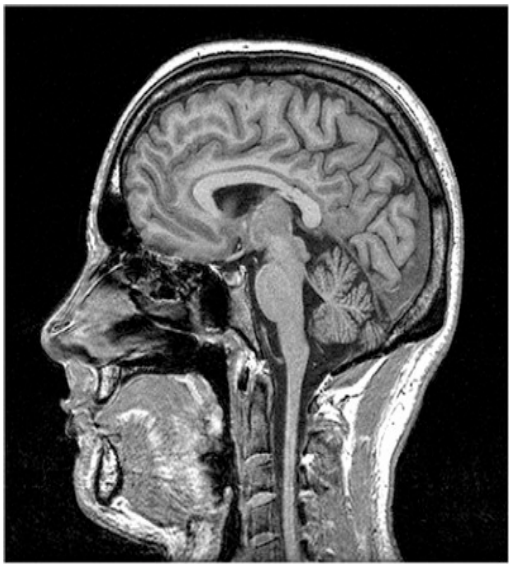
PRINCIPAL INVESTIGATORS: Dr. Sean Deoni and Prof. Declan Murphy

We are studying how the brain develops. Using a large machine, called a magnetic resonance imaging (MRI) scanner, we can take pictures of your brain. To take these pictures, we need you to lie very still inside the MRI scanner. While we are taking the pictures, you will hear clicking and banging sounds. This is normal and nothing to be afraid of. We will give you earplugs and headphones so that the noise isn't too loud. You can even go to sleep in the scanner if you want.

Before going in the scanner, a person called a radiographer will ask you some questions and make sure you are able to go in the scanner safely. They will help you to put in earplugs to protect your ears and put you in the MRI scanner. If you are cold they will give you a blanket to keep you warm. When you are in the scanner, the radiographer will be able to speak and hear you through a speaker built in to the scanner. If you are scared, worried or want to get out, you can ask the radiographer at any time during the scan.

Before the MRI scan, we will also give you some tests to complete. These tests are like ones you may have taken at school. Some may involve asking you what different words mean, or to remember pictures of people or places. In total, these tests will take between 1 and 2 hours, but you will be able to take breaks between them. If you are feeling tired, let us know and we will give you a break. You should not get upset if you cant complete all these tests or don't know the answers, nobody can complete them all, or knows all the answers. You may also meet with someone who will ask you some questions.

Finally, we will also collect some DNA from you. DNA is a part of us that makes us who we are and is different in every person and animal in the world. To collect some DNA, a nurse will gently rub a cotton bud along the inside of your cheek, inside your mouth. This may taste funny, but is harmless and safe.



Since we are interested in how the brain develops, we will ask you to come back 3 more times as you grow and get older.

After reading this sheet with your mum or dad, if you have any questions or don't understand something, you can ask one of the researchers to carefully explain it to you. It is up to you and your parents to decide if you want to have the MRI scans and take the tests. If you want, you can also ask to see the MRI before you decide.

If you decide to take part in the study, you and your mum or dad will sign another sheet and this sheet will be given to you to keep. If you or your parents have any questions, please ask one of the researchers. If you have any questions after you have gone home, here are the names and phone numbers of two researchers you can call at any time:

Asal Shahidiani (020) 7848 0855 Prof. Declan G Murphy (020) 7848 0984

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University of London

Information sheet for participants

Imaging myelination in typical development and in individuals with autism

PRINCIPAL INVESTIGATORS: Dr. Sean Deoni and Prof. Declan Murphy

We are asking you to help us by participating in the following study

We are studying how the brain develops, in particular development of the white matter that builds connections between brain regions. We are studying this in children with and without autism, to find out how the brain develops in children with autism. We want a wide variety of people to take part.

The study might not help you personally, but it may help other people. If you do not want to take part in the study, that is all right and it will not affect your future schooling or healthcare.

You can stop taking part in the study any time. If you are unhappy about any part of what is going on, you can let us know and we will stop immediately.

We will reimburse you for your time, and if you like, we can give you a picture of your brain after the scan!

What will happen if you take part?

We will meet with you and we will start with asking you some questions about your health. We will ask you to fill in a number of questionnaires to help find out how you have been feeling in yourself recently. In addition, we will do several tests. These are simple pen-and-paper tests; for example, we will ask you the meaning of several words and we will ask you to look at some pictures. The testing will take 1½ to 2 hours in total, but we will space them out and give you rests. You should not be upset if you can't do all these tests because nobody can do them all. If you have been diagnosed with Autism in the past, we will also do an interview called ADOS (Autism Diagnostic Observation Schedule), and an interview with your parents, called ADI (Autism Diagnostic Interview).

DNA

We will either take a blood sample or, if you prefer, collect some cheek swab samples for the purposes of DNA extraction. This involves gently rubbing cotton wool buds along the inside of your mouth and is completely painless. The DNA will be stored (but without your name on it) and will be used for future analyses. We will not get individual information from these analyses so we will not be able to give you 'results' of this.

MRI Scan

The next thing we want to do is an MRI (Magnetic Resonance Imaging) scan.

This means you will lie in a large machine that looks a bit like a tube (on the left you see a picture of an MRI scanner). In order to be scanned you will lie down on a table, which slides into the tube. The tube will be open at both ends all the time. We will ask you to lie down as quiet as possible, you can take a little nap if you want! The scanning will last about 45 minutes. With the MRI, we can take pictures of the brain. It has proven to be completely safe. There is a microphone inside the scanner so that you can talk to us at any time you like. If you feel slightly scared being in the scanning machine we can stop the scanning immediately. The MRI scanner makes a lot of noise and we will give you earplugs and headphones to make you as comfortable as possible.

If you have any **metal** pieces in your body then you should **not** go into the scanning machine. For example, you must not have a scan if you:

- have received metal injuries to the eye (caused by metal objects, for example by using a welder)
- have had metallic objects (including clips) inserted into your body during an operation
- have a heart pacemaker
- have ever received a shotgun injury
- have ever had abdominal surgery

The radiographer will go over a list of possible risks with you before you go into the scanner.
MRI scans do not involve any radiation.

In the unlikely event that we find anything unexpected on your brain scan we will contact your GP.

Second, third and fourth scan

If the first scan is successful, we will ask you to come back for a second scan after one year, for a third scan after two years, and for a fourth scan after three years. These scans are exactly the same as your first scan. When you come in for the fourth scan, we will repeat some of the psychological tests that were done with the first scan.

Will I benefit from participating in this study?

We do not expect that you will draw any specific personal benefit apart from a payment to compensate for your time: you will receive £25 for the first visit (including questionnaires and psychological testing) and £30 for the second visit (including the brain scan). For the second and third scan, you will receive £40 each time. If we decide that you are not suitable for taking part in the study, you will still receive £25 for the first visit. Also, all your travel expenses will be reimbursed.

How about confidentiality?

If you agree to take part in this study your General Practitioner (GP) will be informed of your participation. You will not be identified in our computers by name but by a number, and all records obtained while your are in this study, including related health records will remain strictly confidential at all times . The genetic tests using your DNA are purely for research purposes and no information will be revealed to anyone (including your GP) about you. A copy of this 'Information Sheet' and of the signed 'Consent Form' will be given to you to keep. A copy of the consent will be stored at the Institute of Psychiatry.

Remember that you are free to withdraw from the study at any time without giving a reason. This will not affect your current or future medical treatment in any way.

Thank you very much for reading this information. If you are worried about any aspect of this study please contact Professor Murphy (020) 7848 0984 or Asal Shahidiani (020) 7848 0855.
Or write to us at :

The Section of Brain Maturation,
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KING'S College LONDON *Founded* 1829

University of London

Information sheet for parents

Imaging myelination in typical development and in individuals with autism

PRINCIPAL INVESTIGATORS: Dr. Sean Deoni and Prof. Declan Murphy

We are asking you and your son to help us by participating in the following study

- We are studying how the brain develops, in particular development of the white matter that builds connections between brain regions. We are studying this in children with and without autism, to find out how the brain develops in children with autism. We want a wide variety of people to take part.

- The study might not help your son personally, but it may help other people. If you do not want him to take part in the study, that is all right and it will not affect his future schooling or healthcare.

- Your son can stop taking part in the study any time. If he is unhappy about any part of what is going on, he can let us know and we will stop immediately.

- We will reimburse your son for his time, and we will reimburse you and your son for travel expenses. We can provide your son with a picture of his brain after the scan.

What will happen if your son takes part?

We will meet with you and your son and take a medical history. We will administer a number of questionnaires to help find out how your son has been feeling in himself recently. You as a parent (or carer) will be asked to fill in some of these questionnaires. In addition, we will do several psychological tests. These are simple pen-and-paper tests including tests of his ability to use words and memory. The history taking and tests take 1½ to 2 hours, but we will space them out and give your son rests. If your son has been diagnosed with Autism in the past, we will also do an interview with you as parents, called ADI (Autism Diagnostic Interview) and an interview with your son, called ADOS (Autism Diagnostic Observation Schedule).

DNA

We will either take a blood sample or, depending on what your son prefers, collect some cheek swab samples for the purposes of DNA extraction. This will entail gently rubbing cotton wool buds along the inside of the mouth and is completely painless. DNA will be stored anonymously and will be used for future analyses. We will not get individual information from these analyses therefore individual results will not be provided; findings will apply to the group as a whole.

MRI Scan

The next thing we want to do is a MRI (Magnetic Resonance Imaging) scan.

With the MRI, pictures of the brain can be taken. For the MRI scan we will ask your son to lie down on a table that slides into the scanner. The tube will be open at both ends all the time. The whole time in the scanner will last about 45 minutes. The MRI is noisy and we will provide your son with earplugs and headphones to make him as comfortable as possible. There is a microphone inside the scanner so that your son can talk to us at any time he likes. In case he feels anxious we will stop the scanning immediately.

If your son has any **metal** pieces in his body then he should **not** go into the scanning machine. For example, he must not have a scan if he:

- has received metal injuries to the eye (caused by metal objects, for example by using a welder)
- has had metallic objects (including clips) inserted into his body during an operation
- has a heart pacemaker
- has ever received a shotgun injury
- has ever had abdominal surgery

The radiographer will go over a list of possible risks with him before he goes into the scanner.
MRI scans do **not** involve any radiation.

In the unlikely event that we find anything unexpected on your son's brain scan we will contact your GP.

Second, third and fourth scan

If the first scan is successful, we will ask your son to come back for a second scan after one year, a third scan after two years, and a fourth scan after three years. These scans are exactly the same as the first scan. When your son comes in for the fourth scan, we will repeat some of the psychological tests that were done with the first scan.

Will my son and I benefit from participating in this study?

We do not expect that you or your son will draw any specific personal benefit apart from a payment to compensate for your son's time: he will receive £25 for the first visit (including questionnaires and psychological testing) and £30 for the second visit (including the brain scan). You as a parent (or carer) will also receive £10 as a token of gratitude for your time and energy. For the second and third scan, your son will receive £40 each time, and you will receive £10. If we decide that your son is not suitable for taking part in the study, he will still receive £25 for the first visit. In addition, all your travel expenses will be reimbursed.

How about confidentiality?

If you and your son agree to take part in this study your General Practitioner (GP) will be informed of your participation. Your son will not be identified in our computers by name but by a number, and all records obtained while your are in this study, including related health records will remain strictly confidential at all times. The genetic tests using DNA are purely for research purposes and no information will be revealed to anyone (including the GP) about you. A copy of this 'Information Sheet' and of the signed 'Consent Form' will be given to you to keep. A copy of the consent will be stored at the Institute of Psychiatry.

Remember that you and your son are free to withdraw from the study at any time without giving a reason. This will not affect his current or future medical treatment in any way.

Thank you very much for reading this information. If you are worried about any aspect of this study please contact Professor Murphy (020) 7848 0984 or Asal Shahidiani (020) 7848 0855.
Or write to us at :

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KING'S College LONDON
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Consent Form
Imaging myelination in typical development and in individuals with autism

Principal Investigators: Dr. Sean Deoni and Prof. D. Murphy

Participant's Name: ……

The study has been explained to me by:

Prof/Dr/Mr/Ms …….

Please tick each statement as applicable:

I confirm that I have read and understood the information sheet for the above study, and have had the opportunity to ask questions. ☐

I understand what is required from me and my child to participate in this study. ☐

I am willing to be interviewed and to fill in some questionnaires about my child's development. ☐

I agree that DNA extracted from the sample given by my child will be used for research in genetic aspects of autism spectrum disorders and will be destroyed when the study is completed. ☐

I agree to my child having an MRI scan; I agree to be contacted for 3 follow up scans over next 3 years. ☐

I understand that our participation is voluntary and that we are free to withdraw at any time, without giving reason, and without any current or future medical care being affected. ☐

I understand that my records and those of my child may be accessed by responsible individuals from Imperial College and Kings College for audit purposes only. ☐

Signed (participant):…….... Date :……

NAME IN BLOCK CAPITALS: …….

Signed (parent/carer):…….... Date :……

NAME IN BLOCK CAPITALS: …….

Relationship to child: ……

Investigator's signature:……. Date :……..

NAME IN BLOCK CAPITALS: ……