Diffusion Tensor Imaging Exploration of Pediatric Multiple Sclerosis

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Science
University of Toronto

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Abstract

Diffusion Tensor Imaging (DTI) can quantify tissue integrity in normal-appearing white matter (NAWM). NAWM abnormalities present at the earliest time point implicate neurodegeneration operative from the outset of multiple sclerosis (MS).

DTI scans were obtained at first attacks from 6 children later diagnosed with MS and 6 children with monophasic demyelination, and from 6 controls, matched for age. DTI scans were also obtained from 22 children with established MS with clinical onset before age 12 years and compared to age-matched controls. Atlas- and tractography-based image processing methods were utilized.

DTI metrics distinguished MS patients from patients with monophasic demyelination and from controls at the first attack. Differences in NAWM between children with established early-onset MS and controls were only notable when DTI was obtained in adolescence.

DTI provides valuable insights into NAWM in children with MS, although in the youngest patients such changes may require time to develop.
This thesis is dedicated Leonid Rahmilovich Sonkin, a loving grandfather and professor.
Acknowledgements

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Thank you to the children and their families who have volunteered to take part in this project. Without their kindness, this research would not be possible.

Finally, I would like to thank all of my family and friends for the patience and moral support as I worked on this thesis.
# Table of Contents

**SECTION 1: INTRODUCTION**

**SECTIOION 2: BACKGROUND**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Demyelinating Disease</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1 Pediatric Demyelination: A Growing Concern</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2 Clinical Presentations of Pediatric Demyelination</td>
<td>5</td>
</tr>
<tr>
<td>2.1.3 Diagnosing Multiple Sclerosis in Children</td>
<td>7</td>
</tr>
<tr>
<td>2.1.4 Disease Course</td>
<td>9</td>
</tr>
<tr>
<td>2.2 The Power of Neuroimaging</td>
<td>11</td>
</tr>
<tr>
<td>2.2.1 How MRI Works</td>
<td>11</td>
</tr>
<tr>
<td>2.2.2 Neuroimaging as a Diagnostic Tool in Multiple Sclerosis</td>
<td>14</td>
</tr>
<tr>
<td>2.3 Imaging Beyond Lesions</td>
<td>16</td>
</tr>
<tr>
<td>2.3.1 Lesion Load is a Poor Correlate for Clinical Disability</td>
<td>16</td>
</tr>
<tr>
<td>2.3.2 Magnetization Transfer Ratio of Normal-Appearing White Matter in Multiple Sclerosis</td>
<td>17</td>
</tr>
<tr>
<td>2.3.3 Magnetic Resonance Spectroscopy of Normal-Appearing White Matter in Multiple Sclerosis</td>
<td>18</td>
</tr>
<tr>
<td>2.3.4 Brain Atrophy in Multiple Sclerosis</td>
<td>19</td>
</tr>
<tr>
<td>2.3.5 Using Diffusion Tensor Imaging to Measure Normal-Appearing White Matter</td>
<td>20</td>
</tr>
<tr>
<td>2.3.6 Diffusion Tensor Imaging of Normal-Appearing White Matter in Multiple Sclerosis</td>
<td>23</td>
</tr>
</tbody>
</table>
2.3.7 Disease Processes Underlying a Loss of Tissue Integrity in Normal-Appearing White Matter

2.4 Acquiring Diffusion Tensor Images .................................................. 27
   2.4.1 Principles of Diffusion-Sensitive MRI ........................................ 27
   2.4.2 Adding Diffusion to an MRI Sequence ........................................ 28

2.5 Corpus Callosum ................................................................. 29
   2.5.1 Basic Structure and Function ..................................................... 29
   2.5.2 The Corpus Callosum in Adult Multiple Sclerosis ...................... 30
   2.5.3 Corpus Callosum Development in Children ............................... 31
   2.5.4 The Corpus Callosum in Pediatric Multiple Sclerosis ................. 32

SECTION 3: OBJECTIVES, RATIONALE, AND HYPOTHESIS.......................... 33
   3.1 Objectives ................................................................................. 33
   3.2 Rationale .................................................................................. 33
   3.3 Hypothesis ................................................................................ 34

SECTION 4: METHODS ........................................................................... 35
   4.1 Study Participants ................................................................. 35
      4.1.1 Participant Recruitment ......................................................... 35
      4.1.2 Participant Selection ............................................................. 37
   4.2 Image Acquisition ................................................................. 38
      4.2.1 Sequences Acquired ............................................................. 38
      4.2.2 Purpose of Sequences Acquired .......................................... 40
   4.3 Image Processing ................................................................. 41
      4.3.1 Objective 1 .................................................................... 42
      4.3.2 Objective 2 .................................................................... 48
   4.4 Statistical Analysis ................................................................. 54
      4.4.1 Objective 1 .................................................................... 54
      4.3.2 Objective 2 .................................................................... 56

SECTION 5: RESULTS ............................................................................. 59
   5.1 Objective 1 ............................................................................. 59
      5.1.1 Participant Demographics ..................................................... 59
      5.1.2 Abnormal Hemispheric Normal-Appearing White Matter
                      Distinguishes Children with Multiple Sclerosis at the First Attack 60
5.1.3 Potential Tissue Integrity Loss in the Corpus Callosum Normal-Appearing White Matter Does in Children with Multiple Sclerosis at the First Attack 64

5.2 Objective 2 ......................................................... 67

5.2.1 Participant Demographics 67
5.2.2 Diffuse Brain Tissue Integrity Loss in Children with Established Multiple Sclerosis, with Clinical Onset Before Age Twelve Years 69
5.2.3 Age-Dependent Diffusion Tensor Imaging Abnormalities in Children with Established Multiple Sclerosis 71

SECTION 6: DISCUSSION ................................................................. 76

6.1 Key Findings ................................................................. 76

6.1.1 Diffusion Tensor Imaging Features at First Attack Distinguish Children with Multiple Sclerosis from Children with Monophasic ADS 76
6.1.2 Diffuse Brain Tissue Integrity Loss in Children with Established Multiple Sclerosis, with Clinical Onset Before Age Twelve Years 77
6.1.3 Age-Dependent Diffusion Tensor Imaging Abnormalities in Children with Established Multiple Sclerosis 78

6.2 Understanding Abnormal Diffusion Tensor Imaging Metrics ............... 79

6.2.1 The Meaning of Abnormal Diffusion Tensor Imaging Metrics 79

6.3 Biological Explanations of Key Findings .................................... 84

6.3.1 Subclinical Disease in Multiple Sclerosis 84
6.3.2 Age-Related Capacity for Remyelination in Multiple Sclerosis 85
6.3.3 Two Theories 88

6.4 Impact of Findings ............................................................... 89

6.4.1 A Distinction Between Monophasic Acute Demyelinating Syndromes and Multiple Sclerosis 89
6.4.2 Diffusion Tensor Imaging as a Diagnostic Tool 90
6.4.3 The Importance of Studying Young Children with Multiple Sclerosis 90

6.5 Strengths and Limitations of Study .......................................... 92

6.5.1 Study Strengths 92
6.5.2 Study Limitations 93

6.6 Next Steps ................................................................. 94

SECTION 7: REFERENCES................................................................. 96
### List of Tables

<table>
<thead>
<tr>
<th>Table Number</th>
<th>Table Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.1.1</td>
<td>Summary of sequences acquired at the Hospital for Sick Children</td>
<td>38</td>
</tr>
<tr>
<td>4.2.1.2</td>
<td>Summary of sequences acquired at Ospedale San Raffaele</td>
<td>39</td>
</tr>
<tr>
<td>4.4.1.1</td>
<td>Correlations between DTI metrics</td>
<td>55</td>
</tr>
<tr>
<td>5.1.1.1</td>
<td>Demographics of participants studied in objective 1</td>
<td>60</td>
</tr>
<tr>
<td>5.1.2.2</td>
<td>Summary statistics of hemispheric NAWM data comparing participant groups</td>
<td>62</td>
</tr>
<tr>
<td>5.1.3.1</td>
<td>Summary statistics of corpus callosum NAWM data comparing participant groups</td>
<td>65</td>
</tr>
<tr>
<td>5.2.1.1</td>
<td>Separate and pooled demographic characteristics of participants from the</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Hospital for Sick Children and Ospedale San Raffaele</td>
<td></td>
</tr>
<tr>
<td>5.2.1.2</td>
<td>Comparison of patients from the Hospital for Sick Children and Ospedale San</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Raffaele</td>
<td></td>
</tr>
<tr>
<td>5.2.3.1</td>
<td>Summary of the models chosen to fit each DTI metric for each participant</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td></td>
</tr>
</tbody>
</table>
## List of Figures

<table>
<thead>
<tr>
<th>Figure Number</th>
<th>Figure Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.3.1</td>
<td>Patients must have lesions in two of four regions to meet criteria for a diagnosis of MS at the first attack.</td>
<td>8</td>
</tr>
<tr>
<td>2.2.1.1</td>
<td>The spin echo sequence.</td>
<td>13</td>
</tr>
<tr>
<td>2.2.1.2</td>
<td>By varying TR and TE, one can achieve different MRI contrasts.</td>
<td>14</td>
</tr>
<tr>
<td>2.3.5.1</td>
<td>The diffusion tensor ellipsoid describes the probability of water molecule motion in a particular voxel.</td>
<td>21</td>
</tr>
<tr>
<td>2.3.5.2</td>
<td>Higher FA and lower MD values are observed in brain tissues with more myelin, more densely-packed cells, and less extracellular space.</td>
<td>22</td>
</tr>
<tr>
<td>2.5.1.1</td>
<td>A common partitioning scheme subdivides the corpus callosum into 3 thirds.</td>
<td>30</td>
</tr>
<tr>
<td>4.3.1.1</td>
<td>A diffusion tensor matrix with six unknowns represents the diffusion matrix D...</td>
<td>45</td>
</tr>
<tr>
<td>4.3.2.1</td>
<td>A mean FA image with an overlay of its tract skeleton.</td>
<td>50</td>
</tr>
<tr>
<td>5.1.2.1</td>
<td>Hemispheric NAWM FA of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls</td>
<td>62</td>
</tr>
<tr>
<td>5.1.2.2</td>
<td>Hemispheric NAWM RD of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls</td>
<td>63</td>
</tr>
<tr>
<td>5.1.2.3</td>
<td>Hemispheric NAWM AD of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls</td>
<td>64</td>
</tr>
<tr>
<td>5.1.3.1</td>
<td>Corpus callosum NAWM FA of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls</td>
<td>65</td>
</tr>
</tbody>
</table>
5.1.2.2 Corpus callosum NAWM RD of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls

5.1.2.3 Corpus callosum NAWM AD of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls

5.2.2.1 TBSS shows differences in FA between pre-pubertal onset MS patients and controls

5.2.2.2 TBSS shows differences in RD between pre-pubertal onset MS patients and controls

5.2.3.1 MS patient and healthy control FA with age

5.2.3.2 MS patient and healthy control RD with age

6.2.1.1 The changing diffusion tensor ellipsoid as represented by increasing DTI metrics

6.2.1.2 The diffusion tensor ellipsoid in MS disease processes

6.3.1.1 Two possibilities explaining the greater brain tissue integrity loss in MS patients with disease onset at about 12 years of age compared to those patients who have clinical onset of MS at approximately age 6 years

6.3.2.1 The relative sizes of axons and myelin in different myelination scenarios
List of Abbreviations

AD – axial diffusivity
ADC – apparent diffusion coefficient
ADEM – acute disseminated encephalomyelitis
ADS – acute demyelinating syndrome
AIC – Akaike Information Criterion
ANOVA – Analysis of Variance
ANCOVA – Analysis of Covariance
ANIMAL – Automatic Nonlinear Image Matching and Anatomical Labeling
BEDPOSTX – Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques
BET – Brain Extraction Tool
CC – corpus callosum
CIS – clinically isolated syndrome
CNS – central nervous system
CSF – cerebrospinal fluid
DIS – dissemination in space
DIT – dissemination in time
DTI – Diffusion Tensor Imaging
DW – diffusion-weighted
EDSS – expanded disability status scale
FA – fractional anisotropy
FLAIR – fluid-attenuated inversion-recovery
FLIRT – FMRIB’s Linear Image Registration Tool
HC – healthy control
MANCOVA – Multivariate Analysis of Covariance
MANOVA – Multivariate Analysis of Variance
MD – mean diffusivity
Mono-ADS – monophasic acute demyelinating syndrome
MRI – magnetic resonance imaging
MRS – magnetic resonance spectroscopy
MS – multiple sclerosis
MTR – magnetic transfer ratio
NAA – N-acetyl aspartate
NAWM – normal-appearing white matter
OCB – oligoclonal band
ON – optic neuritis
RD – radial diffusivity
RF – radiofrequency
RESTORE – Robust Estimation of Tensors by Outlier Rejection
ROI – region of interest
RRMS – relapsing-remitting multiple sclerosis
SPMS – secondary progressive multiple sclerosis
SyN – symmetric normalization
TBSS – Tract-Based Spatial Statistics
TE – echo time
TFCE – Threshold-Free Cluster Enhancement
TM – transverse myelitis
TR – repetition time
VBM – voxel-based morphometry
Section 1: Introduction

The objective of my thesis is to evaluate normal-appearing white matter (NAWM, non-lesional white matter) abnormalities in pediatric multiple sclerosis patients at the earliest possible time in their disease. Specifically, I used diffusion tensor imaging (DTI) to address two questions: (1) whether abnormalities in NAWM predict future multiple sclerosis (MS) outcome in pediatric patients with acute demyelinating syndromes (ADS) analyzed by DTI at the time of a first clinical attack, and (2) whether NAWM DTI abnormalities occur early in MS pathogenesis, as evidence by findings in patients with MS disease onset before age 12 years. DTI analyses of pediatric MS patients, performed on scans obtained at the first clinical attack, provide a window into NAWM pathology at the earliest detectable time point in the MS disease spectrum. Analysis of the rare subgroup of MS patients with disease onset before age 12 years is also particularly valuable as the very young age of these patients precludes the opportunity for a prolonged period of subclinical disease activity.

The NAWM tissue integrity of the corpus callosum and diffuse white matter were assessed. Three different methods were used for DTI analysis. The first method involved an atlas-based segmentation of the corpus callosum and white matter using several software programs. In the second method, I used tract-based spatial statistics, an
automatic method that showed diffuse abnormalities in the NAWM. Finally, training in the third method, termed tractography, was acquired in Dr. Massimo Filippi's laboratory in Italy.

The key findings of my work indicate that DTI is a sensitive measure of diffuse white matter abnormality in pediatric MS patients, implicating a very early onset of neurodegeneration in this population. These findings have implications for a future role for DTI in identification of children destined for a diagnosis of MS, and potentially for reassurance of families of children likely to experience a monophasic transient inflammatory brain illness. The fact that the DTI abnormalities at MS onset were not apparent in the very youngest patients further implies either an age-specific protection of white matter or that the youngest patients, by virtue of their young age, have experienced only a short subclinical disease period.
Section 2: Background

2.1: DEMYELINATING DISEASE

2.1.1: Pediatric Demyelination: A Growing Concern

The prevalence of pediatric-onset multiple sclerosis (MS) is becoming increasingly recognized worldwide – 5-10% of all MS patients experience the onset of their disease during childhood (reviewed in Venkateswaran & Banwell, 2010). MS is characterized pathologically by multifocal inflammatory injury to myelinated pathways and by progressive neuronal and axonal injury. Inflammatory injury is experienced as clinical MS attacks, defined as discrete episodes of acute neurological impairment lasting for a minimum of 24 hours, and by subclinical disease activity visualized on brain magnetic resonance imaging (MRI). While clinically-evident disease activity defines clinical disease onset, the subclinical aspects of the disease have both a pre-clinical period of uncertain duration, and an ongoing component evidenced by accrual of silent inflammatory lesions and progressive loss of brain integrity. The inflammatory and degenerative aspects combine yielding a clinically relapsing-remitting disease course in the majority of patients
at onset, followed in time by entry into a secondary progressive disease phase characterized by increased physical disability.

Onset of the inflammatory and neurodegenerative processes of MS during childhood may have a particularly deleterious impact in the developing central nervous system (CNS). The CNS undergoes rapid neurogenesis prior to birth and in the first few years of life; while myelogenesis and the maturation of neural networks continue well into adolescence (Verhoeven et al., 2010). As such, demyelination during childhood and teenage years may have a profound effect not only on established myelinated networks, but also on networks that are only partially mature. Studying children with MS at the time of their first attack allows us to assess the presence of subclinical disease by exploring the earliest time point in clinical disease. Also, children with MS onset prior to age 12 years are of particular interest, both due to the age-related state of myelin development, but also based on the fact that the young age of these patients inherently limits the potential period for subclinical disease. The MRI features present at onset and in very young MS patients provide a unique window into early aspects of MS disease.

The difficulty in obtaining research MRI sequences at clinical onset and the rarity of MS onset in very young children has, to date, limited exploration of the MRI features of MS in these patients. The Pediatric MS Program at the Hospital for Sick Children was established in 1999, cares for a large number of pediatric patients with MS, and provides a core resource population for the present thesis. Specifically, my work will focus on the analysis of white matter integrity in the corpus callosum and white matter of scans taken from MS patients at the first clinical attack and in the corpus callosum of very young children with MS (defined by MS onset prior to age 12 years). We believe these projects to be unique.
My thesis will be structured as follows: 1) Background review of the clinical features of pediatric demyelination, 2) Review of conventional MRI methods useful for visualizing demyelination, 3) Review of advanced MRI techniques, including DTI, which are useful in assessing white matter integrity, 4) Overview of the corpus callosum and its development during childhood and adolescence, 5) Summary of the objectives of this study, 6) Description of study design and methods, 7) Study results, and 8) Discussion of the results obtained.

2.1.2: Clinical Presentations of Pediatric Demyelination

A first attack of demyelination in the central nervous system can be either monofocal (localized to one location in the CNS) or polyfocal (multiple sites are involved) (Uitdehaag et al., 2005). In general, the most common presentations are optic neuritis (ON), acute transverse myelitis (TM), and acute disseminated encephalomyelitis (ADEM). This initial demyelinating attack may be a transient monophasic illness or may be the first attack of MS.

Optic Neuritis (ON)

Lucchinetti et al. (1997) performed a retrospective study of 79 patients with ON during childhood, followed for a median of 19.4 years (range 2.7 to 43.2 years). ON was characterized by visual loss, ocular pain, and headache in approximately 30%. Many patients had scotoma (focal loss in the field of vision), and about half of patients had abnormal cerebrospinal fluid (CSF). About 13% of the patients were diagnosed with MS within 10 years of their initial attack of ON, and 26% of these patients were diagnosed
with MS within 40 years of their initial attack (Lucchinetti et al., 1997). The likelihood of a child with an ON presentation to later be diagnosed with MS in other studies varied from 23-56% (Riikonen et al., 1988; Alper and Wang, 2009; Wilejto et al., 2006; Lucchinetti et al., 1997).

**Acute Transverse Myelitis (TM)**

As described by Banwell (2008), pediatric patients with TM present with Lhermitte’s sign (sense of shock in the back and limbs during head flexion) and acute neurological deficits indicative of spinal cord involvement. Bladder and bowel dysfunction is also often observed. Sometimes, patients complain of back pain. In children, TM on MRI tends to vary – lesions can be either longitudinally extensive (span several spinal segments) or restricted to involvement of only one to three spinal segments.

**Acute Disseminated Encephalomyelitis (ADEM)**

ADEM refers to an acute or subacute (rapid onset, but not as sudden as acute) demyelinating event that is both polysymptomatic and includes encephalopathy (a behavioural change and/or an alteration of consciousness) (Krupp et al., 2007). Most children with ADEM experience a monophasic illness. However, approximately 15-20% of children with MS onset before 11 years old present with ADEM-like symptoms at their first attack. At the time of initial illness, it is difficult to distinguish children with monophasic ADEM from those for whom the ADEM illness represents the first attack of MS.
2.1.3: *Diagnosing Multiple Sclerosis in Children*

MS in children is a relapsing-remitting disease characterized by episodes of neurological dysfunction, and ultimately a risk of progressive neurological disability. The diagnosis of MS requires clinical or MRI evidence of dissemination in space (DIS – involvement of more than one CNS location) and dissemination in time (DIT – new attacks or lesions developing over time) (Polman et al., 2011).

**Cerebrospinal Fluid (CSF) Analysis**

A sample of CSF is generally taken in order to exclude infections. Once demyelination is established, oligoclonal banding is a measure used to predict MS outcome. Oligoclonal bands (OCBs) are immune proteins present in the CSF that indicate immune activation in the brain. However, while the presence of OCBs is usually a good marker for MS in adults, OCB positivity in children varies – from 8%-92% of pediatric MS patients in different studies (Ruggieri et al., 2004; Pohl et al., 2004). The discrepancy in OCB frequency likely reflects variability in the laboratory method used, and may also differ based on the age of the child and whether the analysis was taken at a close enough time relative to an attack (Banwell, 2008). Chabas et al. (2010) looked at the CSF profiles of earlier-onset (<11 years) and later-onset (>11 and <18) pediatric MS patients at the time of the first attack. They found that earlier-onset, younger patients were much less likely than older patients to have positive OCBs. The authors suggested that the CSF profile in younger-onset patients might be due to an activation of the innate immune system as opposed to the adaptive immune system in teenagers and adults. This finding supports a potential need to consider earlier-onset and later-onset pediatric MS patients.
**Imaging**

The 2010 McDonald criteria specify certain rules for supporting DIT and DIS using both clinical and MRI data. Unique to the newest version of the McDonald criteria is the ability to diagnose MS on an initial MRI at the time of a first attack, provided that the MRI demonstrates specific features described below. Furthermore, the 2010 criteria permit their usage in the context of pediatric-onset MS patients (Polman et al., 2011). For DIT criteria at the time of a first attack, the MRI must have both asymptomatic gadolinium-enhancing and nonenhancing lesions. For DTI on serial scans, a novel T2 and/or a gadolinium-enhancing lesion must be present. For DIS, a lesion must be present in at least two of the following regions: periventricular, juxtacortical, infratentorial, and spinal cord (Polman et al., 2011) (Figure 2.1.3.1).

![Figure 2.1.3.1](image)

*Figure 2.1.3.1* Patients must have lesions in two of four regions to meet criteria for a diagnosis of MS at the first attack. For a diagnosis of MS, patients must meet DIS criteria. According to the 2010 revision of the McDonald criteria, a lesion must be found in 2 of the following 4 regions ((a) periventricular, (b) juxtacortical, (c) infratentorial, or (d) spinal cord).

In most adolescent patients, a first attack appears similar to adults on MRI (Ghassemi et al., 2008; Waubant et al., 2009; Yeh et al., 2009). However, children with MS onset before age 11 years tend to have larger and more ill-defined lesions at their first attack compared to adolescents with MS onset between age 11 and 18 years (Chabas et al., 2008). Furthermore, patients with MS onset before age 11 years have much better first
attack T2 lesion resolution after 3 months than those with MS onset during their teenage years (92% lesion resolution in children compared to 29% in teenagers). Chabas et al. (2008) suggest that these differences may represent a number of things: nonspecific reactive edema or activation of distinct immune mechanisms.

The unique imaging features that often characterize younger pediatric MS patients provide further rationale for my decision to focus my work in this age group.

### 2.1.4: Disease Course

Almost all pediatric MS patients experience a relapsing-remitting disease course, which means that they experience acute attacks followed by full or partial recovery. Transient disability manifests itself during an attack. Bjartmar and Trapp (2001) explain this to be due to inflammation and acute demyelination. Children tend to recover from an attack and regain function in about 4 weeks, which is shorter than what is usually observed in adults who often require 6-8 weeks (Ruggieri et al., 2004). At the biological level, resolution of an acute lesion relates to resolution of inflammation, reorganization of sodium channels, and some remyelination, which allows the patient to go into a period of clinical remission (Bjartmar and Trapp, 2001).

While periods of remission are clinically asymptomatic, MRI studies have demonstrated that there is subclinical axonal loss in normal-appearing white matter (NAWM, non-lesional white matter) (Bjartmar and Trapp, 2001). This could be due to things including microscopic disease, Wallerian degeneration of those axons that pass through lesions, or a loss of trophic factors that are normally released by myelin during the period of demyelination (Brady et al., 1999; Wilkins et al., 2003). Despite this axonal loss,
relapsing-remitting multiple sclerosis (RRMS) patients remain functional – enough compensatory mechanisms for pathways with lost axons as well as redundant pathways exist (Trapp et al., 1999). However, axonal loss can be compensated for only until a certain threshold is reached, and once it is reached, axonal loss leads to a progression of disability, even in the absence of an actual acute relapse. Upon reaching this threshold, patients are considered to have entered a phase of the MS disease process termed Secondary Progressive Multiple Sclerosis (SPMS) (Trapp et al., 1999).

The exact clinical course of MS is unpredictable and varies largely between individuals. However, pediatric RRMS patients take a longer time to accrue a score of 6 on a measurement quantifying disability for MS patients, the Expanded Disability Status Scale (EDSS). This score signifies the need to use an aid such as a cane to walk 100m. Also, pediatric RRMS patients progress to SPMS after a longer interval, when compared to adult-onset RRMS patients (20 years in pediatric-onset MS patients (Boiko et al., 2002) compared to 7.7 years in adults (Bergamaschi et al., 2001)). However, while it takes more years for pediatric-onset MS patients to reach irreversible physical disability than adult-onset MS patients, pediatric-onset MS patients become disabled at a younger age, leading to a longer life-duration of disability.

Of course, the category “adults” comprises a diversity of age groups – Hurwitz (2010) show that patients with MS onset before 20 years old take longer to reach an EDSS score of 6 than those with MS onset between 20 and 30 years of age, who in turn take longer to reach an EDSS score of 6 than those patients that had a first attack between age 30 and 40, etcetera. No estimation exists for how many years it takes for patients with MS onset before age 12 to progress to SPMS, since this is a rare group of patients. While the onset of MS in a child less than 12 years of age may be associated with heightened capacity
for remyelination, such repair may divert cerebral resources that are needed for age-
expected brain development. We hypothesize that those children who have experienced
their first MS attack before age 12 will fail to reach age-expected benchmarks in
myelination.

2.2: THE POWER OF NEUROIMAGING

2.2.1: How MRI Works

Exciting Spins

The advent of the MRI has revolutionized medicine, providing a window into which
a clinician can look to see what happens inside a living human body. MRI largely relies on
hydrogen atoms, which are present in different concentrations in lipids and water.
Different tissue types consist of various amounts of lipid and water (Pipe, 2009).

Hydrogen atom nuclei have an unpaired proton that gives them the property of
“spin” (Pipe, 2009), which corresponds to a non-zero magnetic moment. When a strong,
static B₀ field that is applied uniformly inside the MRI scanner is activated by the MRI
protocol, a small excess of hydrogen ion spins align with the field instead of opposing it,
creating a net magnetic field (Pipe, 2009).

Once the spins are aligned to the B₀ field, a short, excitatory pulse of magnetization
(the excitation radiofrequency (RF) pulse) is applied (Pipe, 2009). If it is applied at the
resonance frequency, it flips the net magnetization 90° away from their previous
alignment. The resonance frequency, otherwise known as the Larmor frequency, is
described by the following equation (Pipe, 2009):
\[ v = \gamma B \quad (2.2.1.1) \]

\( v \) – frequency, \( \gamma \) – gyromagnetic ratio, \( B \) – strength of magnetic field

As a result of this excitation RF pulse, spins begin to rotate about the main magnetic field like a spinning top – a phenomenon known as “precession” (Pipe, 2009). They do this about \( B_0 \) at the Larmor frequency. As the spins precess, an antenna next to the participant can pick up a current. Over time, net magnetization decays due to dephasing with the time constant \( T_2 \) (after about 100 millisec). Also, with the time constant \( T_1 \) (after about 1 sec), spins will realign themselves with \( B_0 \). The exact values of both \( T_1 \) and \( T_2 \) depend on the tissue. \( T_1 \) and \( T_2 \) differences between tissues are what allow us to visualize contrasts between different tissue types (Pipe, 2009).

Unfortunately, the spins dephase fairly quickly (Pipe, 2009). Therefore, a common solution is to apply a second RF pulse (a refocusing RF pulse). The refocusing RF pulse will flip spins 180°, allowing those spins that precess slower to catch up to the faster ones by putting the faster spins behind the slower ones. If we say that the refocusing RF pulse is applied at time \( t \) after the excitation RF pulse, then the spins become once again in phase at time \( 2t \). MRI protocols that use refocusing RF pulses are called spin-echo pulse sequences, which are important for DTI (Pipe, 2009). A schematic of a spin-echo pulse sequence is shown in figure 2.2.1.1.

Another important sequence is the gradient-echo sequence. The main difference between the gradient-echo sequence and the spin-echo sequence is the absence of a refocusing RF pulse in the former (reviewed in Sharma & Lagopoulos, 2010). Instead, the use of bipolar gradients is incorporated – a positively-pulsed gradient first flips the spins, then a negatively-pulsed gradient dephases them, and then the spins are brought back into phase with a positively-pulsed gradient, generating the gradient echo. Also, the gradient-
An echo sequence specifies an initial excitation pulse to flip spins to an angle $\alpha$, smaller than the $90^\circ$ angle of spin-echo sequences, allowing faster image acquisition by shortening the amount of time it takes for spins to realign to $B_0$ (reviewed in Sharma & Lagopoulos, 2010).

A large variety of pulse sequences exists. However, discussing them all would be beyond the scope of this thesis.

**Figure 2.2.1.1** The spin echo sequence. First, an excitation RF pulse (yellow) rotates the spins $90^\circ$ to the $B_0$ magnetic field, leading them to precess. Variation in the phases of spins, increasing with time, leads to a loss of pixel brightness. Halfway in time between the excitation RF pulse and the spin echo, a refocusing RF pulse flips spins $180^\circ$, allowing slower-precessing spins to catch up to the faster-precessing ones until the spins are once again in phase, leading to a spin echo. Figure adapted from Pipe, 2009.

**Differing Contrasts**

Different image contrasts are achieved by changing Echo Time (TE) and Repetition Time (TR). In spin-echo sequences, TE describes how long it takes until spins precess in phase after an RF pulse (when the signal becomes maximal) (Pipe, 2009). TR refers to the time elapsed in between shots, and it affects the extent to which spins are allowed to realign to $B_0$. A long TR and TE mean that there will preferentially be less signal received from those tissues that have a short $T2$ ($T2$-weighted contrast) whereas a short TR and TE
means preferentially less signal received from those tissues that have a long T1 (T1-weighted contrast). The pulse sequence specifies what contrast is required, telling the scanner when and how to apply an RF pulse or a gradient and when to record data (Pipe, 2009).

![Figure 2.2.1.2](image)

*Figure 2.2.1.2* By varying TR and TE, one can achieve different MRI contrasts. T2-weighted images are obtained by specifying a long TR and a long TE. Conversely, T1-weighted images are obtained by specifying a short TR and a short TE. Figure adapted from Pipe, 2009.

### 2.2.2: Neuroimaging as a Diagnostic Tool in Multiple Sclerosis

Patients with suspected demyelination undergo an MRI session that involves scanning with several different sequences, each using a different contrast. Each sequence
provides the clinician with a different look at the underlying pathology in the brain. T2- and T1-weighted sequences are most commonly used in imaging for demyelination.

**T2-Weighted Imaging**

On T2-weighted images, lipid-rich tissues (e.g., myelinated pathways) appear dark, whereas lesions and CSF appear bright. Imaging with T2-weighted contrast is particularly useful for visualizing lesions (Miller, 2008). However, as both lesions and CSF are bright on T2 scans, it can be difficult delineating periventricular or subarachnoid lesions as they blend in with the bright ventricular or subarachnoid spinal fluid. Fluid-attenuated inversion-recovery (FLAIR) sequences have been developed in order to circumvent the bright-CSF problem of the T2-weighted image, suppressing CSF brightness while retaining lesion brightness (Miller, 2008). CSF suppression is achieved by specifying a long inversion time. However, FLAIR sequences have poorer sensitivity to lesions of the basal ganglia and brain stem (Okuda et al., 1999) and these regions are better visualized using non-attenuated T2-weighted sequences. Another limitation of T2-weighted contrast is that while it is very sensitive to lesion presence, it is not sensitive to lesion type: demyelination, remyelination, microglial clusters, inflammation, and gliosis appear similarly on a T2 image (Miller, 2008). Therefore, while T2-weighted images are useful when describing MS, other sequences must be used in complement.

**T1-Weighted Imaging**

On T1-weighted images, CSF appears dark, whereas lipid-containing tissues appear bright. Areas of tissue loss appear as regions of hypointense signal. This contrast is useful for anatomical delineation. Furthermore, with a 3D T1-weighted gradient echo sequence,
as opposed to a 2D one, some of the lesions that are visible on T2-weighted sequences appear as hypointensities (Miller, 2008). However, 2D T1-weighted sequences show fewer lesions, but more pathological specificity than 3D sequences. Those hypointense lesions that appear on 2D T1-weighted scans suggest edema or a loss of the tissue matrix (Miller, 2008).

In addition, T1-weighted sequences are often used with an injection of intravenous gadolinium chelate, which accumulates in regions of blood-brain barrier leakage (Miller et al., 1988). Regional loss of blood brain barrier integrity is common in acutely inflamed MS lesions. As T1 relaxations are not dominated by macromolecule interactions, small amounts of gadolinium can have considerable effects on enhancing T1 relaxation. Therefore, with gadolinium, bright areas appear specifically around new lesions and areas of active inflammation on T1-weighted images. This is known as gadolinium enhancement (Miller et al., 1988).

2.3: IMAGING BEYOND LESIONS

2.3.1: Lesion Load is a Poor Correlate for Clinical Disability

Although conventional MRI images are useful in the diagnosis of MS (Polman et al., 2011), T2 lesion load does not fare well in explaining clinical disability in MS (Kappos et al., 1999; Brex et al., 2002). This suggests disease activity beyond T2-visible lesions, and that we need novel measures in order to interrogate progression of disease. Many studies have evaluated advanced MRI techniques to analyze NAWM and quantitative means to
measure brain volume. Abnormalities have been found in the NAWM of MS patients, and they correlate well with clinical disability.

2.3.2: Magnetization Transfer Ratio of Normal-Appearing White Matter in Multiple Sclerosis

One quantitative MRI method that has been used to study MS patients is Magnetization Transfer Ratio (MTR). MTR measures proton interactions. During an MTR sequence, an off-resonance pulse is applied, saturating the magnetization of hydrogen atoms bound to myelin and the axonal membrane (whose motion is restricted). The bound hydrogen atoms transfer this magnetization to nearby hydrogen atoms of the extracellular space (whose motion is not restricted). The MTR is a measure of this exchange of magnetization. Because of the transfer of energy, the signal from the bound hydrogen atoms is attenuated. If there is damage to myelin or axons, those atoms that would normally be bound transfer less energy than before, the exchange of magnetization decreases, and the MTR is reduced.

MTR is lower in the NAWM of RRMS patients compared to the white matter of healthy controls (Tortorella et al., 2000), and NAWM MTR correlates with cognitive impairment (Filippi et al., 2000; Rovaris et al., 2000). Serial analyses have shown decreases in MTR with disease progression (Filippi et al., 2000) and that MTR can predict T1 gadolinium-enhancing lesion formation (Filippi et al., 1998; Goodkin et al., 1998). However, no significant differences were found in the MTR of the NAWM of RRMS patients and the white matter of healthy controls in a pediatric population (Mezzapesa et al., 2004;
Tortorella et al., 2006). Therefore, MTR may not be sensitive enough for studying pediatric MS, and I elected not to use MTR as a tool to interrogate brain tissue in my work.

2.3.3: Magnetic Resonance Spectroscopy of Normal-Appearing White Matter in Multiple Sclerosis

Magnetic Resonance Spectroscopy (MRS) is another quantitative imaging method. It allows one to retrieve biochemical information of various metabolites. Using a region of interest approach, an MRS spectrum is produced. The MRS spectrum illustrates peaks relating to different metabolites of interest, each with a known peak frequency. N-acetyl aspartate (NAA) is a metabolite associated with myelin that may also predict axonal loss (Bjartmar et al., 2000). Measuring the ratio of NAA relative to choline (a measure of cell turnover that is unrelated to MS) provides a view into biochemical tissue integrity. Studies of adult RRMS patients have found decreases in NAWM NAA compared to the white matter of healthy adults (Narayanan et al., 1997). Decreased NAA of the NAWM is correlated with clinical disability (Fu et al., 1998; De Stefano et al., 1998), brain atrophy (De Stefano et al., 2002), disease duration (Casanova et al., 2003), and number of relapses (Casanova et al., 2003).

Although few studies of MRS in pediatric MS cohorts have been performed, the available studies have failed to find significant differences between RRMS patient NAWM and healthy control white matter NAA levels (Oguz et al., 2009; Bruhn et al., 1992). Thus, just as with MTR, I felt that MRS would not be a suitable technique for my work.
2.3.4: Brain Atrophy in Multiple Sclerosis

Progressive loss of brain volume is a powerful correlate with cognitive impairment in patients with MS. Measures of brain atrophy are correlated with cognitive dysfunction in adult MS patients (Hohol et al., 1997; Benedict et al., 2002). Brain atrophy is a better predictor of cognitive impairment than lesion burden (Benedict et al., 2004; Christodoulou et al., 2003), and an equal or better predictor of cognitive impairment than NAA in a variety of measures (Christodoulou et al., 2003). Furthermore, brain volume decreases over the course of MS disease (Rudick et al., 1999), consistent with the theory of progressive axonal loss, even during the remission stage of RRMS.

Compared to age-matched healthy controls, pediatric MS patients have smaller heads (Kerbrat et al., 2012), reduced brain volume (Kerbrat et al., 2012; Till et al., 2011a), smaller thalami (Kerbrat et al., 2012; Till et al., 2011; Mesaros et al., 2008), reduced gray matter volume (Till et al., 2011a), and smaller corpus callosi (Till et al., 2011a). Brain volume is correlated with T2 lesion load (Kerbrat et al., 2012; Mesaros et al., 2008). Furthermore, corpus callosum area and thalamic volume can distinguish MS patients with cognitive impairment from those without cognitive impairment (Till et al., 2011a). These results suggest that while brain volume is related to disease severity – it decreases in relation to increasing lesion load, degenerative processes occur early and subclinically in the disease. The average age of the patients in Kerbrat et al.’s (2012) study is 15.2, with the average age of MS onset being 12 (ranging from 4.6 to 17). Their finding of a reduced head size in MS patients suggests that brain volume loss began at a very young age, possibly during a subclinical disease stage when the skull was still growing. Therefore, I suspect that MS patients scanned at the time of their first attack will have abnormal non-
lesional brain tissue integrity, as it is likely that a period of subclinical disease preceded clinical disease in these patients as well.

2.3.5: Using Diffusion Tensor Imaging to Measure Normal-Appearing White Matter

DTI may be a more sensitive measure than MTR and MRS to evaluate tissue microstructure, and may provide complimentary information to measures of brain volume. These are some of the reasons that DTI was chosen as the technique to study the NAWM of MS patients at the time of their first attack and the NAWM of pre-pubertal onset MS patients. As will be described in the next section, DTI anomalies have been found in the NAWM of both adult and pediatric MS patients.

DTI offers an investigation of tissue integrity by quantifying the random motion of water molecules in tissues. Tissue microstructure and other barriers to diffusion affect water motion. Therefore, water molecules experiencing a barrier to diffusion are said to be diffusing in an anisotropic fashion, since tissue barriers will hinder their travel (e.g., molecules travel along the long axis of a myelinated tract).

Using Diffusion Tensor MRI, we also gain a measure of the apparent diffusion coefficient (ADC) of bulk water motion in a voxel from the diffusion tensor ellipsoid, which represents the probability of molecular movement in tissue. Eigenvectors and eigenvalues provide a representation of the diffusion tensor ellipsoid, which in turn represents the distance a given molecule would diffuse from the origin with an equal probability. Eigenvectors define the direction of the diffusion distance, for a given time, along three orthogonal axes ($\varepsilon_1$, $\varepsilon_2$, and $\varepsilon_3$), and eigenvalues define the amount of diffusion, for a given
time, along three orthogonal axes ($\lambda_1$, $\lambda_2$, and $\lambda_3$). A given tensor is defined as parallel to the eigenvector that is associated with the greatest eigenvalue ($\epsilon_1$ and $\lambda_1$), in turn suggesting the fiber's orientation. Since water prefers to diffuse along axons (Moseley et al., 1990) as opposed to against them (Beaulieu, 2002), we can elucidate the main fiber direction in the voxel.

![Figure 2.3.5.1](image)

*Figure 2.3.5.1* The diffusion tensor ellipsoid describes the probability of water molecule motion in a particular voxel. Eigenvectors ($\epsilon_1$, $\epsilon_2$, and $\epsilon_3$) represent the 3 orthogonal directions of water molecule movement, and eigenvalues ($\lambda_1$, $\lambda_2$, and $\lambda_3$) represent the distance a given water molecule would move in these directions.

In fact, this assumption that, in a given time, molecules will diffuse farther along the axon as opposed to against it is the underlying assumption of tractography. By following the principal diffusion direction that is parallel to axons, $\epsilon_1$, the tractography algorithm can recreate major white matter pathways. Therefore, DTI tractography identifies fiber pathways in the brain *in vivo*, non-invasively. In the analysis of pre-pubertal onset MS patients, tractography is used to select for the corpus callosum as a region of interest (ROI).

Two DTI metrics are commonly reported in the literature: mean diffusivity (MD) and fractional anisotropy (FA). MD is an average of the ADC in the 3 diffusion directions,
giving an overall sense of diffusion in a voxel. MD is correlated with cell density in tissues (Gauvain et al., 2001), and can be altered by edema (Benveniste et al., 1992). FA is a complementary measure, quantifying the variability between the three ADCs on a scale of 0 to 1, with 0 representing free water motion or isotropy (e.g., as would be seen within the CSF) and values closer to 1 representing tissue anisotropy (e.g., in highly structured tissue). A reduction in anisotropy (a reduction in FA) suggests a loss of tissue integrity. FA has also been reported to be sensitive to axonal loss and Wallerian degeneration (Pierpaoli et al., 2001), although it can be affected by a variety of structural changes in tissue. Both MD and FA are scalar measures, making them straightforward and relatively objective in comparisons between participant groups. Figure 2.3.5.2 illustrates an increased FA and reduced MD in tissue (b) when compared to tissue (a).

*Figure 2.3.5.2* Higher FA and lower MD values are observed in brain tissues with more myelin, more densely-packed cells, and less extracellular space (e.g., tissue (b)). In such tissues, water molecules are more restricted in their diffusion trajectory. Figure adapted from Scholz, Tomassini, and Johansen-Berg, 2009.

However, anomalies in FA or MD cannot distinguish axonal loss from demyelination. For example, both an increase in diffusivity perpendicular to the fiber direction or a decrease in diffusivity parallel to it would mean a reduction in FA. Therefore, to present more complete data that suggests changes in myelin integrity, it is best to also report axial
diffusivity (AD) and radial diffusivity (RD) in addition to FA and MD. AD represents the amount of diffusion parallel to axons and is said to be sensitive to axonal degeneration (Song et al., 2003). RD is a representation of diffusion perpendicular to the axon, and has been suggested as a measure of myelination (Song et al., 2002; Song et al., 2003; Song et al., 2005; Sun et al., 2006). However, such a representation seems to be an oversimplification of what actually happens in white matter. Budde et al. (2007) compared AD and RD measures with histological samples of experimental autoimmune encephalomyelitis (EAE) (a model of MS) mice. They found that the histological pattern of demyelination did not coincide with an expected increase in RD. In these mice, AD was decreased in both lesions and NAWM and RD was increased in lesions only.

In post-mortem DTI scans of MS patients, all metrics except AD significantly correlated with histological myelin content. FA, MD, and RD were also correlated with axonal loss, but as myelin content and axonal loss were strongly correlated to each other, the authors had difficulty measuring the unique contributions of myelin content and axonal loss on the correlation with FA, MD, or RD (Schmierer et al., 2007; Schmierer et al., 2008). In this thesis, all four DTI metrics (FA, MD, RD, and AD) will be assessed. Changes in these metrics will be interpreted as abnormalities in brain tissue integrity – not necessarily demyelination or axonal loss.

2.3.6: Diffusion Tensor Imaging of Normal-Appearing White Matter in Multiple Sclerosis

Adult MS patients have lower FA and higher MD values in the NAWM as compared to FA and MD values obtained from the white matter of healthy adults (Filippi et al., 2001;
Cercignani et al., 2001; Werring et al., 1999). FA reductions and MD increases of the NAWM are correlated with lesion volume (Filippi et al., 2001), clinical disability (Cercignani et al., 2001; Cicarelli et al., 2001), and cognitive impairment (Rovaris et al., 2002). DTI measures and MRS NAA levels are correlated (De Stefano et al., 2002), as are DTI measures with MTR (Schmierer et al., 2008). Therefore, DTI is congruent with abnormalities detected by MTR and MRS.

DTI may be more sensitive than MTR in pediatric MS. Mezzapesa et al. (2004) and Tortorella et al. (2006) observed differences in the DTI measures of pediatric MS patient NAWM relative to the white matter of age-matched healthy controls, but did not observe significant differences in MTR measures in the same children.

DTI differences have been found comparing the NAWM of children with MS to white matter values from healthy age-matched pediatric controls (Bethune et al., 2011; Vishwas et al., 2010; Tortorella et al., 2006; Absinta et al., 2010; Till et al., 2011b). DTI abnormalities are correlated with lesion volume (Bethune et al., 2011; Tortorella et al., 2006; Absinta et al., 2010) and cognition (Bethune et al., 2011; Till et al., 2011b). Absinta et al. (2010) performed a cross-sectional study involving pediatric established RRMS patients, pediatric patients who have experienced only one demyelinating attack suggestive of multiple sclerosis (clinically isolated syndrome (CIS) patients), adult established RRMS patients, adult CIS patients, and two groups of healthy controls matched to each of the pediatric and adult patients. They observed similar DTI differences in pediatric and adult MS patients relative to their respective age-matched controls. In contrast, neither adult nor pediatric CIS patients differed from healthy controls of their age group. The first objective of this thesis will take this observation one step further, by asking if a similar pattern exists between age-matched healthy controls, pediatric patients
at the time of initial acute demyelination who experience only one demyelinating attack, and pediatric patients with MS at the time of initial acute demyelination.

**2.3.7: Disease Processes Underlying a Loss of Tissue Integrity in Normal-Appearing White Matter**

Conceptually, reduced FA and increased MD may implicate pre-lesional changes, may infer a diffuse neurodegenerative process, or may represent a secondary phenomenon relating to the degeneration of axons that traverse lesions (Wallerian degeneration).

**Normal-Appearing White Matter as a Site of Future Lesions**

Pathological studies have confirmed low-level inflammation in brain regions that look otherwise normal on conventional MRI. Astroglosis, microplaque formation, remyelination, evidence of blood brain barrier breakdown, and vascular hyalinization have all been reported in pathological analysis of the NAWM of MS patients (Allen and McKeown, 1979; Barnard and Triggs, 1974; Gay and Esiri, 1991). Neurofilaments are much more likely to be dephosphorylated in RRMS patients as opposed to controls, and the degree of dephosphorylation is similar in MS NAWM and in MS lesions (Dziedzic et al., 2010). Therefore, the NAWM of MS patients is a site of immune activity and MS pathology.

The claim that abnormalities in the NAWM suggest areas of future lesions is also supported by Werring et al.’s (2000) serial DTI study. DTI and Gadolinium-enhanced T1 images were acquired from MS patients every month for a year. Regions that were NAWM at baseline and evolved into lesions over the course of a year showed a steady and moderate increase in MD before becoming a gadolinium-enhancing lesion. Once the
NAWM region has evolved into a gadolinium-enhancing lesion, a large increase in MD was observed. This suggests that DTI can predict lesions before they appear on conventional MRI.

**Normal-Appearing White Matter Consists of Transected Axons**

There is also evidence that the abnormalities observed in NAWM are due to the degeneration of axons passing through lesions. Trapp et al. (1998) showed that transected axons could be found in the NAWM of histological sections from primary and secondary progressive MS patients. However, Trapp et al.’s (1998) study did not include RRMS patients – the most common form of MS in pediatric patients. Dziedzic et al. (2010) used biopsy samples to histologically study periplaque NAWM in an adult RRMS cohort. In contrast to Trapp et al.’s (1998) result, acute axonal damage and axonal density did not differ in NAWM MS biopsy tissue compared non-MS biopsy tissue. However, Wallerian degeneration was found to be significantly more common in MS NAWM as opposed to white matter from non-MS biopsy tissues (Dziedzic et al., 2010). Howell et al. (2010) found disruptions in the NAWM axoglia that were correlated with the degree of axonal loss and microglial inflammation, suggesting that axoglia disruptions coinciding with microglial inflammation could be a marker for axon injury. Further evidence that axons traversing lesions degenerate is given by studies finding DTI NAWM metrics to be correlated with T2 lesion load (Filippi et al., 2001; Bethune et al., 2011; Tortorella et al., 2006; Absinta et al., 2010). This relation supports the occurrence of axonal degeneration following the appearance of lesions.
Abnormalities of the NAWM in MS may be because of the presence of early lesion formation in the NAWM or because of the degeneration of axons that pass through lesions. In reality, both disease processes likely affect NAWM tissue integrity.

### 2.4: ACQUIRING DIFFUSION TENSOR IMAGES

#### 2.4.1: Principles of Diffusion-Sensitive MRI

The spin echo pulse sequence introduced in the section 2.2.1: How MRI Works is sensitive to diffusion. In this sequence, there are two instances when the receiver next to the patient can pick up a current – after the excitation RF pulse when the spins precess in phase orthogonal to the $B_0$ field, and then again after the refocusing RF pulse brings the spins back in phase after signal decay. Hahn et al. (1950) first observed that the signal after the refocusing RF pulse is weaker than the one following the excitation RF pulse. They suggested that this decrease in signal was due to spin dephasing caused by the diffusion of particles.

Carr and Purcell (1954) explained this dephasing. The magnetic field in the MRI scanner is non-uniform. Spins in different locations experience a different magnetic field and therefore precess at a different Larmor frequency. Thus, when spins move to a new location during image acquisition, they experience a change in phase.
Increasing the magnetic field increases the observed phase change (Carr and Purcell, 1954). The b-value is used to describe the induced sensitivity of a spin to diffusion. This metric is proportional to the square of the gradient strength, and it can be described by the following equation:

\[ b = (\gamma G \Delta)^2 (\Delta - \delta/3) \]  (2.4.2.1)

where:
- \( b \) – b-value
- \( \gamma \) – gyromagnetic ratio
- \( G \) – amplitude of magnetic field gradient
- \( \Delta \) – temporal separation between magnetic field gradients
- \( \delta \) – duration of magnetic field gradient

2.4.2: Adding Diffusion to an MRI Sequence

What sets apart a diffusion-weighted pulse sequence and one that is not diffusion-weighted is a bipolar gradient (Pipe, 2009). It is applied after excitation, but before the signal is sampled. First, this bipolar gradient adds a positive phase to each spin’s precession that depends on its position, and then it adds a negative phase to each spin’s precession, which also depends on the spin’s position. When these positive and negative phases are summed, they do not completely cancel each other out (because the spins have moved), and the signal is attenuated (Pipe, 2009).

To estimate a diffusion coefficient, we must collect two sets of data. The first set is with the b-value set to 0 – the \( S_0 \) (Pipe, 2009). Then, we must also take measures in several directions with a non-zero b-value (i.e., with diffusion weighting) – the \( S_D \). Then, we can measure signal loss in \( S_D \) compared to \( S_0 \) in order to estimate diffusion-related displacement. \( S_D \) must be measured separately for each direction of a total number “n”. So, this experiment must be repeated n times, plus an additional time to measure for \( S_0 \) (Pipe, 2009). The diffusion tensor D can be estimated from the following equation:
\[
\bar{D} = \frac{\ln(S_0/S_D)}{b} \tag{2.4.2.2}
\]

\(D\) = the diffusion tensor, \(S_0\) = signal with no diffusion weighting, \(S_D\) = diffusion-weighted signal, \(b\) = b-value

For diffusion-weighted directions, a b-value of 900-1000s/mm\(^2\) is considered to be an optimal choice for human DT MRI (Kingsley & Monahan, 2004). All DTI scans in this thesis have been acquired using a b-value of 1000s/mm\(^2\). This diffusion weighting is applied for about 40-50ms, which would lead to a diffusion distance of about 12\(\mu\)m.

2.5: CORPUS CALLOSUM

2.5.1: Basic Structure and Function

The corpus callosum (CC) is the largest white matter bundle in the brain. It acts as a bridge between the two hemispheres, connecting them for communication. Such communication creates redundant systems, allowing analogous cortical areas of the other hemisphere to take over if a particular region becomes diseased (Gazzaniga, 2000). Therefore, it is likely an important pathway in multiple sclerosis, where redundant systems allow a patient to compensate for axonal loss (Trapp et al., 1999). Generally, the CC is subdivided into 7 subregions, with each being responsible for connecting a different part of the brain:
Rostrum and Genu: connect the prefrontal cortices. 
Rostral body: connects the premotor and supplementary motor areas. 
Anterior midbody: connects the motor cortex. 
Posterior midbody: connects somatosensory regions and the posterior parietal lobe. 
Isthmus: connects the superior temporal and posterior parietal lobes. 
Splenium: connects the occipital lobes, and the parahippocampal gyri. 
(Witelson SF, 1989)

The CC is easy to identify on an MRI, and it is functionally important. This makes it a good choice as a ROI for comparison in an MS population. Given the relatively low resolution of DTI images, however, in this study, we did not divide the CC into subregions.

2.5.2: The Corpus Callosum in Adult Multiple Sclerosis

Numerous adult DTI studies have found differences in the NAWM tissue integrity of RRMS CC compared to healthy control subjects at cross-sectional time points (Coombs et al., 2004; Hasan et al., 2005; Roosendaal et al., 2009; Fink et al., 2010). Serial DTI studies of NAWM have shown that over the course of several years, adult RRMS patients have significantly greater tissue integrity loss in the CC compared to healthy controls (Bester et
al., 2008; Simon et al., 1999). These specific changes to the CC NAWM have been correlated with functional impairments in sequence-learning tasks (Bonzano et al., 2011), bimanual motor tasks (Bonzano et al., 2008), redundancy gain (Warlop et al., 2008), auditory information processing speed and flexibility (Otzurk et al., 2010; Dineen et al., 2009), upper extremity function (Otzurk et al., 2010), visual perception and memory (Dineen et al., 2009), and verbal learning and memory (Dineen et al., 2009). We can conclude not only that the CC is impaired in MS, but that its impairment also has negative consequences on cognitive function in a wide range of cognitive modalities.

2.5.3: Corpus Callosum Development in Children

While adult studies are informative with regard to a pediatric MS population, it is important to remember that the CC continues to develop until young adulthood (Pujol et al., 1993). Therefore, MS may impact the CC in children differently than in adults with fully myelinated CC structure. Between the ages of 5 and 18, the CC becomes thicker, a phenomenon that is explained by myelination, redirection, and pruning as the number of callosal fibers is fixed at birth (Luders et al., 2010). Keshavan et al. (2002) studied a larger age-range of patients: 7-32. They found age to be positively correlated with area in all regions of the CC except the isthmus. They also found age-related decreases in T1 signal intensity throughout the CC, suggesting that increases in axonal width of CC axons is more common than myelination. Compared to teenagers and young adults, children aged 7-12 years had the most rapid decreases in signal intensity as well as the most rapid increases in CC area (Keshavan et al., 2002).
Giedd et al. (1996) had raised the question of whether CC growth rates differed between boys and girls. They measured the area of the CC on a midsagittal section on MRI of children between ages 5-18 years, and found that while the growth rates did not differ between boys and girls, the growth patterns differed in the genu and rostrum. However, these differences disappeared when the area was adjusted for total cerebral volume. Given the potential differential contribution of sex on brain metrics, all of my work relies on sex-matched data.

**2.5.4: The Corpus Callosum in Pediatric Multiple Sclerosis**

To date, three pediatric studies have examined the impact of MS on the CC NAWM as measured by DTI. Teenage-onset MS patients have lower FA in the CC NAWM compared to healthy controls (Vishwas et al., 2010; Till et al., 2011b). Specifically, teenage MS patients have lower FA in the NAWM of the genu and splenium compared to healthy teenagers (Bethune et al., 2011; Till et al., 2011b). This FA reduction in the genu and splenium is correlated with increased lesion load (Bethune et al., 2011). In addition, reduced FA throughout the CC NAWM is associated with poorer performance on cognitive tasks (Bethune et., 2011) and with poorer math performance (Till et al., 2011b). Therefore, teenage MS patients have disturbances in the CC NAWM, much like adult MS patients do.
Section 3: Objectives, Rationale, and Hypothesis

3.1: OBJECTIVES

(1) To determine whether white matter integrity measured at the time of incident demyelination distinguishes children destined for a diagnosis of MS.

(2) To determine whether abnormalities in white matter integrity occur early in MS disease processes by studying pediatric MS patients who experienced onset of their disease in early childhood.

3.2: RATIONALE

Abnormalities in the NAWM have been observed in both adult and pediatric patients. DTI in particular has shown to be a sensitive technique for identifying abnormalities in pediatric patients. DTI analyses of first attack scans of young adolescents who will later be recognized to have MS and DTI analyses of children with very early onset of MS disease both provide insight onto the early mechanisms of MS. Both objectives of this thesis are the first of their kind.
3.3: HYPOTHESIS

(1) DTI measures of NAWM brain tissue integrity will distinguish children destined for MS, while the NAWM brain tissue integrity in children with monophasic demyelination will be similar to that in healthy controls.

(2) DTI will measure a reduction in age-expected increases of tissue integrity in the CC of patients with MS onset before age 12 years. However, it remains difficult to predict exactly what effect an MS disease onset has on CC development in its most active development stage.
Section 4: Methods

4.1: STUDY PARTICIPANTS

4.1.1: Participant Recruitment

The Hospital for Sick Children

Pediatric patients with acute demyelination were recruited through the Hospital for Sick Children's Pediatric Demyelinating Clinic. Each patient was scanned at multiple time points: within 30 days of their initial attack, at 3 months follow-up, 6 months follow-up, and annually thereafter for up to 7 years. All MS patients met current criteria for a diagnosis of multiple sclerosis (Polman et al., 2011). Children with a monophasic acute demyelinating syndrome (mono-ADS) have experienced no further clinical attacks and have demonstrated no new lesion formation on serial brain MRI for at minimum of 2 years subsequent to their initial attack.

Healthy controls were recruited through community advertising. Each healthy control was scanned at one time point using the same MRI sequences as patients.
Participants with a current or past history of a non-demyelinating neurological disorder were excluded. Participants with a history of alcohol or illegal drug abuse, brain injury, or concussion were also excluded.

The Research Ethics Board at the Hospital for Sick Children has approved this study. Consent and assent forms for MS patients, patients with monophasic ADS, and healthy controls are archived at the Sick Kids Research Ethics Board Office.

**Ospedale San Raffaele**

Collaboration with Dr. Massimo Filippi’s laboratory in Università Vita-Salute San Raffaele in Milan, Italy was established in order to combine data from patients with MS onset before age 12 years to address objective 2. As MS is relatively rare in very young patients, collaboration between investigators is required in order to achieve sufficient patient numbers.

Pediatric patients with MS onset before age 12 years were referred to Ospedale San Raffaele and were recruited consecutively for imaging studies. Following enrollment, all patients were scanned at one time point. Image acquisition was not timed to specific disease duration. All MS patients met current criteria for a diagnosis of multiple sclerosis (Polman et al., 2011) and were relapse- and steroid-free for at least three months.

Healthy controls were recruited through community advertising and referral. Each healthy control was scanned at one time point using the same MRI sequences as patients.

Research Ethics Board approval was received from the local ethics standards committee on human experimentation, and written consent was obtained from the parents of all children enrolled in the study.
4.1.2 Participant Selection

Objective 1: Can DTI Predict MS Outcome at the Time of the First Attack?

For this objective, only those scans that were acquired within 30 days of the presenting demyelinating attack of MS and mono-ADS patients were analyzed. DTI images from age-matched groups of MS patients, mono-ADS patients, and healthy controls were used. An atlas-based segmentation technique was used to segment the corpus callosum and the non-corpus callosal supratentorial white matter.

Objective 2: Is Brain Tissue Integrity of Children with Established Pre-Pubertal Onset Multiple Sclerosis Abnormal?

Patients with established MS, who have had their first demyelinating attacks before age 12 years, were used to answer this question. One cross-sectional DTI image was chosen for each MS patient from the Hospital for Sick Children and from Ospedale San Raffaele. From each research center, a group of healthy controls with an age range similar to that of the MS patient group was selected to create a normal brain atlas. MS patient brains were compared to this atlas in Tract-Based Spatial Statistics (TBSS) analysis. Also, this group of healthy controls was used to create a tractography-guided CC ROI – a mask from which average DTI metrics could be extracted and compared between MS patients and healthy controls.
4.2: IMAGE ACQUISITION

4.2.1: Sequences Acquired

Hospital for Sick Children

All images at the Hospital for Sick Children were acquired on the same 1.5T GE Sigma Excite MRI scanner (GE Healthcare, Milwaukee, WI) with a single channel quadrature headcoil. These images were used to answer both objectives 1 and 2. The table below describes the sequences acquired for this study.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Sequence</th>
<th>TR</th>
<th>TE</th>
<th># Axial Slices</th>
<th>Voxel Dimension</th>
<th>FOV</th>
<th>Matrix</th>
<th># Diffusion Slices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton density/T2-weighted</td>
<td>Dual echo</td>
<td>3500 ms</td>
<td>15 ms</td>
<td>90</td>
<td>1mm x 1mm x 2mm</td>
<td>250 mm x 250 mm</td>
<td>256 x 256</td>
<td>N/A</td>
</tr>
<tr>
<td>T1-weighted</td>
<td>3D spoiled gradient-recalled echo, flip angle 30º</td>
<td>22 ms</td>
<td>8 ms</td>
<td>122</td>
<td>1mm x 1mm x 1.5mm</td>
<td>250 mm x 250 mm</td>
<td>256 x 256</td>
<td>N/A</td>
</tr>
<tr>
<td>DTI</td>
<td>Single-shot spin-echo with EPI readout</td>
<td>8300 ms</td>
<td>79 ms</td>
<td>32</td>
<td>1mm x 1mm x 5mm</td>
<td>250 mm x 250 mm</td>
<td>256 x 256</td>
<td>25 (b = 1000s/mm²) and one non-diffusion weighted slice</td>
</tr>
</tbody>
</table>

Table 4.2.1.1 Summary of sequences acquired at the Hospital for Sick Children
Ospedale San Raffaele

All images at Ospedale San Raffaele were acquired on the same 3.0T Philips Intera MRI scanner (Philips Medical Systems, Best, The Netherlands) with an 8-channel SENSE headcoil. Slices were positioned to run parallel to a line that joined the most inferoanterior and inferoposterior margins of the CC. For DTI sequences, SENSE, a technique useful in scan time reduction, was used with an acceleration factor of 2. These images were used to answer only objective 2. These sequences are summarized in the table below.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Sequence</th>
<th>TR</th>
<th>TE</th>
<th># Axial Slices</th>
<th>Voxel Dimension</th>
<th>FOV</th>
<th>Matrix</th>
<th># Diffusion Slices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton density/ T2-weighted</td>
<td>Dual echo turbo spin-echo</td>
<td>2599 ms</td>
<td>16 ms and 80 ms</td>
<td>44</td>
<td>1mm x 1mm x 3mm</td>
<td>240 mm x 240 mm</td>
<td>256 x 256</td>
<td>N/A</td>
</tr>
<tr>
<td>T1-weighted</td>
<td>3D fast field echo, flip angle 30°</td>
<td>25 ms</td>
<td>4.6 ms</td>
<td>220</td>
<td>0.89mm x 0.89mm x 0.8mm</td>
<td>230 mm x 230 mm</td>
<td>256 x 256</td>
<td>N/A</td>
</tr>
<tr>
<td>DTI</td>
<td>Pulsed-gradient spin echo with EP readout</td>
<td>8775 ms</td>
<td>58 ms</td>
<td>55</td>
<td>1mm x 1mm x 2.3mm</td>
<td>240 mm x 230 mm</td>
<td>112 x 88</td>
<td>35 (b = 1000s/mm²), 1 (b = 900s/mm²), and one non-diffusion weighted slice</td>
</tr>
</tbody>
</table>

Table 4.2.1.2 Summary of sequences acquired at Ospedale San Raffaele
4.2.2: Purpose of Sequences Acquired

Proton-Density/T2-Weighted Image

Fluids appear bright on T2-weighted images. MS lesions, which are edematous, appear bright as well. This makes T2-weighted images well suited for visualizing MS lesions. Therefore, the proton-density and T2-weighted images were used to create lesion masks. The term “mask” refers to a stored selection of a specific region of an image. Lesion masks are files that contain lesion information for each patient. As the aim of this thesis is to study NAWM, lesion masks were used in order to remove lesions from the DTI image.

However, a challenge existed in transferring information that was in the T2-weighted image coordinate system (space) into DTI image space. The two contrasts produce different images, as they use different acquisition parameters. The T2-weighted image had to be registered to the DTI image – it had to be transformed and aligned into the DTI image coordinate system. Once all of the images acquired of a particular participant were aligned, it became possible to transfer information obtained from different contrasts onto the same coordinate system. The solutions that were implemented for T2-DTI registration for each objective will be discussed in the Image Registration subsection.

T1-Weighted Image

T1-weighted images provide robust anatomical visualization of the CC and white matter. Both the CC mask and the Bayes map, which offers information on the segmentation of white matter from the rest of the brain, were registered from a pediatric atlas (Fonov et al., 2011) to the T1-weighted image.
The T1-weighted image had to also be registered to the DTI image. This is not an easy task as these two contrasts are very different. The solution was to use the T2-weighted image as a thoroughfare, performing the registration by first registering the T1-weighted image to the T2-weighted image, which was then registered to the DTI image. This was done because the T2-weighted image contrast is more similar to that of the DTI image (longer TE and TR) than that of the T1-weighted image. The specific methods for transferring the Bayes map and the CC mask into DTI space are discussed in Image Registration subsection.

**Diffusion Tensor Image**

The DTI image was the image of interest in this thesis. Three different methods were used to process DTI images and extract tissue integrity information: (1) an atlas-based approach to segment the corpus callosum and the white matter, (2) TBSS, and (3) tractography.

**4.3: IMAGE PROCESSING**

Images were processed in two different ways in order to address the two objectives of this thesis. However, while done differently, registration was a common theme that allowed me to retrieve meaningful data from the DTI image. The following 5 steps provide an overview of what registration does:

1. Preprocessing: This step involves rescaling images (i.e., altering pixel size so that it is the same between the two images that are registered).
2. Measure of image similarity: Two images are compared for similarity. Their similarity is described by the function $f(image\ A, image\ B)$.

3. Image correspondence: Images are aligned and transformed in an attempt to achieve correspondence between them. Improved correspondence between images is measured as a larger output from $f(image\ A, image\ B)$. The specific registration method used specifies the degrees of freedom allowed for warping image $B$ to image $A$.

4. Determination of a transformation function: The transformation parameters required to transform image $B$ to image $A$ and achieve correspondence are determined. This transformation function provides information on how to resample image $B$ into the space of image $A$.

5. Resampling: Image $B$ is resampled into the geometry of image $A$. Ultimately, this process allows for a transfer of information between images and it also allows one to make comparisons between scans.

**4.3.1: Objective 1: Can DTI Predict MS Outcome at the Time of the First Attack?**

The methods described in this section were used for addressing objective 1.

**Lesion Segmentation**

Lesion maps were created in collaboration with the McConnell Brain Imaging Centre in Montreal, Canada, using a semi-automatic segmentation method (Francis, 2004). This technique relies on a calculation of the probability of a voxel being white matter, gray matter, cerebrospinal fluid, or lesion by assessing the signal intensity of that voxel and
analyzing what that voxel was labeled as in an atlas and in previous T1-, T2-, and proton density-weighted scans of that participant. The decision of how to classify a particular voxel relies on a Bayes rule that evaluates the posterior probability of each tissue class being in each voxel while accounting for both signal intensity in the scan and the expected spatial location of different tissue types. This results in a tissue classification image, creating a primary lesion map. The primary lesion map was reviewed and manually corrected using the Display tool (McConnell Brain Imaging Centre, MNI). The corrected lesion map was resampled from T2 space into DTI image space, and was used to remove lesions for NAWM assessment in later steps. Registration and resampling of the T2 image into DTI image space will be discussed in detail in the Image Registration subsection.

Creating a Corpus Callosum Template Using an Atlas

Atlas-based CC templates were created in collaboration with Dr. Louis Collins’ laboratory at the McConnell Brain Imaging Centre in Montreal, Canada. They have generated an atlas that is appropriate for children aged 4.5-18.5 years (Fonov et al., 2011). Automatic Nonlinear Image Matching and Anatomical Labeling (ANIMAL) (Collins and Evans, 1997) was used to non-linearly register each patient’s T1-weighted image and Fonov et al.’s (2011) age-appropriate template and segment the CC.

ANIMAL automatically transforms atlas labels onto the T1 image space by building a 3D non-linear deformation field that specifies the alignment between the T1 image and the atlas (Collins and Evans, 1997). Anatomical labels were registered onto the segmented structures in the atlas. The deformation field was then used to resample the CC mask into T1 image space. ANIMAL’s automatic segmentation has been validated, and produces results similar to manual labeling (Collins and Evans, 1997). The steps taken to register
the T1-weighted image into DTI space will be discussed in detail in the Image Registration subsection.

**Eddy Current Correction**

Eddy currents are a problem for DTI more so than for conventional MRI sequences. This is because in DTI, gradients must be applied for a considerably longer time than in conventional sequences. A DTI sequence requires large magnetic fields to switch very rapidly, resulting in the creation of eddy currents in the electrically conductive structures of the MRI. The presence of these unwanted magnetic fields leads to a slight discrepancy between the gradient that was actually applied and the one that was prescribed. The result is a geometric distortion of the image. As a first step, eddy currents and simple head motion were corrected using FSL’s (Smith et al., 2004) implementation of \textit{eddy_correct}. This command registers each diffusion-weighted (DW) volume to the b=0 non-diffusion-weighted volume.

**Image Registration**

A combination of several toolkits was used to process DTI images: FSL (Smith et al., 2004), Camino (Cook et al., 2006), AFNI (Cox and Hyde, 1997), and MINC.

After FSL’s Brain Extraction Tool (BET) (Smith, 2002) removed the skull from the DTI image, FSL was used to create an average image of the 25 DW volumes. This average image was used to align the DTI volume. AFNI’s \textit{3dZcutup} disassembled each of the 25 DW volumes, the b=0 image, as well as the average DTI image, into 2D slices. On each of these slices, a 2D rigid body transformation (3 degrees of freedom: 1 in-plane rotation and 2 in-plane translations) in FMRIB’s Linear Image Registration Tool (FLIRT) (Jenkinson and
Smith, 2001) was run. This command linearly registered each DW slice to the average DW slice, using the b=0 image as a weighting volume for the registration cost function – a function that measures how well two images are aligned. The use of a weighting volume increases weighting at important landmark structures such as the ventricles. Two-dimensional FLIRT is a common solution to correct for motion and other linear distortions (e.g., eddy currents) that would affect image quality. Slices were reassembled into a 4D brain volume using 3dTcat and 3dZcat (AFNI), and then split into 26 3D volumes. FLIRT (Jenkinson and Smith, 2001) was run, performing a 3D affine transformation (12 degrees of freedom: 3 translations, 3 rotations, 3 scales, and 3 shears), registering the reassembled 3D volume to the averaged 3D volume, again using the b=0 image as a weighting volume.

Camino’s (Cook et al., 2006) implementation of the Robust Estimation of Tensors by Outlier Rejection (RESTORE) algorithm was used to estimate the diffusion tensor (Chang et al., 2005). From the tensor, we obtained measures of AD, RD, FA, and MD. As shown below in figure 4.3.1.1, the diffusion tensor matrix is symmetrical, so there are only 6 unknowns. Therefore, theoretically, diffusion weighting must be done in only 6 directions in order to estimate the diffusion tensor D. We took DW measurements in 25 different directions for robustness – acquiring more DW images than the minimum afforded the removal of several spoiled images.

\[
D = \begin{pmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{pmatrix}
\]

*Figure 4.3.1.1. A diffusion tensor matrix with six unknowns represents the diffusion tensor D.*
The RESTORE algorithm analyzed the 25 measures, and iteratively reweighted a least-squares regression model to exclude those measures that appeared as outliers (Chang et al., 2005). First, a nonlinear least-squares regression estimated the diffusion tensor, and the model was assessed for a goodness of fit – all data residuals had to lie within the specified confidence interval, which was three times greater than the standard deviation of the background noise, \( \sigma \). Residuals lying beyond this confidence interval, if present, were deemed to originate from outliers. They were removed from the data, and model fitting was restarted without them. This was done iteratively until a convergence criterion was satisfied. Remaining data were fit to a nonlinear least-squares regression model (Chang et al., 2005).

RESTORE has been shown to be superior to least-squares tensor fitting techniques and removes the need for cardiac gating (Chang et al., 2005). Cardiac gating is a technique that times sequence acquisition in order to avoid systole, and the gross diffusion distortions resulting from it. However, RESTORE can remove those voxels distorted when blood is pumped into the brain as outliers, allowing us to greatly reduce scanning time.

Next, ANTs was used to non-linearly align the T2-weighted image to the \( b=0 \) diffusion image. This was done in order to fix non-linear distortions in the DTI image and generate displacement vectors that will later allow us to transfer lesion maps and atlas segmentation information onto the DTI image. The ANTs toolkit contains the tool Symmetric Normalization (SyN). This is one of the best image registration methods available (Klein et al., 2009) that performs volume registration based on diffeomorphic regularization, allowing us to register two scans with differing contrasts: T2 and DTI. We used a mutual information cost function for this alignment. Mutual information is a robust method for measuring the alignment of MRI images with different contrasts (Studholme et
The image being registered is deformed until the amount of corresponding voxels between it and the target image is maximized. Entropy is used to assess registration – it is a measurement of the dispersion and distribution of image gray values. When entropy is low, the two images are well aligned (Mangin et al., 2002). Displacement vectors describing the T2-DTI deformations were computed, and were later applied to segmentation information and lesion maps that were in T2 space in order to resample them into DTI space.

T1 and T2 images were registered linearly and non-linearly using minctracc by collaboration with Dr. Louis Collins’ laboratory at the Montreal Neurological Institute. This was necessary because atlas information that was in T1 space (CC template and Bayes map for white matter segmentation) had to be transferred into T2 space first in order to register it to DTI. Minctracc was used with mutual information as a cost function, trying to align the images in a way as to reduce entropy. The .xfm files storing this registration information were used to transfer the templates into T2 space. Once the templates were in T2 space, nearest neighbor interpolation transformed them into DTI space using the T2-DTI registration information gathered previously. This is one of the easiest methods for interpolation available – for a given voxel, nearest neighbor interpolation chooses one of its closest voxels without being affected by other nearby voxels. If patients had lesions, their lesion maps, which were in T2 space, were also interpolated into DTI space using nearest neighbor interpolation. Finally, average values of FA, MD, RD, and AD were retrieved from the entire non-lesional CC and the entire non-lesional, non-CC (hemispheric) white matter.
4.3.2: Objective 2: Is Brain Tissue Integrity of Children with Established Pre-Pubertal Onset Multiple Sclerosis Abnormal?

Methods for addressing objective 2 are described in this section. This procedure was developed in collaboration with Dr. Massimo Filippi’s laboratory in Università Vita-Salute San Raffaele in Milan, Italy.

Lesion Segmentation

I manually segmented lesions using Jim 5 software (Xinapse Systems Ltd., Northants, UK) on proton density-weighted scans. T2 and T1 images were used for lesion comparison between contrasts. Lesion segmentation was checked and corrected by a trained neurologist.

Tract-Based Spatial Statistics

TBSS (Smith et al., 2006) is a method similar to voxel-based morphometry analysis (VBM), but it addresses VBM’s major problem with misalignment. Similar to VBM, TBSS is automated, is straightforward to apply, and analyzes the entire brain, but at the same time, it is not as sensitive to misalignment as VBM. This is because instead of comparing entire tracts between participants voxel-by-voxel, an FA skeleton is generated. The skeleton is a representation of the centers of all the major tracts that are common to the control participants in the study. When each participant’s FA data is projected onto the common skeleton, the skeleton voxel looks for the center of the nearest relevant tract, and takes the FA value of that participant from there (Smith et al., 2006).
Voxelwise statistical analysis of FA, MD, RD, and AD data was carried out using TBSS (Smith et al., 2006) in FSL (Smith et al., 2004). TBSS was performed separately on two sets of data – children recruited at the Hospital for Sick Children and children recruited at Ospedale San Raffaele. Participants from the two research centers were processed separately due to differences in DTI acquisition.

For each scan, FSL’s eddy_correct was used to correct for eddy currents and the skull was removed from the DTI image using BET (FSL) (Smith, 2002). Next, a diffusion tensor model was fit for each scan using FSL’s dtifit (Smith et al., 2004) to create FA images.

All healthy control FA images were aligned to each other, and the most representative DTI image was chosen as the target image. The algorithm chose the target image by trying to align each of the healthy control FA images to it one by one, and calculated the amount of warping that was required to fit to the target. The image requiring the least amount of average warping was chosen. Once the target was chosen, all patient and healthy control FA data were aligned to it using the nonlinear registration tool FNIRT (Andersson et al., 2007a, 2007b), which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). Then, affine registration was used to transform each child’s FA image into MNI-152 space. MNI-152 is a stereotaxic brain atlas created by averaging 152 healthy brain images. This space works well with the skeletonization process performed in TBSS.

All FA images were merged to create a mean 4D FA image. This mean 4D FA image was skeletonized with a threshold of 0.2. A binary skeleton mask representing the centers of all tracts that were common to this group of control participants was created (Figure 4.3.2.1).
Each participant’s aligned FA data was projected onto the skeleton. This was done in a particular way in order to account for any misalignment between individual scans. For each skeleton voxel, TBSS looked perpendicularly of the tract’s direction for the maximum FA value (Smith et al., 2006). The maximum FA value is in the participant’s tract center, and it was assigned to that skeleton voxel. This perpendicular FA search is constrained in two ways: (1) the projected participant’s FA must be closest to the skeleton voxel to which it is projecting to than to any other skeleton voxel and (2) the maximum distance of the projected FA is further limited by a wide Gaussian function that acts as a weighting function (Smith et al., 2006). This final step was repeated for the other three DTI metrics, projecting MD, RD, and AD data onto the FA skeleton. The resulting data was fed into voxelwise cross-subject statistics.

**Image Pre-Processing for Tractography**

As was done in preparing the images for TBSS analysis, FSL’s *eddy_correct* was used to correct for eddy currents and the skull was removed from the DTI image using BET (FSL) (Smith, 2002). A diffusion tensor model was fit for each scan using FSL’s *dtifit* (Smith
et al., 2004), which estimated the diffusion tensor using linear regression (Basser et al., 1994).

Tractography analysis was performed in the same way, but was optimized separately for data obtained from the Hospital for Sick Children and children recruited at Ospedale San Raffaele because of differences in DTI acquisition protocols.

T2-weighted images and DTI images were each registered to a common image space using the VTK CIGS Registration Toolkit (Hartkens et al., 2002). First, positioning differences between the two sequences were corrected using a rigid transformation, followed by an affine transformation aligning each sequence to the MNI-152 atlas (Mazziotta et al., 2001). Normalized mutual information (Studholme et al., 1999) was used as a similarity metric for the affine registration. Normalized mutual information is similar to mutual information, which has been described previously, but it is normalized for overlap invariance – it corresponds to a ratio between the sum of the image-specific entropies in the two images, and the mutual entropy of the two images. Registration attempts to minimize this ratio, maximizing joint entropy relative to image-specific entropies. This transformation normalized for gross differences in participant anatomy and in head size between participants.

Next, all DTI scans were co-registered as described by Pagani et al. (2010). Each scan was non-linearly registered to a research center-specific FA atlas that was created using control FA images. In order to create this atlas, a control scan was randomly selected and all other scans from that center were non-linearly registered to this selected scan. Next, the average of the registered FA images was resampled using an inverse of the average deformation field. In doing this, a new image with a group-averaged mean shape and mean FA intensity was created. This step was repeated three times to generate a final
center-specific FA atlas (Guimond et al., 2000). Finally all patient and control scans were non-linearly registered to their center-specific atlas.

All non-linear registrations described in the previous paragraph were performed using the adaptive bases algorithm (Rohde et al., 2003). This approach provides a method with good convergence properties that is not very computationally intense. It tries to identify regions that are poorly registered and specifically focuses on realigning these regions. When comparing the adaptive bases algorithm to many other algorithms, it performs similarly (Rohde et al., 2003).

Creating a Corpus Callosum Template Using Tractography

The white matter lesions of MS present a challenge for tractography. The very low FA value within lesions would terminate a tractography trace, or it would cause the trace of the tract to stray away. Many researchers have used methods wherein they created probability maps in control participants, and then applied these maps to MS patients in order to get diffusion parameters in specific tracts. This has been done successfully in analyses of the corticospinal tract in RRMS (Lin et al., 2007) and CIS (Pagani et al., 2005). Likewise, in this thesis, CC ROI masks were created from an average of the CC masks of healthy control brains that represented the age range of the patients enrolled in this study. Two CC ROI masks were created – one was created using healthy participants from the Hospital for Sick Children, and another was created using healthy participants from Ospedale San Raffaele. These ROI masks were applied to the NAWM of their center-specific patient populations.

FSL tools (Smith et al., 2004) was used to prepare each healthy control DTI scan for probabilistic tractography. Bayesian Estimation of Diffusion Parameters Obtained using
Sampling Techniques (BEDPOSTX) estimated the eigenvalues and eigenvectors of each voxel using Markov Chain Monte Carlo sampling.

Tract selection relied on a seed-based approach. A region where the tract of interest is known to traverse was selected, and PROBTRACKX in FDT (Behrens et al., 2003), a probabilistic tractography algorithm, estimated and selected fibers that passed through the selected seed voxels. For the CC, the entire midsagittal section was selected as a seed region. In addition, exclusion masks were created covering areas where the corpus CC certainly does not pass through (e.g., at the fornix-CC junction). Probabilistic tractography can at times estimate tracts to go through regions that are part of tracts adjacent to the tract of interest, and these exclusion masks guided the algorithm away from those regions. From each seed voxel, fiber tracking was initiated (5000 streamline samples, step length 0.5mm, curvature threshold 0.2). The result was a probabilistic map of fibers running from each of the seed voxels. The resulting tract was thresholded at 1000 (i.e., at least 1000 of the 5000 streamlines must pass through each voxel that remains in the probability map).

A concern with creating a single CC tract mask for participants spanning a large range of ages (7.6-15.6 years in Hospital for Sick Children controls and 8.5-17 years in Ospedale San Raffaele controls) was that the CC tract would look differently in participants of different ages and that the mask would not be a good representation of the tract at the more extreme ages in the range. CC tract probability maps were compared between controls of different ages. No visual differences were observed in tract size or thickness across the age range of these controls. Therefore, it was possible to merge the individual CC masks into one, combining all the CC tracts in space to create one stacked 3D image. The merged image was then thresholded, specifying that each voxel must be included in at least 50% of the healthy control tracts in order to be included in the final tract mask.
Finally, the mask was skeletonized – instead of selecting for the entire CC region, the mask was reduced to a thin string of voxels going through the center of the tract. This reduces the possibility of partial volume effects when applying the mask to patient scans – as the tract skeleton is thinner than the actual tract, it allows for some leeway if there is slight misalignment.

T2-weighted lesion masks that had been registered to MNI-152 space (as described above) were applied to each patient’s DTI scan, and lesions were segmented from the image using FSL tools (Smith et al., 2004). FA, MD, RD, and AD values were retrieved from within the skeletonized mask for each patient and healthy control.

4.4: STATISTICAL ANALYSIS

4.4.1: Objective 1: Can DTI Predict MS Outcome at the Time of the First Attack?

Statistical analysis was performed using STATA Release 10.0 (STATA Corporation, College Station, TX). Histograms of each of the DTI metrics (FA, MD, RD, and AD) for both the CC and the hemispheric NAWM were visualized in order to insure sufficient normality for a multivariate analysis of variance (MANOVA) statistical model.

Bartlett’s test for equal variances tested each metric for variance equality between participant groups. This test calculates a $\chi$ statistic describing the differences in variances between groups. A $p$-value less than 0.05 indicates unequal variances. For the hemispheric NAWM measures, all DTI metrics except AD had equal variances across samples ($p>0.05$). For the CC NAWM measures, all metrics showed variance equality ($p>0.05$). Despite the variance heterogeneity in the hemispheric AD measure, the MANOVA model should
remain robust in this case – sample sizes are equal between groups and the means in the hemispheric AD measure are uncorrelated with variance.

Correlations between every pair of DTI metrics were assessed. The table below shows the correlations from both the CC NAWM data and the hemispheric NAWM data.

<table>
<thead>
<tr>
<th>Hemispheric NAWM</th>
<th>ρ</th>
<th>CC NAWM</th>
<th>ρ</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA and MD</td>
<td>-0.6327</td>
<td>MD and RD</td>
<td>0.9869</td>
</tr>
<tr>
<td>FA and RD</td>
<td>-0.7479</td>
<td>MD and AD</td>
<td>0.9549</td>
</tr>
<tr>
<td>FA and AD</td>
<td>-0.3769</td>
<td>RD and AD</td>
<td>0.8944</td>
</tr>
<tr>
<td>CC NAWM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA and MD</td>
<td>-0.5490</td>
<td>MD and RD</td>
<td>0.9492</td>
</tr>
<tr>
<td>FA and RD</td>
<td>-0.7787</td>
<td>MD and AD</td>
<td>0.8797</td>
</tr>
<tr>
<td>FA and AD</td>
<td>-0.0936</td>
<td>RD and AD</td>
<td>0.6855</td>
</tr>
</tbody>
</table>

Table 4.4.1.1 Correlations between DTI metrics.

The metric MD is highly correlated with both RD and AD (ρ > 0.9, or close to 0.9). Such a high correlation suggests that MD is measuring essentially the same biological phenomenon as RD and AD. MD reports the average value of the 3 eigenvalues, AD reports the value of the primary eigenvalue, and RD reports the average value of the 2 other eigenvalues (the eigenvalues that measure diffusivity in the non-primary diffusion direction). RD and AD are MD, but broken down into its components. Reporting all three measures (MD, RD, and AD) does not provide us with more information than if only RD and AD are reported. Therefore, in this analysis, RD and AD will be discussed in lieu of MD. FA was kept in the analysis – it is a popular metric to report in the literature, and while FA is correlated with RD, it is not as highly correlated with RD as MD is.

The statistical test performed was a one-way MANOVA. A separate MANOVA was run for the average value of voxels within the CC NAWM and for the average value of voxels in the hemispheric NAWM. Both sex and age were tested as covariates in a multivariate analysis of covariance model, but neither was a significant predictor of the DTI metric. Therefore, both covariates were excluded from the statistical model. The final
model included FA, RD, and AD as the outcome variables and participant group (MS, mono-ADS, or HC) as the predictor variable. Afterwards, a series of one-way Analysis of Variance (ANOVA) tests were performed for each DTI metric separately. A p-value of less than 0.05 was considered statistically significant. Post-hoc Scheffe tests were used in pairwise comparisons to correct for multiple comparisons.

4.4.2: Objective 2: Is Brain Tissue Integrity of Children with Established Pre-Pubertal Onset Multiple Sclerosis Abnormal?

Statistical Analysis of Tract-Based Spatial Statistics

Statistical analysis of the TBSS data was performed using the randomise tool in FSL (Smith et al., 2004), as recommended by the TBSS manual. Randomise is a Monte Carlo permutation program that can test for significance using a generalized linear model. We specified 5000 permutations with the Threshold-Free Cluster Enhancement (TFCE) option. TFCE automatically looks for clusters within data, enhancing cluster-like structures. The p-value was corrected for multiple comparisons. Age at scan was specified as a nuisance variable. As a result, the program created a statistical skeleton of the image for each of the two tails, and for each DTI metric, where the skeleton’s intensity corresponded to the p-value of the difference between patients and controls. A p-value of less than 0.05 was considered statistically significant.
**Statistical Analysis of Tractography Data**

The goal of this component of the project was to merge data from the Hospital for Sick Children and Ospedale San Raffaele. However, children from the two centers were scanned using different scanners and with different DTI sequence parameters. Analysis was performed with collaborative assistance of Dr. Massimiliano Copetti, who normalized the DTI metrics of the scans acquired at the two centers. Following normalization, differences between Hospital for Sick Children and Ospedale San Raffaele participants were assessed using a Pearson $\chi^2$ test for categorical variables and a t-test for continuous variables. An analysis of covariance (ANCOVA) model with a center-participant group interaction term also tested center heterogeneity for each normalized variable.

For each DTI metric, linear and quadratic models were fitted, modeling the relationship between NAWM CC tissue integrity and participant age for MS patients and healthy controls. Model goodness of fit was assessed using the Akaike Information Criterion (AIC), which guided the choice between a linear model or a quadratic model for that DTI metric. The AIC was calculated for each of the two models, with a penalty included for increasing model complexity. The model for which the AIC value was more negative was chosen.

A linear mixed effects model was used to test the relationship between participant group and age on DTI metrics (FA, RD, and AD). The final model included group (HC or MS), age, and center as fixed effects. Additional separate linear mixed effects models tested the specific relationship between age and DTI metrics for each participant group. Center and age were included in these models as fixed effects. Disease duration was not included in the final model for patients as it was not a significant predictor of DTI metrics. P-values
less than 0.05 were considered significant. All analyses were performed using SAS Release 9.1.3 (SAS Institute, Cary, NC).
Section 5: Results

5.1: OBJECTIVE 1: ROLE OF DIFFUSION TENSOR IMAGING OBTAINED AT ACUTE DEMYELINATION IN PREDICTING FUTURE MULTIPLE SCLEROSIS DIAGNOSIS

5.1.1 Participant Demographics

The demographic features of the participants are delineated in Table 5.1.1.1. The three groups were well-matched for sex and age. The number of days elapsed from the time of initial demyelinating attack to the time of scan did not differ between MS and mono-ADS patients. None of the MS patients were on disease-modifying therapy. All MS patients went on to have a relapsing-remitting disease course.
5.1.2 Abnormal Hemispheric Normal-Appearing White Matter at the Time of a First Attack Distinguishes Children with Multiple Sclerosis from Children with Monophasic Acute Demyelinating Syndromes

In objective 1, I assessed the hemispheric NAWM of first attack scans by measuring FA, RD, and AD. Scans from children with MS were compared to scans from age-matched mono-ADS children taken at the time of their demyelinating attack, and to age-matched healthy controls (HC) using MANOVA.

An overall one-way MANOVA looking at the effect of participant group on FA, RD, and AD measures revealed a significant main effect for participant group, Wilks’ $\lambda = 0.37$, $F(2, 17) = 2.8$, $p < 0.05$. A one-way MANOVA looking at the effects of FA, RD, and AD in the MS group compared to an average of the two control groups (mono-ADS and HC) showed a significant main effect for participant group (MS or mono-ADS/HC), Wilks’ $\lambda = 0.51$, $F(1, 15) = 4.11$, $p < 0.05$. A one-way MANOVA looking at differences in DTI metrics between mono-ADS patients and HC suggested that there were no differences between these two groups – Wilks’ $\lambda = 0.69$, $F(1, 15) = 1.91$, $p > 0.05$. 

Table 5.1.1.1 Demographics of participants studied in objective 1.

<table>
<thead>
<tr>
<th></th>
<th>Healthy Control (n=6)</th>
<th>MS (n=6)</th>
<th>Mono-ADS (n=6)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) ± SD</td>
<td>14.2±1.3</td>
<td>13.9±1.5</td>
<td>13.7±1.5</td>
<td>0.84</td>
</tr>
<tr>
<td>Number of Females (%)</td>
<td>4 (67%)</td>
<td>3 (50%)</td>
<td>2 (33%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Average Number of Days From Onset to MRI Scan</td>
<td>-</td>
<td>21±13</td>
<td>15±6</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Post-hoc, a series of one-way ANOVAs were performed, assessing the effect of participant group (MS, mono-ADS, or HC) on each dependent variable individually (FA, RD, and AD). A test of the effect of participant group on FA produced significant results \[ F(2, 15) = 4.95, p = 0.02 \]. Post-hoc Scheffe test revealed a significant difference in FA between the MS group and healthy controls \( p < 0.01, \text{after adjustment for multiple comparisons} \). The difference between the MS and mono-ADS patient groups did not reach significance \( p = 0.16, \text{after adjustment for multiple comparisons} \) and it did not reach significance between mono-ADS patients and controls \( p = 0.24, \text{after adjustment for multiple comparisons} \). A box and whisker plot of MS, mono-ADS, and HC NAWM FA is shown in figure 5.1.2.1. There was a significant effect of participant group on RD \[ RD: F(2, 15) = 7.59, p < 0.01 \]. A post-hoc Scheffe test revealed significant differences between both MS and HC participants \( p = 0.04, \text{after adjustment for multiple comparisons} \) and between MS and mono-ADS patients \( p = 0.05, \text{after adjustment for multiple comparisons} \), but not between mono-ADS patients and healthy controls \( p = 0.99, \text{after adjustment for multiple comparisons} \). A box and whisker plot of MS, mono-ADS, and HC NAWM RD is shown in figure 5.1.2.2. There was a trend for a significant effect of participant group on AD \[ F(2, 15) = 2.99, p = 0.08 \]. AD values for mono-ADS patients had a high standard deviation due to the presence of an outlier. A box and whisker plot of MS, mono-ADS, and HC NAWM AD is shown in Figure 5.1.2.3. Table 5.1.2.2 summarizes the means and standard deviations of hemispheric NAWM FA, RD, and AD in the 3 participant groups, as well as the Scheffe-corrected p-value for each pairwise comparison. Significant comparisons are bolded.
<table>
<thead>
<tr>
<th>Hemispheric NAWM</th>
<th>HC mean ± SD</th>
<th>MS mean ± SD</th>
<th>Mono-ADS mean ± SD</th>
<th>p-value HC vs. MS</th>
<th>p-value MS vs. Mono-ADS</th>
<th>p-value HC vs. Mono-ADS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>0.32 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.31 ± 0.01</td>
<td><strong>0.005</strong></td>
<td>0.16</td>
<td>0.22</td>
</tr>
<tr>
<td>RD (mm²/s)</td>
<td>0.69 ± 0.02</td>
<td>0.74 ± 0.04</td>
<td>0.68 ± 0.05</td>
<td><strong>0.05</strong></td>
<td>0.04</td>
<td>0.99</td>
</tr>
<tr>
<td>RD (mm²/s)</td>
<td>1.10 ± 0.07</td>
<td>1.17 ± 0.03</td>
<td>1.10 ± 0.07</td>
<td>0.45</td>
<td>0.08</td>
<td>0.54</td>
</tr>
</tbody>
</table>

*Table 5.1.2.2* Summary statistics of hemispheric NAWM data comparing participant groups.

*Figure 5.1.2.1.* Hemispheric NAWM FA of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls. FA is shown on the y-axis, and participant groups are on the x-axis. The edges of the box represent the 25th and 75th percentiles in the FA distribution for each group, and the whiskers (lines) show the range of the distribution (the minimum and maximum values). An asterisk (*) indicates a significant difference from healthy controls at p<0.05. Results show a significantly lower FA in MS patients at onset compared to healthy controls. There is a trend for a reduction in FA in MS patients relative to mono-ADS patients. n=6 in each participant group.
Figure 5.1.2.2. Hemispheric NAWM RD of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls. RD ($10^{-3}$ mm$^2$/s) is shown on the y-axis, and participant groups are on the x-axis. The edges of the box represent the 25th and 75th percentiles in the RD distribution for each group, and the whiskers (lines) show the range of the distribution (the minimum and maximum values). An asterisk (*) indicates a significant difference from healthy controls at p<0.05. A cross (†) indicates a significant difference from mono-ADS patients at p≤0.05. Results show a significantly greater RD in MS patients at onset compared to healthy controls, as well as a significantly greater RD in MS patients relative to mono-ADS at the time of the initial attack. There is a trend for a reduction in FA in MS patients relative to mono-ADS patients. n=6 in each participant group.
Figure 5.1.2.3. Hemispheric NAWM AD of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls. AD ($10^{-3}$ mm$^2$/s) is shown on the y-axis, and participant groups are on the x-axis. The edges of the box represent the 25th and 75th percentiles in the AD distribution for each group, and the whiskers (lines) show the range of the distribution (the minimum and maximum values). An outlier (value greater 1.5x the Interquartile Range from the median) is shown as a point. There is a trend for an increase in AD in MS patients relative to both healthy controls and mono-ADS patients. n=6 in each participant group.

5.1.3: Potential Tissue Integrity Loss in the Corpus Callosum Normal-Appearing White Matter in Children with Multiple Sclerosis at the First Attack

FA, RD, and AD measures were compared from within CC NAWM between first attack scans of children with MS, demyelinating attack scans of age-matched mono-ADS children, and scans of age-matched healthy controls using MANOVA.

A one-way MANOVA looking at the effect of diagnosis on FA, MD, and RD values showed no significant main effect for participant group, Wilks’ $\lambda = 0.44$, $F (2, 17) = 2.2$, $p = 0.08$, but was approaching significance. Differences in CC NAWM integrity in MS patients at the first attack, if they exist, are not as great as those in the hemispheric NAWM. Therefore,
studies with larger sample sizes will be required to detect them. Box and whisker plots showing the distribution of each DTI metric (FA, RD, and AD), comparing the three participant groups, are shown in figures 5.1.3.1, 5.1.3.2, and 5.1.3.3. Table 5.1.3.1 summarizes the means and standard deviations of corpus callosum NAWM FA, RD, and AD in the 3 participant groups.

<table>
<thead>
<tr>
<th>Corpus Callosum</th>
<th>HC mean ± SD</th>
<th>MS mean ± SD</th>
<th>Mono-ADS mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAWM FA</td>
<td>0.52 ± 0.04</td>
<td>0.48 ± 0.03</td>
<td>0.50 ± 0.02</td>
</tr>
<tr>
<td>RD (mm²/s)</td>
<td>0.66 ± 0.05 • 10⁻³</td>
<td>0.72 ± 0.08 • 10⁻³</td>
<td>0.68 ± 0.07 • 10⁻³</td>
</tr>
<tr>
<td>AD (mm²/s)</td>
<td>1.64 ± 0.05 • 10⁻³</td>
<td>1.64 ± 0.09 • 10⁻³</td>
<td>1.60 ± 0.13 • 10⁻³</td>
</tr>
</tbody>
</table>

*Table 5.1.3.1* Summary statistics of corpus callosum NAWM data comparing participant groups.

*Figure 5.1.3.1.* Corpus callosum NAWM FA of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls. FA is shown on the y-axis, and participant groups are on the x-axis. The edges of the box represent the 25th and 75th percentiles in the FA distribution for each group, and the whiskers (lines) show the range of the distribution (the minimum and maximum values). An outlier (value greater 1.5x the Interquartile Range from the median) is shown as a point. There is a trend for a decrease in FA in MS patients relative to both healthy controls and mono-ADS patients, and for a reduced FA in mono-ADS patients relative to healthy controls. n=6 in each participant group.
Figure 5.1.3.2. Corpus callosum NAWM RD of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls. RD ($10^{-3}$ mm$^2$/s) is shown on the y-axis, and participant groups are on the x-axis. The edges of the box represent the 25th and 75th percentiles in the RD distribution for each group, and the whiskers (lines) show the range of the distribution (the minimum and maximum values). There is a trend for an increase in RD in MS patients relative to both healthy controls and mono-ADS patients. n=6 in each participant group.
Figure 5.1.3.3. Corpus callosum NAWM AD of MS and mono-ADS patients at the time of their initial demyelinating attack, and of age-matched healthy controls. AD (10^{-3} \text{ mm}^2/\text{s}) is shown on the y-axis, and participant groups are on the x-axis. The edges of the box represent the 25th and 75th percentiles in the AD distribution for each group, and the whiskers (lines) show the range of the distribution (the minimum and maximum values). There does not appear to be a trend for any differences in AD between MS patients, mono-ADS patients, or healthy controls. n=6 in each participant group.

5.2: OBJECTIVE 2: DIFFUSION TENSOR IMAGING OF CHILDREN WITH ESTABLISHED PRE-PUBERTAL ONSET MULTIPLE SCLEROSIS

5.2.1: Participant Demographics

Table 5.2.1.1 summarizes the demographic distribution of participants from the Hospital for Sick Children, from Ospedale San Raffaele, and from the two centers combined. Neither sex nor age distributions differed between MS patients and HC at either site. All MS patients have an RRMS disease course.
<table>
<thead>
<tr>
<th></th>
<th>Healthy Control</th>
<th>MS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hospital for Sick Children</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Participants</td>
<td>13</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Age at Scan (years) ± SD</td>
<td>12.2±2.2</td>
<td>11±2</td>
<td>0.31</td>
</tr>
<tr>
<td>Age at Onset (years) ± SD</td>
<td>-</td>
<td>9.3±2</td>
<td>-</td>
</tr>
<tr>
<td>Number of Females (%)</td>
<td>11 (85%)</td>
<td>9 (82%)</td>
<td>0.86</td>
</tr>
<tr>
<td>Disease Duration at Scan (years) ± SD</td>
<td>-</td>
<td>1.7±1.5</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ospedale San Raffaele</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Participants</td>
<td>18</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Age at Scan (years) ± SD</td>
<td>12.9±2.6</td>
<td>13.4±2.6</td>
<td>0.61</td>
</tr>
<tr>
<td>Age at Onset (years) ± SD</td>
<td>-</td>
<td>9.4±1.9</td>
<td>-</td>
</tr>
<tr>
<td>Number of Females (%)</td>
<td>9 (50%)</td>
<td>6 (54%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Disease Duration at Scan (years) ± SD</td>
<td>-</td>
<td>4±2.6</td>
<td>-</td>
</tr>
<tr>
<td><strong>Both Research Centers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Participants</td>
<td>31</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>Age at Scan (years) ± SD</td>
<td>12.6±2.7</td>
<td>12.2±2.9</td>
<td>0.63</td>
</tr>
<tr>
<td>Age at Onset (years) ± SD</td>
<td>-</td>
<td>9.4±2</td>
<td>-</td>
</tr>
<tr>
<td>Number of Females (%)</td>
<td>20 (64%)</td>
<td>15 (68%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Disease Duration at Scan (years) ± SD</td>
<td>-</td>
<td>2.9±2.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5.2.1.1 Separate and pooled demographic characteristics of participants from the Hospital for Sick Children and Ospedale San Raffaele.

Table 5.2.1.2 compares MS patient characteristics between the Hospital for Sick Children and Ospedale San Raffaele. Patients from Ospedale San Raffaele were significantly older (p = 0.05) and had longer disease duration (p = 0.02) than patients from the Hospital for Sick Children.
<table>
<thead>
<tr>
<th></th>
<th>Hospital for Sick Children</th>
<th>Ospedale San Raffaele</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>11</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Age at Scan (years) ± SD</td>
<td>11±2</td>
<td>13.4±2.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Age at Onset (years) ± SD</td>
<td>9.3±2</td>
<td>9.4±1.9</td>
<td>0.92</td>
</tr>
<tr>
<td>Number of Females (%)</td>
<td>9 (82%)</td>
<td>6 (54%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Disease Duration at Scan (years) ± SD</td>
<td>1.7±1.5</td>
<td>4±2.6</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Table 5.2.1.2 Comparison of patients from the Hospital for Sick Children and Ospedale San Raffaele*

### 5.2.2: Diffuse Brain Tissue Integrity Loss in Children with Established Multiple Sclerosis, with Clinical Onset Before Age Twelve Years

In objective 2, we assessed patients with established MS who had their first attack before age 12 years. Time from disease onset (age 12 or younger) to age at MRI was 2.87 ± 0.52 years. The purpose of the TBSS analysis was to see whether the NAWM of MS patients generally differed from the white matter of HC in diffuse brain regions. Also, TBSS analysis was performed in order to ensure that the CC NAWM differed between healthy controls and MS patients, and that it was a good choice as a tract for further analysis with tractography. Due to differences in scan acquisition, TBSS was performed separately on participants from the Hospital for Sick Children and participants from Ospedale San Raffaele. Regions where FA is significantly lower (p < 0.05) in patients compared to controls are highlighted in figure 5.2.2.1 and regions where RD is significantly higher (p <0.05) in patients compared to controls are highlighted in figure 5.2.2.2. There were no significant differences between MS patients and controls from either center in AD in any region (data is not shown). In the figures, voxel-by-voxel p-values of significant differences are displayed using a heat map. Results show that MS patients with disease onset before age 12 years have significantly lower FA and greater RD in
diffuse areas of the NAWM, including the CC, the arcuate fasciculus, and the inferior longitudinal fasciculus, but not the corticospinal tract. This validates the analysis of age-related changes in the CC of young MS patients in the next section. Moreover, these differences in regional DTI metrics were very similar in MS patients compared to controls, irrespective of whether the participants were imaged at Ospedale San Raffaele or at the Hospital for Sick Children.

Figure 5.2.2.1. TBSS shows differences in FA between pre-pubertal onset MS patients and controls. Sagittal slices of the mean FA map are shown. A heat map (yellow-red) shows areas where FA is lower in MS patients than in controls (p<0.05). A yellow color suggests a more highly significant difference between participants than a red color. Participants from the Hospital for Sick Children are shown in (a), and participants from Ospedale San Raffaele are shown in (b).
Figure 5.2.2. TBSS shows differences in RD between pre-pubertal onset MS patients and controls. Sagittal slices of the mean FA map are shown. A heat map (yellow-red) shows areas where RD is higher in MS patients than in controls (p<0.05). A heat map shows the significant differences observed at each voxel (a yellow color suggests a more highly significant difference between participants than a red color). Participants from the Hospital for Sick Children are shown in (a), and participants from Ospedale San Raffaele are shown in (b).

5.2.3 Age-Dependent Diffusion Tensor Imaging Abnormalities in Children with Established Multiple Sclerosis

In this analysis, CC tractography was performed on MS patients with disease onset before age 12 years and on age-matched healthy controls. The purpose of this analysis was to evaluate the integrity of NAWM as a function of MS onset in early childhood, with a particular interest in seeing whether differences from healthy children are detectable if the patient is imaged when he or she is still younger than age 12 years (i.e., very young at onset and still young at imaging) vs. detection of differences in NAWM tissue integrity in patients imaged as teenagers several years after the onset of their disease in earlier childhood. Given that the CC matures during early childhood and into adolescence (Keshavan et al., 2002), we elected to focus on the CC and consider age at scan as a variable of interest in the statistical model.
Linear and quadratic models were fit to model age-related changes in FA, RD, and AD metrics within the CC NAWM of patients and the CC white matter of healthy controls. Using the Akaike Information Criterion (AIC) as a tool for model selection, table 5.2.3.1 summarizes the models that were chosen:

<table>
<thead>
<tr>
<th>Patients</th>
<th>Model</th>
<th>Controls</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>quadratic</td>
<td>FA</td>
<td>quadratic</td>
</tr>
<tr>
<td>RD</td>
<td>linear</td>
<td>RD</td>
<td>quadratic</td>
</tr>
<tr>
<td>AD</td>
<td>quadratic</td>
<td>AD</td>
<td>quadratic</td>
</tr>
</tbody>
</table>

*Table 5.2.3.1* Summary of the models chosen to fit each DTI metric for each participant group. In all instances, except to model the RD of patients, a quadratic model fit the data best.

As observed in figures 5.2.3.1 (FA) and 5.2.3.2 (RD), FA was lower, and RD was higher in those healthy controls that were older. However, in MS patients, FA and RD appear to be at a similar value for all patients, irrespective of their age at the time of MRI. As a result, comparison of FA and RD values for the NAWM of the CC were more similar between HC and MS patients when considering the youngest participants, and more disparate when comparing the adolescent participants. The metric AD did not differ between MS patients and healthy controls. While the presence of outliers is noted, we did not have sufficient reasons for removing any patient data from the analysis.
Figure 5.2.3.1. MS patient and healthy control FA (y-axis) plotted against age (x-axis) at MRI. Patients are marked in red and controls in blue. Scatterplot “(a)” shows participants from the Hospital for Sick Children, and scatterplot “(b)” shows participants from Ospedale San Raffaele. A similar pattern occurs at both centers – older controls tend to have a higher CC FA than younger controls; CC FA in MS patient appears similar in all ages, with only the youngest MS patients having an FA similar to that of age-matched controls.
Figure 5.2.3.2. MS patient and healthy control RD (y-axis, units $\times 10^{-3}$ mm$^2$/s) plotted against age (x-axis) at MRI. Patients are marked in red and controls in blue. Scatterplot “(a)” shows participants from the Hospital for Sick Children, and scatterplot “(b)” shows participants from Ospedale San Raffaele. A similar pattern occurs at both centers – in controls, RD tends to decrease with age; in MS patients, RD remains at approximately the same level for all ages.
Linear mixed effects models were fitted to the data. Each DTI metric, modeled separately, was the outcome variable. Age, group (MS or HC), and center (Hospital for Sick Children or Ospedale San Raffaele) were included in the model as fixed effects. A random effect term was included. MS patients had significantly lower FA values and higher RD values in the CC NAWM than controls in the CC white matter (all p-value < 0.01) when corrected for both center and age. AD values did not differ between MS patients and HC. This result replicates our observation from the TBSS analysis in the previous section. Center was a significant predictor for FA (p < 0.05), AD (p < 0.01), and RD (p = 0.05) that the linear mixed effects corrected for. In this overall model, age was not a significant predictor of DTI metrics.

Subsequently, subgroup analyses were performed, stratifying the data by group (MS or HC). The model was re-estimated for each DTI metric. In this analysis, an age effect was observed in healthy controls for FA and RD, but not AD. Older healthy controls had higher FA values (p < 0.05) and lower RD values (p < 0.01) in the CC white matter than younger healthy controls. In contrast, an age effect was not observed in the CC NAWM for patients with MS.
Section 6: Discussion

6.1: KEY FINDINGS

6.1.1: Diffusion Tensor Imaging Features at First Attack Distinguish Children with Multiple Sclerosis from Children with Monophasic ADS

DTI scans obtained at onset demonstrate abnormalities of hemispheric NAWM that distinguish children destined for a diagnosis of MS from children with monophasic demyelination and from healthy children. These findings implicate diffuse pathological changes in otherwise normal-appearing tissue, and indicate that such pathology is evident even at the earliest stage of the disease. Since it is likely that diffuse injury requires time to accrue, the presence of diffusely abnormal white matter also implicates a subclinical disease phase that precedes the first clinical attack. In contrast, the absence of such changes in children with monophasic ADS implicate a more acute biology, and a fundamental distinction in the underlying processes of transient brain inflammation.

Significant differences in DTI metrics between MS patients, mono-ADS patients, and healthy controls were not observed in the CC, although there was a trend (as illustrated in the box-and-whisker plot in figures 5.1.3.1 and 5.1.3.2). We suspect that we were simply
underpowered to detect small corpus callosal differences (mean FA ± SD: MS – 0.48±0.03, HC – 0.52±0.04, mono-ADS – 0.50±0.02), but it is also possible that a longer period of time is required to detect CC abnormalities than is required to detect change in hemispheric NAWM. Studies of larger pediatric MS cohorts, studied at variable time points (typically several years) from disease onset, have observed significant differences in the CC FA between MS patients and controls. For example, Vishwas et al. (2010) observed a 16% decrease in the mean FA of MS patients relative to healthy controls. In a cohort imaged from our own center (none of whom were included in this thesis), Bethune et al. (2011) reported an approximately 8.8-11.4% decrease throughout the CC in RRMS patients relative to controls.

Taken into context with the hemispheric NAWM data and published pediatric data, the CC appears to be relatively spared in MS patients early in clinical disease. It is possible that the CC is a tract that becomes afflicted later than other regions in the brain. In order to address this, a longitudinal study would be needed in order to assess the rate of tissue integrity loss in non-lesional CC tissue in pediatric MS patients.

6.1.2: Diffuse Brain Tissue Integrity Loss in Children with Established Multiple Sclerosis, with Clinical Onset Before Age Twelve Years

TBSS analysis showed diffuse brain tissue integrity losses in children with pre-pubertal MS onset, several years following clinical onset. Abnormalities in MS patients were observed in FA and RD metrics, but not in AD, throughout the brain – in the CC, arcuate fasciculi, and in the inferior longitudinal fasciculi. That CC abnormalities were observed in the overall MS patient cohort relative to healthy controls reinforces our choice
to study age effects within this tract. This analysis will be described in the following section. In addition, a study comparing age-related DTI metrics within the acruate fasciculi and the inferior longitudinal fasciculi is underway.

Patients recruited from both the Hospital for Sick Children and Ospedale San Raffaele showed DTI abnormalities in the same tracts. The finding of overall diffuse NAWM tissue integrity loss in MS patients is in line with other studies that have shown DTI abnormalities in pediatric MS patients relative to healthy controls (Bethune et al., 2011; Vishwas et al., 2010; Tortorella et al., 2006; Absinta et al., 2010; Till et al., 2011b).

6.1.3: Age-Dependent Diffusion Tensor Imaging Abnormalities in Children with Established Multiple Sclerosis

We demonstrate that the FA values in the CC of healthy children aged 7-17 years increases as a function of age, while RD values decrease, a finding previously reported (Verhoeven et al., 2010). In contrast, patients with established MS imaged at the same age as healthy controls did not show lower FA and increased RD in the older patients, leading to an increasingly apparent discrepancy between MS patients and controls with age. We demonstrate that, within the CC, children with MS imaged prior to age 12 years appear to have “healthier” NAWM in the region, while older children with MS (12-17 years old) differ considerably from age-expected DTI metrics in the CC. This finding may help explain why we did not reach significance in our CC studies under Objective 1. If CC NAWM abnormalities require time to develop (whether subclinical or clinical disease duration), then patients presenting with a first clinical attack may vary in their subclinical disease period and thus the likelihood of CC abnormalities at this initial time point.
Alternatively, or concurrently, if age at onset and age at imaging are relevant, then CC features must be considered as a function of age. FA and RD values were plotted against age separately for the healthy controls enrolled at HSC and HSR participants, and similar slopes were obtained from both centers. Furthermore, these slopes are in line with the reported DTI growth curves that were observed by Verhoeven et al. (2010), who imaged a large age range of healthy children, supporting the validity of our data.

6.2: UNDERSTANDING ABNORMAL DIFFUSION TENSOR IMAGING METRICS

6.2.1: The Meaning of Abnormal Diffusion Tensor Imaging Metrics

Both FA and RD showed to be sensitive measures for detecting differences in the brain tissue microstructure between children with MS and healthy controls. RD was more likely to differ in MS patients relative to control groups than FA (as illustrated by studying patients at clinical onset), and AD did not differ between different participant groups. In order to understand why some metrics were sensitive while others were not, it is important to understand what change in the diffusion tensor ellipsoid each metric is measuring.

Figure 6.2.1.1 shows a schematic of how an increase in FA, MD, RD, and AD would affect a sphere. Imagine that the tract is traversing up/down. As shown in (a), an increase in FA means an increase in anisotropy, which is reflected in the variability of diffusion distances in the x, y, and z directions. An increase in FA can occur if the ellipsoid becomes elongated or flattened. MD, a commonly reported measure in the literature, can be thought of as referring to the size of the ellipsoid – an increase in MD means molecules can diffuse
a larger distance in a given time. MD is an average of diffusion in the x, y, and z directions, so an increase in MD will not specify if diffusion distance is increasing in one, two, or all three directions. An observed increase in MD can reflect either an increase in RD (as shown in (c), an increase in diffusivity perpendicular to the axon), or an increase in AD (as shown in (d), an increase in diffusivity parallel to the axon direction). This is why in this thesis I focused on describing RD and AD instead of MD.

When I compared first attack DTI scans of mono-ADS and MS patients, I observed that RD significantly differed between the two groups whereas FA only trended towards significance. RD may be more sensitive than FA because FA is less specific – an increase in FA can represent one of two outcomes (figure 6.2.1.1 (a) – the ellipsoid can either become more sausage-shaped or more discus-shaped). An increase in RD specifically represents the diffusion tensor ellipsoid becoming more discus-shaped (figure 6.2.1.1 (c)).
Figure 6.2.1.1 The changing diffusion tensor ellipsoid as represented by increasing DTI metrics. The tract direction is imaged as traversing in the up/down direction. In (a) are shown two possible outcomes for increasing FA – the ellipsoid is becoming either sausage-shaped or discus-shaped. As shown in (b) an increasing MD means an increase in the average distance molecules diffuse. An increasing RD, as shown in (c), shown an increase in diffusivity perpendicular to the axon direction, and an increasing AD, as shown in (d), refers to an increase in diffusivity parallel to the main axon direction.
Schmierer et al. (2008) compared post-mortem DTI metrics with histological measures in the NAWM of MS patients. They found that both RD and FA were significantly correlated with axonal loss and demyelination, whereas AD was not correlated with these measures. Figure 6.2.1.2 shows a schematic of the diffusion tensor ellipsoid in healthy tissue and in several examples of MS-related disease processes. A healthy tract with myelinated axons and an elongated diffusion tensor ellipsoid is shown in (a). When the space between axons increases (shown in (b) with axonal loss or edema or in (c) with demyelination), the diffusion tensor ellipsoid becomes wider. This translates as an increase in RD and a decrease in FA without a change in AD.

It appears that DTI cannot separate between changes in tissue occurring due to axonal loss or demyelination. Schmierer et al. (2008) found axonal loss and demyelination highly correlated with each other histologically, making it impossible to distinguish which of the two measures RD or FA was better at measuring. An increase in RD or a decrease in FA can represent demyelination, edema, or axonal loss – an overall loss in brain tissue integrity.

Schmierer et al. (2008) found that post-mortem DTI could not predict histological measures of gliosis in MS. Gliosis is an astroglial response leading to the proliferation of astrocytic processes that is stimulated by permanent tissue injury. As shown in figure 6.2.1.2 (d), it is difficult to predict how gliosis would translate into DTI metrics. While increasing cellularity due to the infiltration of activated astrocytes alone should lead to a decrease in RD and may affect FA, this is unlikely to be the only process in diseased nervous tissue. Inflammation would also lead to edema (increasing RD and reducing FA), and the tissue underlying the area where the astrocytes infiltrate likely has some demyelination and axonal loss (increasing RD and reducing FA). Therefore, the overall DTI
metrics in such tissue would depend on the weighting of the impact of all of these disease processes. However, gliosis is significantly more severe in lesions as opposed to our area of interest – the NAWM (Schmierer et al., 2008).

Figure 6.2.1.2 The diffusion tensor ellipsoid in MS disease processes. (a) in healthy tissue, the diffusion tensor ellipsoid is elongated. However, the ellipsoid widens in the case of (b) axonal loss or edema or (c) demyelination, resulting in increasing RD and decreasing FA. In (c) gliosis, it remains difficult to predict the change in DTI metrics that occurs due to the multiple disease processes that coincide with glial scarring.

The reduced FA and increased RD that were observed in most of our patients with MS can implicate a variety of disease mechanisms – microscopic disease processes, diffuse axonal degeneration, or Wallerian degeneration. It is difficult to predict the role that each of these mechanisms plays in reducing brain tissue integrity in our patients. However, all of these processes likely play and important role in the pathology of MS, and they are likely intertwined.
6.3: BIOLOGICAL EXPLANATIONS OF KEY FINDINGS

6.3.1: Subclinical Disease in Multiple Sclerosis

The diffuse pathological changes in the hemispheric NAWM of MS patients at their first attack suggest an already ongoing period of subclinical disease. In previous work by our group, we demonstrated a significant difference in age-expected brain size in children with MS, also suggesting that the MS disease process has been active for considerable time (Kerbrat et al., 2012). Specifically, despite a mean age of onset of 12 years (range 4.6-17 years) and an average age of 15.2 years at MRI, children with MS had a reduced head size compared to age-matched controls. Given that head size is determined predominantly prior to age 10 years, these findings implicate a very early age of MS onset.

In our study of pre-pubertal onset MS patients, there are two possible explanations for the age-increasing disparity between MS patients and healthy controls. As shown conceptually in figure 6.3.1.1 (a), it is possible that both a child with MS onset at age 6 years and a child with MS onset at age 12 years have had a similar duration of subclinical disease, but the older child has had a much more rapid progression in brain tissue integrity loss (in order to be at the age-expected DTI metric that we observed for MS patients). Figure 6.3.1.1 (b) shows another scenario. It is possible that both the child with clinical MS onset at age 6 years and the child with clinical MS onset at age 12 years have had a subclinical onset of MS at the same age of 6 years, but in the older child, MS was not noticed until that child reached age 12 years.
Figure 6.3.1.1 Two possibilities explaining the greater brain tissue integrity loss in MS patients with disease onset at about 12 years of age compared to those patients who have clinical onset of MS at approximately age 6 years. (a) a scenario where both patients experience a similar duration of subclinical disease activity, but the patient with MS onset at age 12 years has more rapid brain tissue integrity loss. (b) both patients have had MS disease onset at a similar age, but MS went undetected for a much longer time period in the older patient.

6.3.2: Age-Related Capacity for Remyelination in Multiple Sclerosis

As reviewed by Franklin and ffrench-Constant (2008) a demyelinated axon experiences one of two possible fates – remyelination or degeneration. Yet, even in the remyelination scenario, the regenerated myelin is thinner and smaller than the normal myelin generated during development. The recapitulation hypothesis suggests why this is so – in a developing nervous system, newly formed myelin associates with a growing axon, and as a result, myelin and axons grow together, as shown in figure 6.3.2.1 (a). In contrast,
when a mature axon is remyelinated, as shown in figure 6.3.2.1 (d), the axon does not grow significantly, and neither does the myelin associated with it – it remains small (Franklin and Hinks, 1999).

Figure 6.3.2.1 The relative sizes of axons and myelin in different myelination scenarios. (a) in development, newly-formed myelin sheaths grow together with small axons until reaching a normal myelinated state (b). However, when (c) a mature axon is demyelinated, (d) newly formed myelin is much smaller and thinner than then already-mature axon. Adapted from Franklin and Hinks, 1999.

Keshavan et al. (2002) observed increasing axonal widths in the CC of healthy children aged 7-12 years, whose rate of growth slowed down significantly in adolescence. This may offer an explanation as to why we observed normal DTI metrics in MS patients under age 12 years, but did not in those MS patients that were older. When the youngest children in our pre-pubertal study remyelinated axons following injury, as their axons were still undergoing a rapid growth phase, their regenerating myelin was generated concurrent with active axonal growth, potentially contributing to a more normal age-expected microstructure. In contrast, the teenagers in this study had increasing divergence from normal in DTI metrics, possibly due to less successful remyelination that led to
axonal loss or a greater proportion of remyelinated axons of the type shown in figure 6.3.2.1 (d), where the myelin sheath is thinner. If axons in these children have thinner myelin sheaths, this would also translate to a reduction in FA and an increase in RD compared to normal, as more space would exist between individual axons in a tract.

Following demyelination, remyelination is the default reaction by the CNS, and despite an inflammatory environment that is not conducive to remyelination in MS disease, many patients remyelinate demyelinated areas (reviewed in Franklin & ffrench-Constant, 2008).

Studies of remyelination in rodent models of toxin-induced demyelination (Shields et al., 1999) found that younger rats (aged 2 months, about pubertal age) remyelinated faster than older rats (aged 9-12 months), although both age groups were able to fully remyelinate the lesioned area. However, a toxin was used to induce demyelination in that study, and it does not exactly recapitulate the chronic inflammatory milieu of MS.
6.3.3: Two Theories

The results observed in this thesis can be explained by two theories:

(1) Differing Duration of Subclinical Disease in MS Patients of Different Ages:

This theory proposes that younger patients with MS, having had a shorter subclinical disease, have experienced less time for diffuse tissue insult, and have had less tissue to remyelinate.

(2) Differing Capacity for Remyelination in MS Patients of Different Ages:

This theory suggests that pre-pubertal aged MS patients are better able to remyelinate following demyelination, and therefore do not lose brain tissue integrity as rapidly as children with MS who experience demyelination as teenagers.

It is likely that both theories play a role in the results that we observed. Further studies will be necessary in order to elucidate the role of each theory in pediatric MS. Also, histological studies in MS animal models of different ages will be useful for understanding the presence of an age-related capacity for remyelination in MS.
6.4: IMPACT OF FINDINGS

6.4.1: A Distinction Between Monophasic Acute Demyelinating Syndromes and Multiple Sclerosis

My finding that NAWM at the first clinical attack distinguishes patients later diagnosed with MS from those with mono-ADS supports the construct that the initial attack is indeed the sentinel clinical attack of an established MS disease process.

By studying pediatric patients with ADS (aged 12-16 years) as opposed to adults, I was able to minimize the possible duration of subclinical disease, as younger age precludes long subclinical disease duration. Secondly, I was able to use scans that were obtained much closer to the initial demyelinating attack (average 3 weeks) than in most published adult studies attempting to study MS at clinical onset (averaging between 2.5 and 19 weeks in different studies (Fernando et al., 2005; Rocca et al., 2007; Iannucci et al., 2000)). I have also matched carefully for sex and age, thereby controlling for the effects of these risk factors for MS, and all participants were scanned using the same imaging protocol.
6.4.2: Diffusion Tensor Imaging as a Diagnostic Tool

The ability for DTI to detect a difference in patients that will be diagnosed with MS at the first attack from those that will only experience one demyelinating attack suggests that, given further studies assessing DTI's predictive power, it could be used as a tool in the differential diagnosis for demyelination. DTI may be useful as a complement to other techniques that aid in diagnosing MS in children. Also, in contrast to conventional MRI imaging, DTI can assess non-lesional white matter, which would allow the clinician to understand an aspect of the disease that is not assessed by other tools.

6.4.3: The Importance of Studying Young Children with Multiple Sclerosis

From our analysis of children with MS onset before age 12 years, we observed that those children that were younger than 12 years old at the time of the scan did not demonstrate the diffuse NAWM pathology found in those patients that were teenagers at the time of scan. This raises several possible theories, as discussed. Future studies should focus on children with MS under 12 years old in order to understand the early mechanisms of MS disease. Stemming from their young age, these children have an even shorter subclinical disease duration than adolescents with MS. Therefore, once these children are identified in the clinic, studying them allows for a thorough study of early disease evolution.

Furthermore, our finding of normal brain tissue integrity on DTI in young children may be explained by an improved ability for young children to remyelinate axons, as their
axons are still in their growth stages. Further study of remyelination mechanisms of young children with MS will be necessary to confirm this.

Axon survival depends on the receipt of trophic factors from myelin (Wilkins et al., 2003). Therefore, the study of remyelination must be important if we want to understand how to prevent axonal loss. As reviewed by Trapp and Nave (2008), there is compelling evidence for axonal loss in connection with demyelination, as evidenced by studies showing progressive brain atrophy in MS patients and progressive axonal loss in mice genetically modified to have an altered myelin structure. Axonal loss may contribute to disability in adults with SPMS. Therefore, developing neuroprotective medicine is a current therapeutic goal.

As reviewed by Franklin and ffrench-Constant (2008), remyelination depends on diverse molecular interactions between oligodendrocyte precursor cells, growth factors, inflammatory molecules, and others. Epigenetic modification also plays an important role. Remyelination failure may result from the failure of oligodendrocyte precursor cells to differentiate and mature properly. It is a complex process that is not yet fully understood.

Studies of young children with successful remyelination may lead to an improved understanding of remyelination failure in adults, and can help in developing therapies that will improve remyelination and reduce disability related to axonal loss in older MS patients.
6.5: STRENGTHS AND LIMITATIONS OF STUDY

6.5.1: Study Strengths

The novelty of my thesis rests on analysis of a rare population of MS patients – young children diagnosed prior to puberty, and pediatric patients imaged at the time of the first MS attack. By analyzing DTI scans of children at the time of acute demyelination, I was able to assess losses of tissue integrity in the NAWM at the earliest time in clinical disease, which can predict which children will experience relapses in the future. The available literature regarding pediatric-onset MS has largely focused on adolescents, and thus our study expands this knowledge to MS patients experiencing onset of disease at an even more active time in brain development. I have shown that those MS patients that are still undergoing age-related brain development are a particularly interesting group to study – they have a different impact of MS on the integrity of NAWM.

In addition, this thesis established a novel collaboration between the DT MRI imaging teams of the Canadian and Italian multiple sclerosis study groups. This collaboration allowed me to analyze a sufficient amount of high quality images from patients with MS who had their first demyelinating attack prior to puberty. The need for international collaborations in rare diseases is paramount.
6.5.2: Study Limitations

The DTI images acquired at the Hospital for Sick Children were of relatively low resolution – 5mm thick slices. This means that many voxels in the DTI image may represent a mixture of different tissues, making it difficult to assess the integrity of specific white matter tracts. However, we only assessed large tracts such as the CC and overall white matter, which should not be greatly affected by this concern. Also, I compared the results obtained from participants from Hospital for Sick Children with those recruited at Ospedale San Raffaele, whose DTI images were acquired with a slice thickness of 2.3mm. Healthy controls from both research centers showed similar DTI metrics for a given age in the CC, suggesting that our findings from 5mm-thick images are valid for this large tract. However, if at a later time we want to assess smaller brain structures, it would be necessary to acquire a higher resolution DTI image.

We were unable to image children younger than seven years old for objective 2. DTI measures the microscopic diffusion of water and is therefore greatly affected by participant movement. While it would be interesting to image pre-school aged children with MS, children younger than about 7 years old cannot stay still enough for a movement-sensitive sequence such as DTI.

When studying children with established MS who had their first attack before age 12 years, I was limited to a cross-sectional study design. I attempted to assess the effect of age on the interplay between DTI metrics and MS disease. However, as I plotted FA/RD metrics of different patients of different ages instead of the same patients as they grew, I was limited by the inferences that I could make regarding tract development or changes in DTI metrics over time. Future studies of pre-pubescent-onset MS patients, however,
should use a longitudinal study design.

6.6: NEXT STEPS

The first priority will now be to replicate the findings of the present thesis in a large cohort of children with ADS, and in particular, in a larger cohort of very young MS patients analyzed at the time of their incident attack. The rarity of MS onset prior to puberty will necessitate that the latter studies are performed through collaborative networks. The collaboration with the Italian group is the first step towards such an effort.

To better appreciate the pathobiological insights provided by DTI, it will now be necessary to perform studies that more directly interrogate white matter function. In other words, does the diffuse abnormality in NAWM present even at onset in older children and teenagers with MS yield measurable deficits in clinical metrics such as processing speed, or in functional MRI measures of cerebral activation?

A future goal is to determine if DTI, or other future MRI metrics, can serve as a diagnostic test for MS. Diagnostic tests are tools that allow physicians to accomplish one or more of the following five goals: establish probability of disease, screen for disease, predict disease prognosis, monitor therapy, and/or confirm disease absence (Elavunkal et al., 2011). A good diagnostic test should accurately predict both the presence of a condition when it is truly present (sensitivity), as well as the absence of a condition when it is truly absent (specificity). It may be possible to develop DTI as a non-invasive diagnostic test for MS that could accomplish all of the above goals except screening for disease, which would be prohibitively expensive. With an approximate annual incidence of MS worldwide of 2.5/100,000 per year (World Health Organization and Multiple Sclerosis International
Foundation, 2008), screening asymptomatic populations would require MRI scans in a very large number of people in order to identify individuals prior to MS onset, which is far too costly. I have shown that DTI can distinguish MS in patients at the first demyelinating attack; also, DTI measures are correlated with cognitive impairment in adult and pediatric MS patients (Rovaris et al., 2002; Bethune et al., 2011). In order to develop DTI as a diagnostic test, it would first be necessary to establish DTI norms for white matter metrics of many healthy controls, and NAWM metric ranges for many patients with monophasic ADS and MS. It would be necessary to study participants with a wide range of different ages, and, in those patients who have experienced demyelination, a wide range of times elapsed from the initial demyelinating attack. Afterwards, it will be possible to test the predictive sensitivity and specificity of DTI for predicting MS outcome.

Finally, it will be important to evaluate the trajectory of DTI findings in children with MS and in children with monophasic ADS, and to determine how the developmental changes in FA and RD change as a component of normative development. Our national pediatric study employs a longitudinal design, with DTI acquired not only at baseline, but also at 3, 6, and 12 months and annually for up to 8 years. Analysis of these serial scans is now underway.
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