Diffusion Tensor Imaging Biomarkers of Stem Cell Therapy Efficacy in Pediatric Cerebral Palsy

by

Anastasiya G Batrachenko

Graduate Program in Medical Physics
Duke University

Date:_______________________

Approved:

___________________________
Allen W Song, Supervisor

___________________________
Chunlei Liu

___________________________
Nan-Kuei Chen

___________________________
Robert Reiman

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Graduate Program of Medical Physics in the Graduate School of Duke University

2012
ABSTRACT

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Abstract

Cerebral palsy (CP) is a non-progressive sensory-motor disorder and is one of the most costly and debilitating chronic pediatric conditions, affecting 2-3 out of 1000 children in the United States. To better understand the underlying mechanisms that result in the devastating motor deficits in CP patients, quantitative assessment of brain regions and connectivity with respect to the severity of injury is critical. It is generally known that motor control can be largely influenced by the integrity of the corticospinal tract (CST) connecting the primary motor cortex (pre-central gyrus) to the brainstem and the spinal cord. Thus, we further sought to establish CST volume as a sensitive and reliable biomarker that can reveal functional deficit in individual patients through comprehensive quantitative analyses in 29 consented human subjects (1-6 years of age, mean 2.67±1.36 years) diagnosed with hemiplegic, diplegic, or quadriplegic CP. The proportional reduction of bilateral CST volume with increased disease severity was observed for diplegic and quadriplegic patients (i.e. with bilateral motor deficits). Furthermore, CST volume with respect to disease severity in hemiplegic patients (i.e. with unilateral motor deficits) exhibited more complex patterns of asymmetry, revealing evidence for alternative neuromechanisms of motor control, such as compensation and neuronal plasticity by the unaffected hemisphere. In all cases, other diffusion metrics such as fractional anisotropy (FA) and mean diffusivity (MD) within the CST did not
display significant correlation with disease severity. It is thus concluded that individual CST volume, accurately derived from DTI tractography, is one of the strongest non-invasive imaging biomarkers which could potentially help understand the differential causes for motor deficits and compensation in bilateral CP. It could also be further adopted to assess the underlying mechanism for functional recovery in individual patients undergoing cellular therapies. In addition to motor disability, numerous other neurodevelopmental differences are associated with CP and contribute greatly to the disease morbidity. Therefore, we further examined global changes in WM connectivity with respect to CP disease severity with a whole brain connectome analysis in a cohort of 18 pediatric patients with bilateral CP. As expected, reduction in overall and mutual connectivities throughout the motor regions, including the primary and supplemental motor areas, basal ganglia, and brainstem, was associated with the extent of primary motor disability. In addition, our results show diffuse and significant reduction in global inter-regional connectivity, measured as fiber volume, throughout the entire brain in relation to disease severity. This novel finding has not been previously reported in the CP literature and brings CP into line with multiple other pediatric neuropsychiatric disorders that are thought to involve network-level structural and/or functional disruptions early in development.
Dedication

To my family.
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1. Cerebral Palsy (CP) Overview and Study Motivation

Cerebral Palsy (CP) refers to a group of heterogeneous disorders characterized by impairment of movement and posture with resultant limitation of activity (Bax et al., 2005). Despite advances in obstetric and perinatal medicine, CP remains the most common motor disorder affecting 2-3 out of every 1000 children in the USA (Clark et al., 2003; Cauraugh et al., 2010). Contributing to the disease burden in these patients are common comorbid deficits in sensation, cognition, communication, behavior, and seizures (Bax et al., 2005; Jones et al., 2007). A variety of non-progressive disturbances in the developing fetal or infant brain may lead to CP and an individual’s clinical presentation is likely determined by the spatial pattern and extent of grey and white matter (WM) involvement (Volpe 2003; Folkerth et al., 2005). Cerebral palsy is one of the most disabling and costly chronic conditions of childhood, with an estimated annual cost of care of $8.2 billion for affected children in 2002 (Koman et al., 2003).

1.1 Causes and Classification of Pediatric CP

Both full-term and premature infants can develop cerebral palsy as a result of brain injury sustained during the prenatal, perinatal, or postnatal period. Most affected full-term infants have sustained a prenatal neurologic insult, such as an in-utero stroke, or a hypoxic-ischemic injury at the time of delivery (Clark et al., 2003). It often arises from white matter (WM) damages during in utero or neonatal development, but can also be caused by injuries or developmental abnormalities of gray matter (GM) with
secondary white matter involvement. In response to the need to have a standardized system for classifying the severity of movement disability among children with CP, the five-level Gross Motor Function Classification System (GMFCS) was developed, with level I patients being completely ambulant and exhibiting very mild motor disturbances, and level V patients requiring full assistance in all daily tasks (Morris 2006). Outcomes of the motor testing in cerebral palsy can also be summarized as the CP severity that ranges among mild, moderate, and severe, approximately equivalent to GMFCS scores of I, II-III, and IV-V, respectively.

1.2 Current Treatments

Unfortunately, no curative therapy for cerebral palsy exists today. Current treatment modalities focus on maximizing functional abilities and quality of life through several approaches. These include non-pharmacologic measures, such as physical, occupational and speech therapies, orthotics, optimal nutrition, constraint therapy and use of adaptive devices; pharmacologic interventions such as oral pharmacological agents, botulinum toxin injections, and oral or intrathecal baclofen; and surgical options including dorsal rhizotomy and various orthopedic procedures to relieve the possible spastic, ataxic, and dyskenetic symptoms (Koman et al., 2003; Sutcliffe et al., 2009; Porter et al., 2010; Rutherford et al., 2010). Autologous stem cell therapy has recently shown potential to repair ischemic tissue and stimulate plasticity in the surrounding brain areas.
(Goldman 2005; Daadi et al., 2010), making it a promising treatment tool in pediatric CP, with the first 2nd phase clinical trial currently underway at Duke University.

1.3 Anatomical Neuroimaging in Cerebral Palsy

Improved sensitivity of neuroimaging techniques in children with CP is still needed to aid clinicians and researchers with subgroup classification, prognosis, targeted treatment strategies and treatment monitoring. The latter has become increasingly necessary as more neuroprotective and rehabilitative treatment trials are underway. Thus far, neuroimaging data have shown early promise as treatment response biomarkers in neuroprotective therapeutic hypothermia (Porter et al., 2010; Rutherford et al., 2010), and rehabilitative trials within this population (Trivedi et al., 2008; Sutcliffe et al., 2009). Magnetic resonance imaging (MRI) is particularly well-suited for brain visualization because of its ability to assess the differences in water distribution within the soft tissues, rendering it a powerful tool to characterize the possible brain injury and recovery in pediatric CP. Brain abnormalities in CP revealed by MRI include, but are not limited to periventricular white matter thinning (a.k.a. periventricular leukomalacia or PVL), ventricular enlargement, cortical atrophy, and porencephaly.

Standard clinical neuroimaging identifies gross patterns of injury in 70-90% of cases and provides some useful prognostic information, but even within these cases a lot of clinical heterogeneity exists (Woodward et al., 2006, Korzeniewski et al., 2008, Towsley et al., 2011, Martinez-Biarge et al., 2011, Benini et al., 2012).


1.4 Diffusion Tensor Imaging in Cerebral Palsy

1.4.1 Background

Diffusion weighted imaging measures the extent of water diffusion within a given voxel, modeling the spatial distribution of the diffusing molecules as a three-dimensional ellipsoid, a.k.a diffusion tensor. The tensor eigenvalues can be used to derive various metrics characterizing the local diffusive properties of tissues, such as the predominance of the primary directionality of diffusion – or fractional anisotropy (FA), and the average extent of water diffusion along all three axes of the ellipsoid – or mean diffusivity (MD). White matter in the brain is highly organized into bundles that restrict the local diffusion of water molecules along the direction of the WM tracts. The direction and extent of these tracts can then be modeled by determining the likely water diffusion paths (or streamlines) solved from the continuous diffusion tensor field acquired to cover the whole brain. The resulting diffusion trajectories approximate the coherently organized brain white matter pathways, which found to be consistent with known post-mortem anatomy (Mori et al., 1999; Basser et al., 2000). The method’s reliability does diminish in locations of crossing or merging fiber tracts, and is also greatly susceptible to the signal-to-noise output of the imaging protocol. Still, this method is currently able to provide the most robust quantification of connectivity and continuity of neural pathways in vivo (Basser 2000). Developments in diffusion tensor imaging (DTI) and tractography can thus provide more reliable delineation of the major nerve fiber
pathways in pediatric CP brains without dependence on the traditional anatomical landmarks, together with comprehensive information to assess the connectivity within these pathways and among the cortical regions they connect (Basser et al., 1994; Basser et al., 2000, Le Bihan et al., 1995; Mori et al., 1999).

1.4.2 DTI and Tractography Applications in CP

Diffusion contrast may reveal potential connectivity impairments in brain structures that may appear normal in conventional T1- and T2-weighted images in the presence of significant functional deficits. DTI and tractography are particularly well-suited for the detection WM micro-structural alterations (Gerig et al., 2004; Mori et al., 2006) that are thought to contribute heavily to the clinical manifestations of CP. Some diffusion based studies have shown relatively diffuse changes in multiple WM regions of interest (ROIs), particularly in patients born prematurely, using mean diffusivity and fractional anisotropy methods (Thomas et al., 2005, Zhang et al., 2005; Korzeniewski et al., 2008). However these studies are restricted methodologically, confined largely to diffusivity and anisotropy measures of WM integrity in a priori ROIs.

Tract-based analysis of WM provides more comprehensive insights into possible mechanisms of disruption for specific functional abilities in pediatric CP. DTI and structural connectivity analysis in CP patients has been used to generate potential imaging biomarkers to characterize injuries to sensory-motor pathways with respect to behavioral deficits (Hoon et al., 2002; Thomas et al., 2005; Zhang et al., 2005; Nagae et al.,
It has been shown that group analysis of white matter tractography can be used in the assessment of micro- and macroscopic axonal injuries through modeling the compactness, coherence, and the overall extent of WM pathways.

### 1.4.3 Whole Brain Connectome Analysis

Even studies utilizing tractography methods frequently limit assessment of brain injury to the identification of an a priori tract as an ROI for diffusivity measurements. Further advances in connectivity imaging in the recent years gave rise to the so-called brain connectome and its related analysis (Sporns et al., 2005), in which brain regions are delineated by the overall connectivity to the rest of the brain, as well as by the connectivity to other individual brain regions. Such a whole-brain analysis provides a standardized and informative means to characterize brain connectivity with recent increased applications in normal and diseased brains - although none of these studies, to our knowledge, have examined fiber tract characteristics of WM in the whole brain and inter-regional WM connectivity (Sporns et al., 2005). Given the neuropathologic evidence of diffuse gross and ultrastructural WM pathology in CP patients, we investigated the spatial pattern and extent of WM involvement using fiber tractography analysis of the whole brain.
1.5 Present Study Aims

Based on the described magnetic resonance imaging based advances, we sought to examine the general connectivity impairments in pediatric brains with CP in an effort toward identifying the most sensitive and reliable brain imaging biomarker that is related to the functional manifestation of the disease in individual patients. Given the neuropathologic evidence of diffuse gross and macrostructural WM pathology in CP patients, we investigated the spatial pattern and extent of WM involvement using fiber tractography analysis of the whole brain in children with spastic bilateral CP. The discovered biomarkers could be further used in bilateral and unilateral CP to reveal repair mechanisms and treatment effectiveness following cellular therapy, which is currently underway at Duke University.
2. Study Design

Patients in this report are enrolled in an ongoing randomized, placebo-controlled study of the efficacy of autologous cord blood infusions in patients with CP [IND 14360] at Duke University Medical Center. Eligibility includes children from 1-6 years of age with CP with a Gross Motor Function Classification System Level (GMFCS) of I-IV at study entry and a qualified banked autologous cord blood unit. Children are evaluated at baseline and in follow-up by a team of neurologists, psychologists, developmental pediatricians, physical therapists, occupational therapists, speech therapists and neuroradiologists with an extensive battery of tests including neurofunctional testing and MRIs. Children are treated with an intravenous infusion of autologous cord blood cells or placebo at baseline and 1 year post initial treatment in a blinded, cross-over design. The results presented in this report represent the findings from the baseline MRI exam of these patients. The remaining findings will be reported in later years during and after the cellular therapy. Written informed consent was obtained from each patient’s parents before any studies were performed.

Figure 1: Patient Enrollment and Evaluation
2.1 Subject Recruitment

At present, forty patients have undergone MRI scans, although several were excluded from this analysis due to excessive motion artifacts occurring in non-sedated or light patients. Further exclusions were made in cases of extreme subject brain deformities that created warping mismatches in the non-linear registration pipeline. The remaining cohort analyzed in this report consists of 32 children 1-6 years of age (mean of 2.67±1.36 years), out of which 11 exhibited unilateral motor deficit (hemiplegia) and 18 exhibited bilateral motor impairment (diplegia or quadriplegia), as summarized in Table 1. The outcome of the neurological motor testing was summarized as the CP severity score that ranged from mild (score of 1) to moderate (scores between 2 and 3, reported by neurologists with a composite score of 2.5) to severe (scores between 4 and 5, reported with a composite score of 4.5). All children under 5 years of age were sedated with a combination of midazolam (0.5mg/kg) and either dexmetatomidine (1-2 mcg/kg) or propofol (1-2 mcg/kg) administered as an intravenous bolus prior to entering the magnet and either as incremental bolus or continuous drip during the scan.

The Kruskal-Wallis test confirmed that the average ages of subject in the mild, moderate, or severe CP subgroups were not significantly different in either bilateral or unilateral CP patient categories (Liptak et al., 2001). Subject demographical details and CP severity distribution are summarized in Table 1.
Table 1: Demographic summary of the CP patient cohort.

<table>
<thead>
<tr>
<th>CP Type</th>
<th>Patients</th>
<th>Age</th>
<th>Gender</th>
<th>Mild CP (1)</th>
<th>Moderate CP (2.5)</th>
<th>Severe CP (4.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemiplegia</td>
<td>11</td>
<td>3.2±1.5</td>
<td>5 F, 6 M</td>
<td>6</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Quadriplegia &amp; Diplegia</td>
<td>18</td>
<td>2.33±1.17</td>
<td>6 F, 12 M</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

2.2 MRI Protocol

All subjects were scanned in a supine, head first position with a 3 Tesla GE HD scanner (Waukesha, WI) and an 8-channel head coil, 31.25 KHz bandwidth, oblique acquisition, and SENSE factor of 2. The field of view (FOV) was set at 19.2cm to accommodate head size of children up to 6-8 years of age, while minimizing the scan time for each pulse sequence.

Table 2: MRI protocol summary for Duke CPAC study

<table>
<thead>
<tr>
<th>Anesthetized</th>
<th>Older Awake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical: T1, T2/PD (10 min)</td>
<td>T1, T2/PD weighted (10min, movie allowed)</td>
</tr>
<tr>
<td>Susceptibility (15min)</td>
<td>rest fMRI (5min, no movie, eyes closed)</td>
</tr>
<tr>
<td>Rest fMRI (5min + 4min of high-res EPI)</td>
<td>motor fMRI (5min, visual instruction)</td>
</tr>
<tr>
<td>DTI (6min)</td>
<td>DTI (6min, movie allowed)</td>
</tr>
</tbody>
</table>

2.2.1 Structural Imaging

2.2.1.1 T1 Protocol

T1-weighted images used for WM volume quantification were obtained with an inversion-prepared 3D fast spoiled-gradient-recalled (FSPGR) pulse sequence with an
echo time (TE) of 2.5 ms, an inversion time (TI) of 450 ms, and recovery time (TR) of 6.5 ms, and flip angle of 12°, at 1mm³ isotropic resolution.

2.2.1.2 T2 and Proton Density (PD) Protocol

Additional anatomical PD and T2 images were acquired via the flow-compensated fast spin echo (FSE-XL) pulse sequence with echo train length (ETL) of 24 echoes, minimum full TE of 7.9ms (PD image), effective TE of 120ms, TR of 4500ms, and isotropic 1mm³ resolution.

2.2.2 Susceptibility Imaging

Susceptibility maps were reconstructed from the 3D, SPGR acquisition with TE of 40ms, TR of 51ms, and flip angle of 15°. Spatial resolution was kept at 1mm³, and phase FOV (right-left spatial direction) was set to 0.75 of the frequency FOV to achieve shorter scan time.

2.2.3 Functional Imaging

2.2.3.1 Resting State fMRI

High order shimming was done prior to the functional data acquisition. Resting state protocol involved a single shot, 2D gradient echo pulse sequence with echo planar imaging (EPI) signal read-out with TE of 27ms, TR of 2500ms, and a 77° flip angle. Slice thickness was set at 3mm together with a 64x64 acquisition matrix. Slices in each volume were acquired sequentially in an inferior-to-superior progression. Total of 100 volumes were collected for each subject.
2.2.3.2 High Resolution IR-prepared EPI

High resolution T1-weighted EPI sequence was developed at the Brain Imaging and Analysis Center (Nan-Kuei reference) in order to minimize differences in acquisition artefacts between the functional images and the patient’s anatomical scans used for high resolution localization of observed brain activity. The high resolution IR-prepared EPI was built as a 2D 2-shot spin echo excitation and EPI acquisition. The minimum TE was 18.3 ms, TR – 5000 ms, inversion time – 1000 ms. Slice thickness was set at 3 mm with a 256x256 acquisition matrix. The resulting image was then used as a registration template for high resolution localization of the activation maps generated from the resting state scan.

2.2.4 Diffusion Tensor Imaging

Diffusion weighted images were acquired using a 25-direction gradient encoding scheme at \( b = 1000 \) s/mm\(^2\) and with 3 non-diffusion-weighted images to ensure a robust baseline. An echo time (TE) of 70.5 ms and a repetition time (TR) of 12000 ms were used. Isotropic resolution of 2 mm\(^3\) was achieved using the 96 x 96 acquisition matrix.

2.5 Analysis Pipelines

All imaging data was processed on the Brain Imaging and Analysis Center (BIAC) Linux cluster, using commercial software for fiber tracking, as well as in-house Python and MATLAB toolkits for statistical analysis and graphics. Non linear image registration steps were carried out via the DiffeoMap graphical user interface.
3. CST as the Non-Invasive Imaging Biomarker of Motor Function in Pediatric CP

One of the defining manifestations of cerebral palsy is primary motor dysfunction (Bax et al., 2005). Corticospinal tract (CST) is the principal white matter pathway responsible for transmitting neuronal signals from the primary motor cortex to the brainstem, controlling all further muscle motion. In case of the motor cortex injury/malformation or a hypoxic-ischemic insult within the connecting white matter, corticospinal pathway volume and, potentially, diffusive properties are expected to be affected (Nagae et al., 2007; Yoshida et al., 2010; Rose et al., 2011). The extent of the primary motor pathway disruption is expected to be influenced by the extent of the original WM or GM injury (Volpe 2003; Folkerth et al., 2005), which would consequently affect behavioral deficits observed among CP patients.

3.1 Tracking Selection

3.1.1 Brain Normalization, Tract Selection and Quantification

White matter tractography was performed via the fiber assignment by continuous tracking (FACT) algorithm (Mori et al., 1999; Toussaint et al., 2007). To ensure reliable and precise delineation of ROIs in diffusion weighted image space, a single-subject atlas Eve, based on the Johns Hopkins – MNI template (Oishi et al., 2010), was chosen because of its robust diffusion contrast. These standard ROIs were then warped to each patient’s DTI image space via the Large Deformation Diffeomorphic Metric
Mapping (LDDMM) algorithm (Miller et al., 2005; Faria et al., 2010). The following ROIs: brainstem, posterior limb of internal capsule (PLIC), and the superior portion of the pre-central gyrus, were selected to reliably delineate the motor pathway. CST volumes were then quantified, together with the average fractional anisotropy (FA) and mean diffusivity (MD) metrics.

3.1.2 Correlational Analysis

CST volumes (in mm$^3$) were estimated from the total number of voxels traversed by the tract in each patient (Toussaint et al., 2007). Total CST volumes in bilateral CP patients (computed as the sum of right and left CST volumes) and CST volumes within each hemisphere in unilateral CP patients were subsequently normalized by the total WM volume to remove global effects such as age-dependent brain development. The CST volume fraction, along with its average FA and MD, was then examined against the CP severity scores.

3.1.3 CST Assessment Results

3.1.3.1 CST Delineation

DTI tractography was carried out to delineate the CST through the primary motor region, posterior limb of the internal capsule and brainstem. The complete warping, ROI standardization, and tracking procedure is illustrated in Fig. 2.
Representative delineations of a healthy CST, that from a 2 year old patient diagnosed with spastic diplegia, and that from a 2 year old with spastic quadriplegia, are shown in Fig. 3, qualitatively demonstrating the critical role that the CST plays in modulating primary motor function.

3.1.3.2 CST as Potential Biomarker in Bilateral CP

Quantitative correlational analyses were subsequently carried out in individual patient groups between the CST volume and the severity of motor deficits. In diplegic and quadriplegic patients (i.e. with bilateral motor deficits), the normalized total CST...
volume fraction is shown to be significantly and inversely correlated with behavioral CP severity (Fig. 3), with an \( r^2 = 0.769 \) and \( p < 0.0001 \).

Figure 4: Normalized CST volumes with respect to the CP severity score in diplegic and quadriplegic patients with bilateral motor deficits.

To complete the evaluation of other diffusion metrics in the CST, the averaged FAs and MDs were also examined against the motor deficits, as summarized in Table 2.

Table 3: Average CST diffusivity metrics in bilateral CP.

<table>
<thead>
<tr>
<th>Bilateral CP Severity</th>
<th>CST FA</th>
<th>CST MD (x10^{-3}mm^2/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>0.437±0.208</td>
<td>0.763±0.305</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.438±0.216</td>
<td>0.806±0.351</td>
</tr>
<tr>
<td>Severe</td>
<td>0.419±0.171</td>
<td>0.869±0.401</td>
</tr>
</tbody>
</table>

While the group averaged mean FAs and MDs in general exhibit respective trends of decrease and increase with progressing disease severity scores, which have also been found in previous reports (Thomas et al., 2005; Yoshida et al., 2010; Faria et al., 2011), the relatively large standard deviations make these metrics less sensitive to serve as biomarkers that can be used to evaluate causes and treatment outcome in an individual patient.
3.1.3.3 CST as Potential Biomarker in Unilateral CP

The findings in hemiplegic patients (i.e. with unilateral motor deficit), however, exhibit a more complex pattern (Fig. 5).

Figure 5: Corticospinal tract volume asymmetry (left) and CST volume in individual hemispheres (right) with respect to CP severity score in hemiplegic patients with unilateral motor deficits. Numerical labels (i.e. 15, 17) represent the most extreme patient outliers in correlation between CST volume reduction and motor deficit in mild and moderate CP, respectively.

While there is a general trend showing a lower CST volume fraction on the affected side, there is not a linear correlation between the volume reduction and CP severity scores. This is largely caused by a wide range of volume reductions in patients with a mild motor deficit – potentially attributed to the compensatory mechanism by the opposite hemisphere. However, discrepancies between the CST volume reduction/asymmetry and motor deficit are seen at both ends of the severity spectrum. That is, one subset of patients has significant asymmetry but only display mild unilateral motor impairment, while another subset experiences significant unilateral motor impairment without displaying significant CST volume asymmetry. These anomalies, which led to the overall reduction of linear correlation between CST volume reductions
and motor deficits, are illustrated in the two representative cases at the both ends of the CP spectrum in Fig. 5. The top panel shows the FA map and the tracked CST in a hemiplegic subject (patient 15, 2 years 9 month old) with mild functional symptoms, despite an almost complete interruption in the CST in the affected hemisphere, and hence extreme volume asymmetry, due to a unilateral damage to the superior corona radiata. On the other hand, the bottom panel in Fig. 5 depicts a hemiplegic patient (patient 17, 3 years 8 month old) with severe unilateral motor functional deficits, yet with comparatively much more symmetric CST.

Figure 6: Top: Axial FA map (a), coronal FA map (b), and delineated CST (c) in one hemiplegic patient (Patient 15) with mild contralateral motor impairment despite severe interruption in the CST on the right side, suggesting functional compensation by the ipsilateral CST. WM damage is visible in the FA maps in the superior corona radiate. Bottom: Axial FA map (a), coronal FA map (b), and delineated CST (c) in another hemiplegic patient (patient 17) with significant contralateral motor deficit despite much less impaired CST, likely indicating damages upstream in the gray matter or downstream in the neuromuscular systems.
On the other hand, if the full pre-central gyrus ROI is used for delineation of the primary motor tract, the full CST volume asymmetry becomes much more prominent in the moderately affected hemiplegic subjects, as indicated in Figure 7.

![Hemiplegic Full CST Volume Asymmetry](image)

**Figure 7:** CST volume asymmetry in hemiplegic patients with full pre-central gyrus employed as ROI for tract selection.

Stronger full CST asymmetry in more severe unilateral CP could emphasize the injury localization to the lateral portion of the primary motor gyrus in cases of middle cerebral artery stroke. It could also indicate partial functional reorganization of the inferior part of the motor cortex to assume control of limb and torso movement.

In hemiplegic patients, the FAs and MDs of the affected and unaffected CST were evaluated. The average unilateral FA and MD values in the mild and moderate cases of unilateral CP are summarized in Table 4.
Table 4: Average CST diffusivity metrics in unilateral CP.

<table>
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<tr>
<th>Unilateral CP Severity</th>
<th>Affected CST</th>
<th>Unaffected CST</th>
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<tr>
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<td>FA</td>
<td>MD</td>
</tr>
<tr>
<td>Mild</td>
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<td>0.823±0.328</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.438±0.141</td>
<td>0.821±0.352</td>
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</table>

The averaged mean FA and MD metrics once again exhibit respective general trends of increase and decrease between the affected and unaffected CSTs, in concordance with the previously published literature (Thomas et al., 2005; Yoshida et al., 2010; Faria et al., 2011). Yet, just as in the bilateral CP analysis, the relatively large standard deviations make these metrics less sensitive as of cerebral palsy causes and treatment outcomes in individual patients.

3.1.4 Discussion

Efforts were made in this study to determine the most sensitive imaging metric that can characterize the differentiating structural impairments in individual CP patients. Through a standardized group-based whole-brain connectome analysis, it was found that the most functionally relevant connectivity impairments in pediatric CP involve areas linked to the CST. It was thus postulated that the CST volume can be used as a sensitive non-invasive imaging biomarker for individual evaluations and can help differentiate the neuromechanisms of motor function deficits and potential compensation.
Through quantitative analyses of CST volume, it was found that in bilateral CP patients with either di- or quadriplegia, age-normalized CST volumes exhibited a strong linear relationship with progressing disease severity scores. This finding is highly significant, indicating that the CST volume in bilateral CP patients can be used reliably as a sensitive biomarker for CP severity. While it is already significant to help better understand the underlying disease mechanism, this biomarker is particularly valuable during the course of cellular treatment currently underway in our laboratory. It is anticipated that these non-invasive measurements will shed light on the repair and recovery mechanisms for motor function in pediatric CP patients.

In unilateral CP patients with hemiplegia, normalized CST volume did not correspond consistently with CP severity scores, although there was a general reduction of CST volume in the affected hemisphere. The absence of a clear relationship between impairment and CST volume was largely due to patients with great reduction of CST volume but relatively mild motor impairment, and patients with mild reduction of CST volume but more severe motor deficit. Nevertheless, the individual CST volume would still provide a valuable measure to help differentiate the various potential causes and compensation mechanisms for motor deficits, and guide more specific follow-up clinical exams better corresponding to the actual disease mechanism. More specifically, in hemiplegic patients who displayed extreme CST volume asymmetry between hemispheres but only mild unilateral motor impairment, a potential compensatory
neuronal mechanism involves utilization of the healthy ipsilesional motor pathways. It is known that neuronal plasticity plays an important role in structural and functional development of the brain post injury. In children with CP, reorganization of central motor pathways has been observed using transcranial magnetic stimulation (TMS), electromyography (EMG), and functional MRI (fMRI) (Carr et al., 1993; Staudt et al., 2000; Fujii et al., 2003; Carter et al., 2010). It has also been shown that in stroke patients with a unilateral lesion involving the motor tracks, motor control of the affected limbs is provided by compensatory corticospinal projections from distant regions in the ipsilateral, healthy hemisphere (Staudt et al., 2000; Staudt et al., 2002; Chen et al., 2003; Staudt et al., 2006; Johnston 2009).

For patients with significant unilateral motor deficit yet relatively symmetric CST volumes between hemispheres, potential damages upstream in the contralateral cortical GM layers or downstream in the ipsilateral neuromuscular systems could both contribute to the observed functional impairment (Carr et al., 1993; Ito et al., 1996; Kandell et al., 2000; Yin et al., 2000; Kadhim et al., 2003; Folkerth et al., 2005). Certainly, additional damage to supplementary sensory-motor WM pathways involved in sensory processing and feedback integration, such as the accompanying focal injury to the posterior thalamic radiation found in patient 17, could have further contributed to subject’s impaired ability to discern and perform the required motor tasks. Indeed, the presence of multiple WM pathway impairments has been reported in literature (Nagae
et al., 2007; Scheck et al., 2012), suggesting the need for a concurrent whole-brain connectivity analysis to achieve a comprehensive assessment of brain injury and functional recovery in hemiplegic CP patients.
4. Whole Brain Connectome Assessment

4.1 Rationale

Cerebral palsy (CP) is a heterogeneous group of non-progressive motor disorders caused by brain injury during early development. Although the predominant feature of CP is motor disability, it has been well-established that numerous other neuro-developmental differences are associated with CP and contribute greatly to the disease morbidity (Volpe 2003; Bax et al., 2005). Grey and white matter (WM) injury has been described in CP with both focal and global components that are not restricted to motor regions (Hoon et al., 2005; Rutherford et al., 2010; Scheck et al., 2012). These widespread disturbances are thought to be related to the neurologic basis of the broad symptomatology often seen in children with CP. Here we examine global changes in WM connectivity with respect to CP disease severity in a whole brain connectome analysis in a cohort of 18 pediatric patients with bilateral CP to test the hypothesis that white matter connectivity would show deficits proportional to disease severity throughout the brain. Structural connectome analysis was used to classify the connectivity patterns of cortical fibers between anatomically defined brain regions in effort to define the scope of impaired brain circuits related to motor impairment and developmental delays in CP. The relationship between large-scale inter-regional structural connectedness and CP disability was investigated using a whole-brain connectome analysis.
4.2 Methodology

The relationship between functional motor deficits and white matter connectivity was assessed in a whole-brain connectome analysis in patients with bilateral CP. White matter tractography was performed via the fiber assignment by continuous tracking (FACT) algorithm (Mori et al., 1999) implemented in the DTK tracking toolbox (Wang et al., 2007). The Johns Hopkins University (JHU) standardized parcellation scheme (Faria et al., 2010) was subsequently applied to the bilateral CP patient tractograms to assess volume and diffusivity metrics of the resulting cortico-cortical connections. Fiber pathways were filtered to preserve only the streamlines originating and terminating in cortical and deep GM or brainstem ROIs, and measures of inter-ROI connectivity for the entire brain were obtained. All tract streamline and traversed voxel counts were normalized by the total WM volume based on each subject’s high-resolution T1-weighted images (Smith et al., 2004; Woolrich et al., 2005). The number of streamlines between each pair of ROIs was used as the connectedness metric in a rank correlation of white matter “connectivity” with CP severity score using the Kendall-τ-b rank correlation test (Provenzale et al., 2008). A matrix of Kendall-τ rank correlation coefficients describing how connectivity varied with CP severity score, as well as a matrix of p-values describing the significance of these coefficients was obtained for the group. The significance matrix was corrected for multiple comparisons using false discovery rate correction as implemented by FSL’s FDR algorithm (Genovese et al.,
The Kendall-τ statistic quantifies the association between two variables, here CP severity score and “connectivity” defined as the number of streamlines connecting two brain regions. The resulting coefficient ranges between -1 and 1, a score of 1 indicating that there is a perfect agreement and a score of -1 meaning that there is a perfect disagreement between the variables. This connectivity analysis was performed for both inter-regional streamline counts (mutual connectivity) and the total number of streamlines associated with each ROI (total connectivity).

4.3 Whole Brain Connectome Results

Analysis of all streamlines related to a given node, or total connectivity, was assessed in relation to CP severity. Those ROIs that had a reduction in total connectivity (number of streamlines associated with that region) in correlation to CP severity were in putative sensory-motor regions. These nodes include primary and supplemental motor areas, basal ganglia, and brainstem as shown in figure 7 (red nodes). Red nodes have a deficit in total connectivity to all other brain regions that significantly correlated with CP severity score (p<0.023, corrected for multiple comparisons) and node size is proportional to the total number of connections associated with each region. This finding was based on the whole brain analysis and not confined to a priori ROIs. This data is consistent with other studies frequently finding differences in the WM related sensory-motor networks in patients with CP (Thomas et al., 2005; Yin et al., 2000; Scheck et al., 2012).
Figure 8: Whole brain connectome of patients with bilateral CP. Red nodes are regions that show a significant deficit (p<0.023, corrected) in total connectivity to all other brain regions, correlated with CP severity score. Red edges depict mutual connectivity deficits that are correlated with CP score, and connect two red nodes. Blue edges depict mutual connectivity deficits that are significantly correlated (p<0.026, corrected) with CP severity score. Node size is proportional to the total number of connections associated with each region.

4.4 Discussion

This section of the study demonstrates reductions in whole brain WM connectivity that relate to disease severity in young children with CP. The inter-regional connectivity, as measured by streamline count, is reduced diffusely throughout the brain with increasing CP severity. Global streamline counts and the diffusivity measure, FA, also showed reductions associated with increasing motor disability.
As expected, there is a reduction in total connectivities throughout the motor regions including primary and supplementary motor areas, basal ganglia, and brainstem (Fig. 7), contributing to motor deficits which are the most significant and apparent clinical observations in CP. These results are consistent with the most common findings of altered diffusivity measures in tracts associated with sensory-motor networks (Scheck et al., 2012). Furthermore, reduced mutual connectivity is observed between the frontal lobe and the primary and supplementary motor area within both brain hemispheres, as well as among the subsections of the frontal lobe itself, possibly causative of the widespread developmental deficits observed in patients with most severe grade of CP (see appendices B and C). Further studies, including graph theory assessment of the cortical connectivity networks, are necessary for comprehensive understanding of the extent of the structural injury and possible compensating mechanisms in pediatric cerebral palsy.
5. Conclusions and Future Directions

We conclude that CST volume in individual patients can be used as a sensitive biomarker to help characterize the differentiating causes and potential compensation mechanisms in CP patients. In patients with bilateral motor deficit, the CST volumes correlated significantly and linearly with the disease severity, thereby providing a non-invasive and potentially quantitative measure indicative of disease mechanism and severity. In patients with unilateral motor deficit, the individually divergent patterns in the correlation between the CST volume and the disease severity would help differentiate various potential causes for motor impairments and also provide the basis for alternative compensatory mechanisms such as neuronal plasticity. With increased subject enrollment and progression of our cord blood stem cell therapy, this non-invasive imaging biomarker in CST volume could provide individual and quantitative measures to help characterize the neural correlate of CP pathology, understand the repair mechanism following treatment, and contribute to the design of the most effective cellular therapy strategy.

Both histopathologic and structural neuroimaging studies have further frequently demonstrated wide-spread WM damage in patients with CP (Hoon et al., 2005; Rutherford et al., 2010; Scheck et al., 2012). The majority of DTI studies in CP restricted their analysis to a priori selected WM regions or tracts with putative sensorymotor connections and did not include other WM regions in their analyses.
Within these studies, reduced WM integrity in the corticospinal tract or the thalmocortical projections are repeatedly demonstrated (Hoon et al., 2002; Hoon et al., 2009; Yoshida et al., 2010; Rose et al., 2011; Scheck et al., 2012). Based on the interdisciplinary and multi-modal evidence of wide-spread WM damage in CP, we further developed whole brain connectivity measures, which would be more sensitive to broad WM disturbances and correlated with disease severity. The whole-brain structural connectome analysis in patients with bilateral CP showed specific decreases in total connectivity to regions putatively associated with sensory-motor functionality that correspond to increasing motor disability in pediatric CP patients. More interestingly, we also demonstrated global reductions in inter-regional connectivity with respect to disease severity in frontal and temporal parts of the brain that provide potential insight into possible disruption of network development responsible for other co-morbidities of CP, such as cognitive and language impairments.

In order to better understand neuronal and axonal repair mechanisms occurring in CP brains due to growth and development, frequency of physical therapy, and the timing of the stem cell infusion, individual patient follow-up from baseline to the end of the study will be done to assess any potential primary sensory-motor connectivity restoration and re-routing, along with any possible changes in the additional structural, susceptibility, or functional MRI contrasts, mentioned in Section 2.2. Whole brain connectome correlation of individual diffusivity metrics with respect to the neurological
and cognitive performance, together with graph theory analysis of the baseline WM connectivity patterns and consequent reorganization, will also shed light on neuronal plasticity mechanisms in both bilateral and unilateral CP patient groups. The immediate next step is to explore how selective vulnerability in long-range vs. short range connectivity relates to morbidity in CP, which is already currently underway in our laboratory, and to furthermore apply the connectome methodology to understand more comprehensively the structural or physiological injury and compensation patterns with respect to the corresponding global functional abilities in children with unilateral CP.
Figure 9: Matrix representation of the whole brain assessment of mutual WM connectivity in bilateral CP. Color bar represents (1-p) statistic output from the Kendall-Tau test of tract volume reduction with increased CP severity.
### Table 5: Cognitive Assessment of Patients with Bilateral CP

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<th>Subject</th>
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## Appendix C

### Table 6: Language Assessment of Patients with Bilateral CP

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<th>Subject</th>
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References


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