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Diffusion tensor imaging detects axonal injury and demyelination in the spinal cord and brain of a murine model of globoid cell leukodystrophy

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Abstract

Globoid cell leukodystrophy is an inherited neurodegenerative disorder caused by a deficiency of the lysosomal enzyme galactosylceramidase. In both human patients and the authentic murine Twitcher model, pathological findings include demyelination as well as axonal damage in both the central and peripheral nervous system. Diffusion tensor imaging (DTI) has emerged as a powerful noninvasive technique that is sensitive to these white matter disease processes. Increases in radial diffusivity ($\lambda_{\perp}$) and decreases in axial diffusivity ($\lambda_{\parallel}$) correlate with histopathological evidence of demyelination and axonal damage, respectively. Compared to age-matched, normal littermates, DTI of optic nerve and trigeminal nerve in end-stage Twitcher mice displayed a statistically significant increase in $\lambda_{\perp}$ and decrease in $\lambda_{\parallel}$ consistent with previously characterized demyelination and axonal damage in these regions. In the Twitcher spinal cord, a statistically significant decrease in $\lambda_{\parallel}$ was identified in both the dorsal and ventrolateral white matter relative to normal controls. These results were consistent with immunofluorescent evidence of axonal damage in these areas as detected by staining for nonphosphorylated neurofilaments (SMI32). Increase in $\lambda_{\perp}$ in Twitcher spinal cord white matter relative to normal controls reached statistical significance in the dorsal columns and approached statistical significance in the ventrolateral region. Reduced levels of myelin basic protein were detected by immunofluorescent staining in both these white matter regions in the Twitcher spinal cord. Fractional anisotropy, a nonspecific but sensitive indicator of white matter disease, was significantly reduced in optic nerve, trigeminal nerve, and throughout the spinal cord white matter of Twitcher mice relative to normal controls. This first reported application of spinal cord DTI in the setting of GLD holds potential as a noninvasive, quantitative assay of therapeutic efficacy in future treatment studies.

Keywords

globoid cell leukodystrophy; Twitcher mouse; diffusion tensor imaging; radial diffusivity; axial diffusivity; axonal damage; demyelination; spinal cord

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INTRODUCTION

Globoid cell leukodystrophy (GLD, Krabbe disease) is an inherited autosomal recessive lysosomal storage disease caused by deficiency of galactosylceramidase (galactocerebroside β -galactosidase, GALC).^{1,2,3} GALC is necessary for the normal metabolism of galactolipids found in myelin such as galactosylceramide and psychosine (galactosylsphingosine).³ Without sufficient GALC activity, psychosine accumulates to toxic levels causing apoptosis of oligodendrocytes and Schwann cell impairment with resultant myelin loss within both the peripheral and central nervous systems.^{4,5,6,7,8} A component of axonal damage and loss is also present in affected white matter regions.^{3,9,10,11,12} In the most common early infantile form, human patients undergo progressive neurological deterioration starting at three to six months of age with death typically occurring by two years of age.^{2,3}

The Twitcher mouse (*twi/twi*) harbors a spontaneous mutation in the GALC gene and mirrors the pathology and clinical course of the infantile form of the human disease.^{3,10,13,14} As such, the Twitcher mouse has proven to be extremely valuable as a tool to study both the pathology of GLD as well as the efficacy of various therapeutic strategies including from bone marrow transplantation, substrate reduction, and gene therapy approaches.^{14,15,16,17,18,19,20} These treatment studies typically rely on functional parameters as well as biochemical and qualitative histopathological analysis to assess therapeutic benefit.

Magnetic resonance imaging has been explored as a noninvasive tool to assess pathology in the brain of both human Krabbe disease patients as well as the Twitcher mouse.^{11,12,21,22,23,24} The MRI technique of diffusion tensor imaging (DTI) holds particular promise in this regard with its ability to quantify directional, anisotropic diffusion of water in tissues.²⁵ In numerous other models, separation of directional diffusivity data obtained by DTI into components that are parallel (axial, $\lambda_{||}$) and perpendicular (radial, $\lambda_{\perp}$) to white matter tracts has been shown to correlate with histopathological evidence of axonal and myelin damage, respectively.^{26,27,28,29,30,31,32,33,34} Fractional anisotropy (FA), the normalized standard deviation of the measured diffusivities, decreases with either increased $\lambda_{\perp}$ or decreased $\lambda_{||}$ and is, therefore, a sensitive marker of white matter injury without specificity to axonal injury or demyelination.³³

A few studies have utilized the ability of DTI to interrogate white matter pathology of the brain in the setting of GLD. In a small number of untreated Krabbe patients, statistically significant decreases in relative anisotropy were identified throughout various white matter regions of the brain compared to normal children.²¹ In these same patients, the sensitivity of DTI in identifying disease-specific white matter abnormalities was superior to that of conventional MRI sequences.²¹ Therapeutic effect was also suggested by DTI within the brains of several Krabbe patients following stem cell transplantation.^{23,24} In the Twitcher model, a study utilizing diffusion-weighted imaging (DWI) demonstrated statistically significant abnormalities in apparent diffusion coefficient (ADC) parallel to the trigeminal and optic nerves consistent with histopathological evidence of axonal damage in these nerves.¹¹

In the current study, DTI was used to detect statistically significant abnormalities in FA, $\lambda_{||}$, and $\lambda_{\perp}$ in the optic and trigeminal nerves of end-stage Twitcher mice compared to normal, age-matched littermates, consistent with known axonal damage and demyelination in these tracts. Throughout white matter of the Twitcher spinal cord, abnormalities in FA, $\lambda_{||}$, and $\lambda_{\perp}$ reached statistical significance except in the case of $\lambda_{\perp}$ in the ventrolateral white matter. These DTI findings correlated with immunofluorescent evidence of moderate axonal damage and mild, patchy demyelination throughout the Twitcher spinal cord white matter. As the first reported application of DTI within the spinal cord of either the Twitcher mouse

or Krabbe patients, this study establishes a potential noninvasive, quantitative assay for disease monitoring as well as for investigating the efficacy of future therapeutic strategies.

MATERIALS AND METHODS

Animals

Mice heterozygous for the Twitcher mutation (*twi*+) on a congenic C57Bl/6 background were maintained by MSS at Washington University School of Medicine. Strict brother-sister matings of the heterozygous animals were used to obtain mice homozygous for the Twitcher mutation (*twi/twi*) as well as normal control mice (+/+). These genotypes were determined in newborn mice by a polymerase chain reaction method specific for the Twitcher mutation.³⁵ All animal procedures were carried out in accordance with the regulations established by the IACUC at Washington University School of Medicine.

DTI

Five Twitcher mice and five normal control littermates underwent *in vivo* DTI at 38±1 days of age, essentially at the mean lifespan of mutant mice in the colony. Animals underwent sequential spine and brain imaging with isoflurane/oxygen anesthesia (5% induction and 1% maintenance) delivered by a custom nose cone that also allowed respiratory-gated acquisition.³⁶ The mice were placed in a custom holder designed to immobilize the spine and isolate respiratory motion.³⁴ An actively detuned radiofrequency transmitter (6 cm internal-diameter cylinder with a 10 cm length) and receiver coil pair was used. The receiver coil designed to fit around the spine of the mouse was constructed with a 9 mm × 16 mm internal diameter. The receiver coil designed to encompass the mouse brain had a 20-mm diameter. The entire preparation was placed in an Oxford Instruments 200/330 magnet (4.7 T, 40 cm clear bore) equipped with a 10 cm inner-diameter, actively shielded Magnex gradient coil (up to 60 G/cm, 200 μs rise time). Core temperature was maintained at 37 °C with circulating warm water. The magnet, gradient coil, and gradient power supply were interfaced with a Varian NMR systems (Palo Alto, CA, USA) INOVA console controlled by a Sun Blade 1500 workstation (Sun Microsystems, Santa Clara, CA, USA).

Sagittal scout images of the brain and coronal scout images of the spine were acquired. Vertebral segments were identified using the ilium as a reference. Multiple transverse slices through the brain as well as segments T11–T13 of the spine were obtained using a Stejskal–Tanner spin-echo diffusion weighted sequence³⁷ with the following acquisition parameters: TR~1500 ms (determined by the respiratory rate of the mouse), TE= 37 ms, number of excitations= 4, slice thickness= 1.0 mm, field of view= 1 cm × 1 cm for spinal cord and 3 cm × 3 cm for brain, data matrix= 128×128 (zero-filled to 256×256) for spinal cord and 256 × 256 (zero-filled to 512 × 512) for brain. Diffusion-sensitizing gradients were applied in six orientations: (G_x,G_y,G_z)= (1,1,0), (1,0,1), (0,1,1), (−1,1,0), (0,−1,1), and (1,0,−1) with a gradient strength= 11.25 G/cm, duration (δ)= 7 ms, and separation (Δ)= 18ms, to obtain *b* values of 0 and 1,000 s/mm². Acquisition time was approximately one hour for each of the brain and spine scans.

Data analysis

A weighted linear least-squares method was used to independently estimate diffusion tensors for each pixel from the diffusion-weighted images as previously described.³⁸ The eigenvalue decomposition was then applied to the tensor, yielding a set of eigenvalues ($\lambda_1 \geq \lambda_2 \geq \lambda_3$) and eigenvectors for each pixel. Diffusion indices maps including the $\lambda_{||}$, $\lambda_{\perp}$, FA, and mean diffusivity (<D>) were generated by applying the following equations for each pixel:

$$\begin{aligned} \langle D \rangle &= (\lambda_1 + \lambda_2 + \lambda_3) / 3 \\ \lambda_{||} &= \lambda_1 \\ \lambda_{\perp} &= (\lambda_2 + \lambda_3) / 2 \\ FA &= \frac{\sqrt{3} \sqrt{(\lambda_1 - \langle D \rangle)^2 + (\lambda_2 - \langle D \rangle)^2 + (\lambda_3 - \langle D \rangle)^2}}{\sqrt{2} \sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \end{aligned}$$

Statistical analysis

Regions of interest (ROIs) encompassing the dorsal spinal cord white matter, ventrolateral spinal cord white matter, optic nerves, trigeminal nerves, and corpus callosum were drawn manually on the DTI parameter maps using ImageJ v1.37 software.³⁹ The boundary between white matter and cerebrospinal fluid was identified on ADC maps, and the boundary between white matter and gray matter was identified on FA maps. T2-weighted images ($b = 0$) were also employed as anatomical proof. Mean FA, $\lambda_{||}$, and $\lambda_{\perp}$ was calculated for each mouse in each ROI containing 30 voxels for the optic nerve, 200 voxels for the trigeminal nerve, 80 voxels for the corpus callosum, 60 voxels for the dorsal spinal cord white matter, and 600 voxels for the ventrolateral spinal cord white matter. Signal-to-noise ratio (SNR) was calculated as the signal intensity of the ROIs divided by standard deviation of background noise from $b = 0$ images. The SNR was approximately 50 for all ROIs. Statistical analysis of the Twitcher group versus the normal control group was performed with a two sample t-test using Origin 7.5 SR2 v7.5817 (Origin Lab Co., MA, USA).

Histological analysis

Immediately after imaging, mice were perfusion fixed with 4% paraformaldehyde in 1% phosphate-buffered saline. The crania and vertebral columns were excised, fixed overnight, and decalcified for 24 hours. Fixed tissues were embedded in paraffin and sectioned on a microtome at a thickness of 3 μ m. Vertebral segments were used as a reference for spinal cord sectioning. After the sections were deparaffinized and rehydrated, antigen retrieval was performed in 1mM EDTA at 95 °C –1000°C in a water bath. Sections were blocked in 2% blocking buffer (Invitrogen, Carlsbad, CA, USA) for one hour at room temperature and incubated with a 1:1000 dilution of polyclonal anti-myelin basic protein (MBP) antibody (Sigma Chemical Company, St. Louis, MO, USA) or a 1:5000 dilution of monoclonal anti-dephosphorylated neurofilament H (SMI32; Sternberger Monoclonals, Inc., Lutherville, MD, USA) at 4°C overnight. After rinsing, a 1:300 dilution of goat anti-mouse or anti-rabbit IgG conjugated with Cy3 (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) was applied. After washing, sections were covered in Vectashield Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA, USA). Digital images were taken on a fluorescence microscope (Nikon, USA) using identical light intensity and exposure time settings for sections stained with the same primary antibody. The digital images used identical intensity scales.

RESULTS

Brain DTI

In the brains of Twitcher mice, qualitative DTI abnormalities relative to normal controls were identifiable within the optic nerves and trigeminal nerves on parameter maps (Fig. 1). While FA and $\lambda_{||}$ in these cranial nerves appeared decreased in Twitcher mice, $\lambda_{\perp}$ appeared increased compared to normal controls. Other regions of the brain such as the corpus callosum failed to demonstrate a difference in DTI parameters between the groups (Fig. 1c).

Consistent with these qualitative imaging findings, DTI parameters in the optic nerves and trigeminal nerves demonstrated statistically significant ($p < 0.05$) decrease in FA and $\lambda_{||}$ and statistically significant ($p < 0.05$) increase in $\lambda_{\perp}$ in Twitcher mice ($n=5$) compared to age-matched, normal littermates ($n=5$) (Table 1). Other regions of the brain such as the corpus callosum showed no statistically significant difference between groups in FA, $\lambda_{\perp}$, or $\lambda_{||}$.

Spinal Cord DTI

In DTI parameter maps of dorsal white matter and ventrolateral white matter of the spinal cord lumbar enlargement area (T11, T12, T13), qualitative decrease in FA and $\lambda_{||}$ and increase in $\lambda_{\perp}$ were suggested in Twitcher animals relative to normal controls (Fig. 2).

Quantification of these parameters in ROIs that separately encompassed the ventrolateral white matter and dorsal white matter (Fig. 2) was performed in a group of Twitcher mice ($n=5$) and normal littermates ($n=5$) (Table 2). Compared to normal controls, Twitcher mice showed a statistically significant ($p < 0.05$) decrease in FA and $\lambda_{||}$ in both the ventrolateral white matter and dorsal white matter. In the dorsal white matter, Twitcher mice demonstrated a statistically significant increase in $\lambda_{\perp}$ ($p < 0.05$) relative to controls. A trend ($p = 0.052$) for increased $\lambda_{\perp}$ in the ventrolateral white matter of Twitcher mice compared to normal animals was strongly suggested.

Histological analysis

Immunofluorescence for nonphosphorylated neurofilament H (SMI32), a marker for axonal injury, was performed on the ventrolateral and dorsal white matter of the spinal cord segments imaged by DTI (Fig. 3a). In both regions, increased staining was seen in Twitcher mice relative to normal control animals, consistent with moderate axonal damage.

Immunofluorescent staining for MBP in both the ventrolateral and dorsal white matter of the spinal cord showed a generalized mild decrease in intensity with focal areas of further reduced signal in Twitcher mice compared to normal littermates (Fig. 3b). Relatively slight widening of the spaces between axons in the Twitcher mice compared to control animals was observed consistent with perineural edema seen in this disease. Although myelin levels appeared lower in Twitcher mice, the majority of axons continued to display considerable MBP staining. These findings indicate relatively mild and patchy demyelination in the dorsal and ventrolateral white matter of the Twitcher spinal cord.

DISCUSSION

This study contributes to continually mounting evidence that DTI is not only a sensitive test for white matter disease but also can further predict the underlying pattern of histopathology. Numerous previous reports in a wide range of models have correlated decreased $\lambda_{||}$ with axonal damage and increased $\lambda_{\perp}$ with myelin loss.^{26,27,28,29,30,31,32,33,34} In the current study, decrease in $\lambda_{||}$ and increase in $\lambda_{\perp}$ within regions of the spinal cord and brain of Twitcher mice were identified, consistent with evidence of axonal damage and demyelination in these areas.

The DTI data obtained in the brain of Twitcher mice in this report add to findings from an earlier study that used diffusion-weighted imaging to calculate ADC in the optic and trigeminal nerves of this mouse model.¹¹ In this previous study, a statistically significant decrease in ADC parallel to the optic and trigeminal nerves was identified in Twitcher mice relative to normal controls. Likewise, DTI measurements in the current report displayed a statistically significant decrease in $\lambda_{||}$ in Twitcher mice. This abnormality again likely reflects axonal damage manifested by apparent loss of axon straightness in these tracts, as previously demonstrated by electron microscopy and confirmed in our own experience (data

not shown).¹¹ Previously published calculations of ADC perpendicular to the optic and trigeminal nerves suggested increases in Twitcher mice relative to controls without statistical significance despite mild to moderate evidence of demyelination in these nerves by electron microscopy.¹¹ The DTI measurements in the current study were, however, able to demonstrate a statistically significant increase in $\lambda_{\perp}$ in Twitcher mice compared to normal littermates in these nerves. This finding presumably relates to improved sensitivity to demyelination with the current DTI technique.

In the spinal cord, statistically significant decreases in $\lambda_{\parallel}$ were identified in ROIs encompassing essentially the entire ventrolateral white matter as well as the dorsal column white matter of Twitcher mice relative to normal controls. Concordantly, evidence of axonal injury was demonstrated in these regions immunofluorescently by SMI32 staining. Patchy areas of decreased anti-MBP immunofluorescent staining superimposed on a background of mildly reduced signal were also identified throughout the white matter of the Twitcher spinal cord. While a statistically significant increase in $\lambda_{\perp}$ was identified in the dorsal white matter, the increase in $\lambda_{\perp}$ in the ventrolateral white matter did not reach statistical significance ($p=0.052$). These DTI results are likely due to the heterogeneous and overall mild severity of demyelination in the Twitcher spinal cord as evidenced by immunofluorescence in the current study. This patchy distribution of demyelination which spares a significant portion of myelin sheaths in the Twitcher spinal cord has been described in previously published experience with histochemistry,^{10,40} anti-MBP immunostaining,¹⁴ and electron microscopy.⁴⁰

While demyelination is the most often discussed pathological feature of GLD, the above described results call attention to axonal injury as a component of the disease process. In all Twitcher ROIs displaying decreased FA, an indicator of general white matter disease, a statistically significant decrease in $\lambda_{\parallel}$ consistent with axonal damage was also present. It should be noted that several other groups have reported histopathological evidence of axonal injury in the both the brain^{10,11,12} and spinal cord¹⁰ of the Twitcher mouse. Importantly, the presence of axonal injury has also been documented in the brain and spinal cord of human Krabbe patients as well.^{3,9}

To the best of our knowledge, this study comprises the first reported application of spinal cord DTI in the setting of GLD, including either the Twitcher model or human patients. At the time of the current submission, no other studies of spinal imaging of any type in the Twitcher mouse appeared in the published literature. In human Krabbe patients, two descriptions of lumbosacral nerve root enhancement following intravenous gadolinium administration^{41,42} and incidental notes of both cervical spinal cord thinning⁴³ and thickening⁴⁴ on standard MRI were found in case reports. Conventional T1- and T2-weighted sequences otherwise appear to have revealed no spinal cord abnormalities in this disease. Given several reports of the spinal cord as occasionally the earliest site of pathology in Krabbe patients, DTI may have a clinical role in disease diagnosis and monitoring.^{41,45,46}

The statistically significant DTI abnormalities in the optic nerve, trigeminal nerve, and spinal cord of Twitcher mice establish this imaging method as a noninvasive, quantitative assay for the therapeutic efficacy of treatment strategies. Studies involving bone marrow transplantation, substrate reduction, and gene therapy in the Twitcher model have typically relied on qualitative histopathology and functional parameters to evaluate efficacy.^{14,15,16,17,18,19,20} In human patients, a stem cell transplantation study has suggested the utility of DTI in detecting clinical benefit in the brains of Krabbe patients.^{23,24} Given these results, DTI will likely be sensitive in detecting treatment effects in the Twitcher mouse as well. This hypothesis will be especially interesting to test in the spinal cord, particularly

with treatments such as intrathecal delivery of therapeutic agents as has been performed in other mouse models of lysosomal storage disease.^{47,48}

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ABBREVIATIONS

ADC	apparent diffusion coefficient
DTI	diffusion tensor imaging
DWI	diffusion-weighted imaging
FA	fractional anisotropy
GALC	galactocerebroside β -galactosidase
GLD	globoid cell leukodystrophy
MBP	myelin basic protein
ROI	region of interest
SNR	signal-to-noise ratio

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Figure 1.

Representative *in vivo* DTI maps of optic nerves (a), trigeminal nerves (b), and corpus callosum (c) with insets demonstrating manually segmented ROIs. In the optic (a) and trigeminal nerves (b), qualitative increase in $\lambda_{\perp}$ and decrease in FA and $\lambda_{||}$ is suggested in the Twitcher animal compared to the normal, age-matched littermate. No discernable difference in these parameters is detectable between the Twitcher and normal mouse within the corpus callosum (c).

Figure 2.

Representative *in vivo* DTI maps of the spinal cord at the T11, T12, and T13 levels with manually segmented ROIs for dorsal and ventrolateral white matter displayed. Qualitative increase in $\lambda_{\perp}$ and decrease in FA and $\lambda_{||}$ is suggested in the dorsal and ventrolateral white matter of the Twitcher animal compared to the normal, age-matched littermate.

Figure 3.

Immunofluorescent detection of dephosphorylated neurofilament H (SMI32, a) and myelin basic protein (MBP, b) in the dorsal white matter (DWM) and ventrolateral white matter (VWM) of the spinal cord. Extensive increase in SMI32 positive axons (green color, a) consistent with axonal damage is present in both the DWM and VWM of the Twitcher cord compared to background staining in the normal control cord. Although a mild generalized decrease in MBP staining is seen in the Twitcher cord relative to the control cord (red color, b), the majority of Twitcher axons continue to display myelin sheaths. Focal areas of further decreased MBP staining are identified in the DWM and VWM of the Twitcher mouse but not the normal control indicating mild, patchy demyelination (b, insets).

Table 1

DTI parameters from the optic nerve, trigeminal nerve, and corpus callosum ROIs displayed in Figure 1. Means $\pm$ SD are compared between a group of Twitcher mice (n=5) and age-matched, normal littermates (n=5). Statistically significant increase in $\lambda_{\perp}$ and decrease in FA and $\lambda_{\parallel}$ is detected in Twitcher mice compared to controls in the optic nerve and trigeminal nerve, but not in the corpus callosum.

Optic Nerve (n = 5, Mean $\pm$ SD)			
	Normal	Twitcher	P
FA	0.88 $\pm$ 0.08	0.65 $\pm$ 0.09	0.011
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.29 $\pm$ 0.06	0.41 $\pm$ 0.06	0.042
$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	1.89 $\pm$ 0.13	1.15 $\pm$ 0.09	0.0086
Trigeminal Nerve (n = 5, Mean $\pm$ SD)			
	Normal	Twitcher	P
FA	0.82 $\pm$ 0.05	0.46 $\pm$ 0.05	1.4 E-05
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.34 $\pm$ 0.06	0.57 $\pm$ 0.06	3.9 E-04
$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	2.07 $\pm$ 0.06	1.42 $\pm$ 0.07	3.5 E-07
Corpus Callosum (n = 5, Mean $\pm$ SD)			
	Normal	Twitcher	P
FA	0.56 $\pm$ 0.10	0.55 $\pm$ 0.05	0.91
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.46 $\pm$ 0.07	0.44 $\pm$ 0.03	0.70
$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	1.41 $\pm$ 0.16	1.36 $\pm$ 0.09	0.55

Table 2

DTI parameters from the dorsal and ventrolateral white matter ROIs of the spinal cord displayed in Figure 2. Combined means $\pm$ SD from the T11, T12, and T13 levels are compared between a group of Twitcher mice (n=5) and age-matched, normal littermates (n=5). In the dorsal and ventrolateral white matter, statistically significant decrease in FA and $\lambda_{||}$ is detected in Twitcher mice compared to controls. Increase in $\lambda_{\perp}$ reaches statistical significance in the dorsal white matter of Twitcher mice relative to normal littermates but not in the ventrolateral white matter (p=0.052).

Dorsal White Matter (n = 5, Mean $\pm$ SD)			
	Normal	Twitcher	P
FA	0.88 $\pm$ 0.05	0.75 $\pm$ 0.07	0.011
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.24 $\pm$ 0.02	0.29 $\pm$ 0.05	0.041
$\lambda_{ }$ ($\mu\text{m}^2/\text{ms}$)	1.65 $\pm$ 0.13	1.38 $\pm$ 0.05	0.0097
Ventrolateral White Matter (n = 5, Mean $\pm$ SD)			
	Normal	Twitcher	P
FA	0.85 $\pm$ 0.05	0.73 $\pm$ 0.04	0.022
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.28 $\pm$ 0.03	0.32 $\pm$ 0.03	0.052
$\lambda_{ }$ ($\mu\text{m}^2/\text{ms}$)	1.69 $\pm$ 0.12	1.45 $\pm$ 0.02	0.0092