Longitudinal Changes in White Matter Fractional Anisotropy in Adult-Onset Niemann-Pick Disease Type C Patients Treated with Miglustat

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Abstract Niemann-Pick disease type C (NPC) is a rare neurometabolic disorder resulting in impaired intracellular lipid trafficking. The only disease-modifying treatment currently available is miglustat, an iminosugar that inhibits the accumulation of lipid metabolites in neurons and other cells. This longitudinal diffusion tensor imaging (DTI) study examined how the rate of white matter change differed between treated and non-treated adult-onset NPC patient groups. Nine adult-onset NPC patients (seven undergoing treatment with miglustat, two not treated) underwent DTI neuroimaging. Rates of change in white matter structure as indexed by Tract-Based Spatial Statistics (TBSS) of fractional anisotropy were compared between treated and untreated patients. Treated patients were found to have a significantly slower rate of white matter change in

the corticospinal tracts, the thalamic radiation and the inferior longitudinal fasciculus. This is further evidence that miglustat treatment may have a protective effect on white matter structure in the adult-onset form of the disease.

Introduction

Niemann-Pick disease type C (NPC) is a rare, autosomal recessive, neurovisceral disorder involving alterations in intracellular lipid trafficking. Mutations in the NPC1 or the NPC2 genes result in impaired sterol cycling and accumulation of glycosphingolipids and unesterified cholesterol in neural and other tissues. The adult-onset form often presents with psychiatric symptoms, including psychosis, depression and cognitive difficulties (Walterfang et al. 2006). Progressive neurological symptoms include dysphagia, dysarthria and ataxia, and loss of first vertical, then horizontal, saccades (Sevin et al. 2007).

The accumulation of lipids in neurons results in changes to dendritic and axonal morphology (Zervas et al. 2001). Axonal transport mechanisms are also altered, and hyperphosphorylated tau accumulates within neurons (Suzuki et al. 1995). Cerebellar purkinje cells are particularly vulnerable to the damaging effects of accumulated GM2 and GM3 gangliosides. Murine models have found that some cerebellar purkinje populations last longer than others, with those in the cerebellar vermis generally the last to be affected (Taniguchi et al. 2001). Cross-sectional MRI investigations in humans with adult-onset NPC have found significant reductions in thalamic and hippocampal volumes, and both cerebellar grey and white matter volumes compared to age- and gender-matched controls (Walterfang et al. 2010, 2013).

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Currently, the only disease-specific treatment for NPC is the iminosugar, miglustat, an inhibitor of glucosylceramide synthase. This reduces glycolipid storage in neurons and has been shown in a controlled clinical trial of juvenile, adolescent and adult-onset NPC to slow disease course (Patterson et al. 2007). Feline models of NPC have found increased purkinje cell survival in the cerebella of treated cats (Stein et al. 2012; Zervas et al. 2001).

Although there have been a number of human neuroimaging studies of NPC, there have been few longitudinal studies. To date, three longitudinal studies have been able to use brain imaging methods, including volumetric methods, diffusion tensor imaging and spectroscopy, to examine treated and untreated patient groups (Bowman et al. 2015; Masingue et al. 2017; Sedel et al. 2016); these have shown a restoration of abnormal markers of neuronal loss and axonal integrity, and a slowing of cerebellar volumetric changes, respectively. A diffusion tensor-based analysis of one patient treated with miglustat suggested a reduction in white matter abnormalities after 1 year of treatment (Scheel et al. 2010), and a French study that examined 11 patients over time showed an improvement in FA in the callosum and corona radiata (Masingue et al. 2017), although no control sample of untreated NPC patients was used.

In the current study, a diffusion-weighted imaging analysis was undertaken using FSL's Tract-Based Spatial Statistics (TBSS) package to explore possible differences in

the rate of change in fractional anisotropy (FA) between treated and untreated NPC patients.

Methods

Nine adult-onset NPC patients (five male, four female) were recruited and scanned at the Royal Melbourne Hospital, Melbourne, Australia between 2000 and 2012. The clinical and imaging data are demonstrated in Table 1. The mean age of the patients at baseline was 33.2 years (SD 9.8 years), with mean age of onset of neurological symptoms 27.9 years (SD 10.7 years). NPC diagnosis was confirmed with biochemical analysis of fibroblasts cultured from skin biopsies, including cholesterol esterification rate and staining of perinuclear cholesterol. Further details of patient characteristics can be found in Bowman et al. (2015).

Seven NPC patients received miglustat treatment from baseline. Pre-treatment MRI data points were available for one patient (Patient 3), and one patient refused miglustat treatment and thus served as a natural control (Patient 7). Oral miglustat treatment consisted of 200 mg tds, with the exception of one patient who received 100 mg tds (Patient 9). Three patients (Patients 3, 5 and 6) received oral atypical antipsychotics from baseline and throughout the follow-up period. Patient 3 received 10–30 mg/day and Patient 6 received 30–50 mg/day olanzapine. Patient 5

Table 1 Genetic, clinical and imaging details of treated and untreated patients

Treated patients	Diagnosis	Age at baseline/age at final follow-up (years)	Follow-up period (months)	Scan intervals (months from baseline)	Gender	Age of neurological onset
1	Filipin: variant	49.3/52.2	34	0, 7, 13, 26, 34	M	47.0
2	Genetic: S940L S954L	34.7/36.9	21	0, 6, 13, 21	F	29.0
3	Filipin: variant	34.5/38.3	45	0, 6, 14, 22, 30, 37, 45	F	26.0
4	Genetic: G992R R1186H	45.4/47.8	29	0, 9, 15, 29	F	41.0
5	Genetic: G992R c.3591+4delA	21.1/25.4	52	0, 7, 12, 19, 25, 32, 40, 52	M	19.0
6	Genetic: R518W N1156S	32.8/34.0	14	22, 28, 36	M	25.0
8	Genetic: I1061T I1061T	18.6/23.5	58	0, 10, 15, 18, 22, 26, 31, 38, 46, 58	M	14.0
9	Genetic: R518W N1156S	35.2/36.1	11	0, 11	F	31.0
Untreated patient						
Pre-Tx 6	Genetic: R518W N1156S	31.0/31.5	6	0, 6	M	25.0
7	Genetic: G992R R1186H	31.2/33.1	23	0, 23	M	19.0

received 100 mg/day quetiapine. Additionally, Patient 7 was receiving 500 mg/day oral sodium valproate.

Diffusion tensor imaging (DTI) was undertaken via a 25-direction (one b0 image) echoplanar imaging sequence with 20 axial 5-mm slices, a b-value of 1,000, TE/TR 90/6,000 ms a flip angle of 90°, a matrix size of 256 × 256 and voxel dimensions of 0.938 × 0.938 mm.

Participants' fractional anisotropy (FA) maps were aligned into a common space using FSL's FNIRT registration package (Smith et al. 2004). A mean FA image was then created and thinned, to isolate a mean "skeleton" of white matter tracts common to all participants. All skeletonised FA (sFA) images were then aligned to the mean skeleton, creating a skeleton for each participant at each time point. Rate of change of FA over time in each patient was calculated as the beta coefficient of the linear regression of the skeletonised FA data across time. Linear regression across the four-dimensional skeletonised data was performed using the polyfit function in MATLAB. This produced a skeleton for each patient containing the rate of change of white matter FA over time in each voxel. Between-group analysis was performed on these skeletons using Randomise in FSL. Randomise uses permutation-based non-parametric inference using a general linear model design (Winkler et al. 2014). Images were prepared using FSL's Threshold-Free Cluster Enhancement (TFCE) at $p = 0.95$.

Results

A number of regions were identified where in untreated NPC patients' fractional anisotropy was changing significantly faster than in treated patients. These include the corticospinal tracts, the thalamic radiation and the inferior longitudinal fasciculus (Fig. 1).

Discussion

Previous cross-sectional studies have found significant differences in fractional anisotropy between adult-onset NPC patients and age-matched controls (Walterfang et al. 2010; Masingue et al. 2017). Bilateral widespread reductions were found in most major white-matter tracts in the patient groups, including in both projection and association fibres. The thickness and shape of the corpus callosum are also found to be significantly reduced in NPC patients compared to control groups, and the degree of these changes correlated with illness measures in both adult (Walterfang et al. 2011) and paediatric (Lee et al. 2014) patients. One of the few longitudinal studies of NPC disease progression in treated and untreated patients found

that cerebellar white matter was lost at a significantly faster rate in untreated patients than in patients treated with miglustat (Bowman et al. 2015). Similarly, a magnetic resonance spectroscopy (MRS) study in treated and untreated patients found a normalisation of the Cho/NAA ratio in the white matter of the centrum ovale with miglustat treatment (Sedel et al. 2016).

Axonal structure is known to be altered in NPC and can include axonal swelling, spheroid formation and abnormal axonal branching and growth of collaterals (March et al. 1997; Sarna et al. 2003; Zervas et al. 2001). Such disruptions to healthy axonal growth may reflect the abnormal transport of cholesterol and other lipid products to distal axons. Studies have also found a reduction in myelin-specific proteins that may suggest a disruption to myelin-producing biochemical pathways in oligodendrocytes (German et al. 2002; Goodrum and Pentchev 1997).

Miglustat decreases abnormal and destructive glycolipid storage in intracellular compartments through the inhibition of glycosylceramide synthase, and thus the reduction in ultrastructural changes to axons and impairments in myelination may be how treatment with miglustat impacts upon white matter changes in the NPC patient group.

The results of this investigation, the only study to statistically compare both treated and untreated patients over time, reveal that white matter change over time as measured by fractional anisotropy is significantly slower in NPC patients treated with miglustat compared to untreated patients, and is concordant with previous findings suggesting that it slows cerebellar volume loss. Whilst our study is limited by its small sample size, the statistical significance of our results in even this modest sample suggest that this is a true biological effect of treatment, and larger samples may yet demonstrate a more widespread and obvious protective effect on white matter structures from miglustat treatment.

Synopsis

Treatment with miglustat may affect the rate of alteration of white matter structure in progression of Niemann-Pick disease type C.

Author Contributions

Mark Walterfang designed, conceived and implemented the study, including patient recruitment, assessment and data collection. Elizabeth Bowman analysed neuroimaging data. Patricia Desmond assisted with study design and data collection. Dennis Velakoulis assisted with study design and implementation. All authors contributed to preparation and finalisation of the submitted manuscript.

Fig. 1 Areas of difference in treated vs untreated patients. Areas of significantly decreased rate of change in FA in treated compared to untreated patients. The *left column* shows sagittal, *middle column* coronal and *right column* axial images. The *top row* demonstrates a significant difference in the left corticospinal tract, the *middle row*

showing changes in the left corticospinal tract and the right inferior longitudinal fasciculus and the *bottom row* demonstrating significantly different change over time in the right thalamic radiation and the right inferior longitudinal fasciculus

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Mark Walterfang accepts full responsibility for this work, the conduct of the study, had access to the data and controlled the decision to publish.

Competing Interests

Mark Walterfang has served on a scientific advisory board and as a consultant for and received funding for travel from Actelion Pharmaceuticals Ltd, the manufacturer of miglustat. Elizabeth Bowman has received salary support through an unrestricted educational grant to the University of Melbourne from Actelion Pharmaceuticals Ltd. Patricia Desmond and Dennis Velakoulis declare no competing interests.

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Ethics Approval and Patient Consent

This study was approved by the Melbourne Health Research and Ethics Committee. All subjects provided informed consent.

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