Longitudinal TBSS reveals progressing demyelination in the mouse model of progressive neurodegenerative disease EPM1

O. H. Manninen[1], T. Laitinen[2], O. Kopra[1], O. Gröhn[2], and A-E. Lehesjoki[1]
[1]Folkhälsan institute of Genetics and Neuroscience Center, University of Helsinki, Helsinki, Finland, [2]Department of Neurobiology, University of Eastern Finland, Kuopio, Finland

Introduction - Unverricht-Lundborg disease is an autosomal recessive neurodegenerative disorder (EPM1, OMIM 254800), with onset in humans at the age of 6 to 18 years. It is characterized by severe incapacitating stimulus-sensitive myoclonus, tonic-clonic epileptic seizures and ataxia. Mutations in the gene encoding Cystatin B (CSTB) underlie EPM1, but many of the neurological processes leading to observed symptoms remain unknown. In order to gain a better view and understanding of the neurological changes underlying the array of symptoms in the EPM1, we used a murine model of the disease, the *Cstb* gene -targeted mouse (the *Cstb-/-* mouse model) to study the disease [1]. EPM1 patients have been shown to undergo gray matter changes [2], and track based spatial statistics *[3]* (TBSS) analysis of diffusion tensor imaging (DTI) data from both adult EPM1 patients [4], and adult (6 months) *Cstb-/-* mice [5], have shown decreased fractional anisotropy (FA) in several brain regions especially in cerebellum and thalamus, with mouse histology revealing reduced myelin in these areas. In this study we wanted to determine how the white matter (WM)-changes progress with the disease, and whether the the lower myelin presence at 6 months was caused by demyelination or dysmyelination. To accomplish this we performed a TBSS analysis of FA comparing the *Cstb-/-* wild type controls at timepoints of 2, 4 and 6 months of age. TBSS has recently been implemented to longitudinal follow-up in patients with neurodegenerative changes [6], but to our knowledge the approach has not been implemented to an animal model.

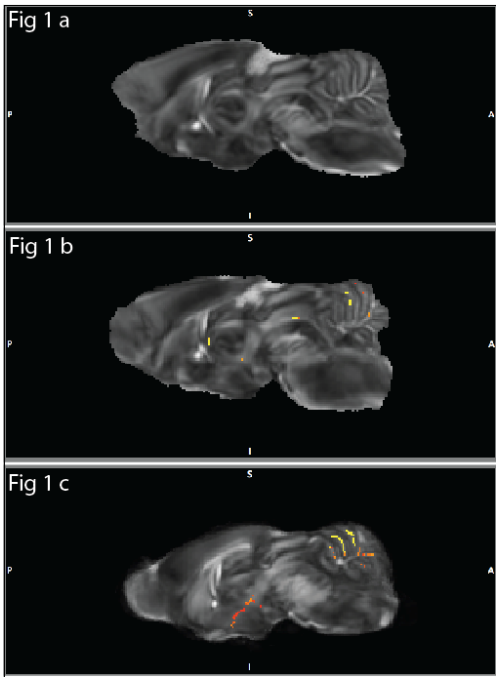

Figure 1. Development of WM-lesions in Cstb-/- mice: *FA decrease in Cstb-/- mice as compared to Cstb+/+ controls analyzed with TBSS. Areas of significantly lower FA (p<0.05) shown in red and yellow, projected on the study specific mean FA images. a) Age 2 months, b) Age 4 months, c) Age 6 months*

Materials and Methods - For the *ex vivo* DTI we used 2 months old (9 Cstb -/-, 7 Cstb +/+), 4 months old (9 Cstb -/-, 6 Cstb +/+) and 6 months old animals (9 Cstb -/-, 4 Cstb +/+). Mice were perfused intracardially with 4 % PFA and prior to imaging the fixed brains were immersed in Fomblin. DTI was performed in a 9.4 T vertical magnet interfaced to a Varian DirectDrive console. Data acquisition was done using a 3D fast spin echo sequence (TR = 1000 ms; TE = 28 ms; echo train length = 2, data matrix 128x64x64, zero padded to 256x128x128; FOV 20x10x10 mm3). Six 3D data sets with diffusion weighting (diffusion time 17 ms, *b*-value 1000 s/mm2) in six non-collinear directions and one data set without diffusion weighting were acquired. Measurement time was 16 h.

DTI data were corrected for eddy current distortions with affine (linear) alignment with *flirt* [6], which is included in the FSL software package [7] that provides all the tools used in the analyzing of the data. Diffusion tensor was calculated with the *dtifit* program for whole brain volumes of each data set. We applied free-search of the best registration target for the each compared data set to minimize the image warping required for other volumes. Calculated best target was used in subsequent TBSS-scripts as a template into which final transformations were targeted to. Following registration, mean FA-image was created and thinned to represent mean FA-skeleton, creating a common skeleton onto which individual animals´ FAs were projected. Finally, skeletonised individual FA-maps were fed into voxelwise cross-subject statistical analyses. Null distribution was built over 5000 permutations and results are given as $t > 2.7$, $p < 0.05$, multiple comparison corrected, cluster-level inference.

Results and discussion - The TBSS comparison of FA between *Cstb-/-* and *Cstb+/+* mice revealed a decrease in FA-values in the cerebellum, thalamus, cortices and corpus callosum, but no significant (Threshold-Free Cluster Enhancement $p<0.05$ with multiple comparison correction) changes in FA-values were detected at 2 months of age (**FIG 1 a**). At 4 months, TBSS detected significant FA-change at cortices, external capsule, thalamus and cerebellum (**Fig 1 b**). As previously reported, *[5]* TBSS detected FA difference in 6 months old animals, with areas of significant changes in the cerebellum and thalamus growing in size (**Fig 1 c**). The results illustrate progressing WM-degeneration due to lack of CSTB, showing that TBSS of FA can detect temporal development of WM-lesions in animals with progressing neurodegenerative diseases.

The data also indicate that until 2 months of age, the WM is relatively normal in *Cstb-/-* mice, showing no lesions detectable with FA TBSS. At 4 months cerebellum and thalamus had discernable WM-lesions and the degeneration progresses to 6 months of age, indicating that WM-lesions in *Cstb-/-* mice are a result of demyelination, and not dysmyelination, corresponding with the neurodegenerative disease course in EPM1. WM-lesions also appear to be secondary in the disease as the changes at 4 months are preceded by myoclonus in *Cstb-/-* mice at 1 month of age [1] . As TBSS of DTI data in adult EPM1 patients [3] has also shown FA changes at cerebellum and thalamus, our data suggest that the WM-changes seen in EPM1 patients could also result from demyelination.

References: *[1] Pennachio L.A. et al., Nat Genet 20 (1998) 3:251-258, [2] Koskenkorva P. et al, Neurology. 73 (2009) 8:606-611, [3] Smith S.M. et al., NeuroImage 31 (2006) 1487-1505, Smith S.M. et al., Nature Protocols (2007) 2 499-503, [4] Koskenkorva P. et al., RSNA 2008 abstracts, # SSE17-01, [5] Manninen O.H.H et al., ISMRM 2009 # 545, [6] Raz E. et al. Radiology 257 (2010) 2:448-454, [6] Jenkinson M. et al., NeuroImage (2002) 17 825-841, [7] http://www.fmrib.ox.ac.uk/fsl/*