Tract based spatial statistics in mouse model of neurodegerative Unverricht-Lundborg (EPM1) disease

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Introduction - Unverricht-Lundborg disease is an autosomal recessive neurodegenerative disorder (EPM1, OMIM 254800), characterized by onset in humans at the age of 6 to 18 years, 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 resulting in observed symptoms remain unknown. Diffusion tensor imaging (DTI) provides great differentiation of anatomical structures and can detect pathological changes in many disease models. Unfortunately, neurodegenerative diseases often exhibit widespread changes that affect different brain areas but the changes are not easily observable with conventional MRI with interest (ROI) based quantification of MRI parameters.

In order to gain a better view and understanding of neurological changes underlying the array of symptoms in the EPM1, we subjected the murine model of the disease, the *Cstb* gene -targeted mouse (the *Cstb-/-* mouse model) to MR DTI [1]. Instead of the quantification of changes in DTI parameters with conventional hand drawn ROIs, we implemented a recently introduced whole brain voxelwise statistical analysis method, tract based spatial statistics (TBSS) [2,3]. The TBSS of DTI parameters between controls and affected animals provides a black box statistical comparison, subjecting data to a group level whole brain statistical voxel-by-voxel comparison. Method has previously been successful in human applications [4,5], but as of today, there are no published reports in mice.

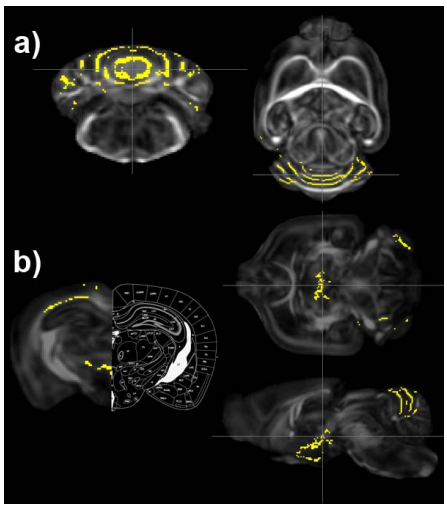

Figure 1. *FA decrease in Cstb-/- mice as compared to controls as analyzed with TBSS. a) reduced FA in corpus callosum, external capsule and several different thalamic and hypothalamic nuclei. b) reduced FA in the cerebellum.*

Materials and Methods - For the ex vivo DTI imaging we used 6 months old *Cstb-/-* (n=9) and control (n=4) mice. Mice from late stages of the disease were selected in order to provide robust changes and the widest possible view into the affected areas with the TBSS analysis.

Mice were intracardially perfused with 4 % PFA and the fixed brains were immersed in Fomblin. DTI was done in a 9.4 T vertical magnet interfaced with 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 orthogonal directions and one reference data set without diffusion weighting were obtained. Measurement time was 16 h.

Diffusion weighted 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 following analyses. 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 whole 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 (background image in all panels in **Fig. 1**) and thinned to represent mean FA-skeleton, creating a common skeleton onto which individual animals' FAs were projected and 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 control mice revealed reduced FA at several brain areas in the *Cstb-/-*mice. FA was significantly lower at cerebellum (**FIG 1 a**) and a significant reduction was also observed at corpus callosum, external capsule and several different thalamic and hypothalamic nuclei (**Fig 1 b**). The cerebellar granule cells have been histologically confirmed to undergo an apoptotic cell death in the *Cstb-/-* mice [8], and the MRI of EPM1 disease patients has also revealed morphological changes at the cerebellum, especially at the cerebellar vermis [9]. This indicates that the observed changes in FA at cerebellum are characteristic to the disease and reflect ongoing pathological processes, as cell death directly affects the free moment of water at cell level. Medial and reticular nuclei of the thalamus have previously been histologically shown to be affected in the *Cstb-/-* mice [8]. The neurodegeneration in thalamus is hypothetized being a downstream result of cerebellar granular cell loss potentially leading to excessive excitatory inputs to the thalamic nuclei.

The findings clearly indicate that many of the neurodegenerative changes present in the *Cstb-/-* mouse are detectable with DTI utilizing FA TBSS, and the setup can reveal both brain regions that are known to be affected in EPM1 as well as anatomical regions that have not previously been associated with the disease. Furthermore, as the TBSS is originally implemented into the patient DTI data, histology confirmed findings in mouse model can also in future be linked with patient data, and thus provide valuable clinical insight into the disease.

References:*[1] Pennachio L.A. et al., Nat Genet 20 (1998) 3:251-258, [2] Smith S.M. et al., NeuroImage 31 (2006) 1487-1505, [3] Smith S.M. et al., Nature Protocols (2007) 2 499-503, [4] Cader S. et al., NeuroImage (2007) 36 19-27, [5] Douaud G. et al., Brain (2007) 130 2375-2386, [6] Jenkinson M. et al., NeuroImage (2002) 17 825-841, [7] http://www.fmrib.ox.ac.uk/fsl/, [8] Shannon P et al. J Neuropathol Exp Neurol (2002) 61 1085-1091, [9] Mascalchi M. et al., Neurology (2002) 58 1686-1689*