Statistical shape analysis on 3D MRI of the ventricular system of the Cyln2/Rsn double knock-out mice

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Introduction

Many neurological disorders are characterized by structural brain changes that are valuable imaging markers to quantify the state of a disease in e.g. neurodegenerative disorders such as Alzheimer disease and Huntington disease. In segmentation analyses, the volumes of different brain structures are then compared between normal and diseased subjects. However, complementary to these volume analyses one could analyse the change in the shape of a brain structure and attribute these changes to the underlying cellular, molecular and even genetic causes. We are interested in a rare human neurodevelopmental disorder, Williams-Beuren Syndrome (WS, or WBS), which is caused by the hemizygous deletion of more than 20 genes on chromosome 7q11. In search of genotype-phenotype correlations, different mouse models were previously developed (for review, see Hoogenraad et al BioEssays 2004), in which single genes were knocked out and the phenotype of the mice was compared to symptoms in WS patients. In the case of the Cyln2 gene knock out, growth deficiencies, learning disabilities, reduced corpus callosum and enlarged ventricle volumes were found, that occur also in WS patients. The Cyln2 gene encodes the microtubule binding protein CLIP-115, which is highly related to another important regulator of the cytoskeleton, CLIP-170 (encoded by the Restin (Rsn) gene). In this study we measured not only the ventricle volumes of Cyln2/Rsn double knock-out (DKO) mice, but we also aimed at quantifying the change in the shape of the ventricles as compared to normal controls.

Material and methods

MRI: Five double *CYLN2* KO and five wild type mice were anaesthetised with 5% isoflurane induction and 1 to 1.5 % isoflurane maintenance (in a mixture of 30% O_2 and 70% NO_2) and their temperature (37.0 ± 0.5) °C and breathing rate (100BPM) was kept constant during the experiments. MRI imaging was performed at a 300 MHz Bruker system with horizontal bore magnet (Bruker, Ettlingen, Germany). A 20mm diameter surface RF coil was used for both transmitting and receiving. Scouting gradient echo images in the 3 orthogonal directions were acquired to guide the positioning of the 3D slab of the stereotactically positioned mouse head. High resolution coronal slices of the mouse brain were obtained using a 3D Fast Spin Echo sequence with an echo train length of eight, reducing the imaging time to 43 minutes. MR signals of a 3D volume of (20 x 20 x 20) mm^3 were acquired within a (256 x 128 x 64) matrix, TR = 2500 ms and first TE = 14 ms. MRI data was reconstructed to an image matrix of (256 x 256 x 256), containing 256 coronal slices of 78 μm thickness with an in plane resolution of (78 x 78) μm^2.

Data-analyses: The ventricles of the ten subjects were manually segmented from MRI 3D data sets and volumes were measured using AMIRA. Subsequently, for each ventricle, a surface-based representation was obtained by triangulating the boundary of the segmentation. Position and orientation normalization were performed through iterative closest point surface alignment of each ventricle with a chosen template ventricle; this in order to analyze only shape and size differences. A point wise correspondence was established between the surfaces by warping the template surface to each of the other surfaces. This warp was obtained by transforming each vertex of the template surface to the closest point on the target surface. In this way, a group of 10 points was obtained for each vertex of the template surface, containing the position of the vertex together with the positions of the corresponding points on each of the other 9 surfaces. A statistical test was conducted at each vertex, comparing measures derived from these 10 positions. Of specific interest was the within group variation of the Euclidian distance of the points to the group mean, compared between the control group (5 measurements) and the KO group (5 measurements). A parametric F-test has been used at each vertex since it is assumed that the underlying distribution is approximately Gaussian. This results in a p-value for each vertex of the template; these are further corrected for multiple comparisons by allowing a false discovery rate of p=0.05. Then, the corrected p-values are visualized on the mean ventricle surface through color mapping, hereby allowing a localization of the between-group differences in variation.

Results

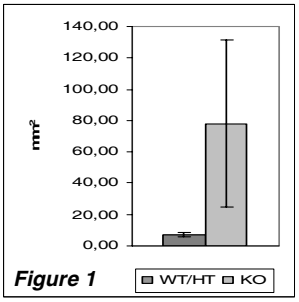
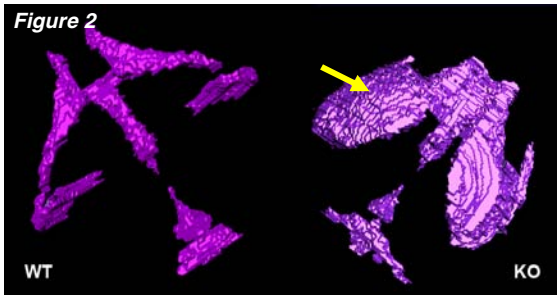


Figure 1



Segmentation of the ventricle volumes in control and DKO clearly showed significant (p=0.01) increased volume in the DKO mice as compared to control animals (*figure 1*).

Visual inspection of the ventricle volumes (*figure 2)* indicated clear changes in the lateral ventricle (arrow), though this was not as clear for aquaduct and fourth ventricle.

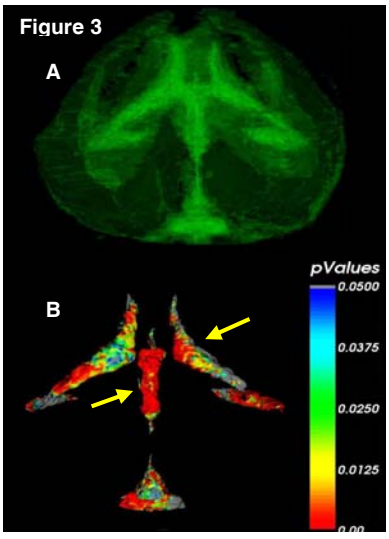


Figure 3B, shows the result of the alignment of the ventricle surfaces to the template surface (the 10 surfaces are visualized transparently on top of each other). After warping, the within group position variance was assessed (Figure 3A), showing that the variances were significantly different between the control and DKO animals, specially in the aquaduct and lateral ventricle (yellow arrows) where the highest significance values were observed.

Discussion

We segmented the ventricular system of this Cyln2/Rsn double knock-out mouse, showing enlarged volumes of the entire ventricular system.

After warping, statistical analysis were performed on shape showing that although the shape of the lateral - fourth ventricle and aquaduct in double DKO is significantly different from controls, the highest significance levels are observed in the aquaduct.

This change in ventricle size and shape might be explained by the fact that the genes being knock-ed out encode cytoplasmic linker proteins (CLIP115 and CLIP-170), which might have an important function in the cilia in the ventricles. Dysfunctioning of the cilia might block the normal flow of the cerebral spinal fluid (CSF) enlarging specific parts of the ventricles. Since the ventricle enlargement is more obvious than in the Cyln2 knock out mice, we speculate that CLIP-170 might compensate for loss of CLIP-115 in WS patients.