Progress Report: Neural Correlates of Executive Functioning in Cystinosis: a Structural MRI Study

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a. Hypotheses and Specific Aims

Our hypotheses and specific aims of this project are:

1.) Patients with cystinosis have a reduced volume of the frontal lobe with respect to impairments in executive functioning (EF).
Aim: To assess the frontal lobe volume including its subdivisions with respect to EF in cystinosis patients and matched controls using ROI analyses.
2.) Patients with cystinosis show volume decreases in brain areas connected to the frontal lobe in correlation with EF impairments.
Aim: To identify the focal gray and white matter decreases adjacent to the frontal lobe in patients with cystinosis with respect to EF using voxel-based morphometry.
3.) Patients with cystinosis display decreased fiber integrity within the subdivisions of the frontal lobe with respect to EF impairments
Aim: To assess fractional anisotropy indices as measures of fiber integrity in the subdivisions of the frontal lobe in cystinosis patients and controls in correlation to EF scores.

b. Progress and Results

Ad 1.) – 3.):

Magnetic Resonance Imaging (MRI) database:
The MRIs of all patients and controls were thoroughly inspected for image quality and structural abnormality. Some scans showed structural changes that can be interpreted as normal variants (e.g., Cavum septum pellucidum et vergae) whereas others had structural abnormalities (e.g., Chiari 1 Malformation) or motion artifacts. As of now, these subjects were not included in the volumetric analyses, which resulted in a total number of 20 cystinosis patients and 20 control subjects. However, some of these scans (i.e., the ones with normal variants) might still be usable for specific MRI analyses, for instance region-of-interest analyses.

Neurobehavioral analyses:
Demographic statistics showed no differences in the 20 cystinosis patients versus 20 controls with respect to age, gender, handedness and socioeconomic status (SES). All cystinosis patients had significantly reduced test scores as compared to controls in the 5 indices of the Delis-Kaplan Executive Function System (D-KEFS), a standardized set of tests used for the

assessment of EF. Overall, cystinosis patients scored in the low average range with strongest impairment in the Trail Making Condition of the D-KEFS ($p < 0.0001$).

MRI analyses:
Data Pre-Processing:
All T1-weighted images were reoriented so that the anterior and posterior commissures were located on a horizontal line using the 6 parameter (rigid body) transformation in Statistical Parametric Mapping (SPM). In a second step, the T1-weighted images were spatially normalized into a standard space as defined by the MNI template. This was achieved using 12-parameter affine transformations and nonlinear deformations (i.e. linear combination of 3-dimensional discrete cosine transform basis functions). Spatially normalized images were “modulated” in order to preserve the total amount of signal in the images. Therefore, areas that were expanded during warping were correspondingly reduced in intensity. In a third step, the spatially normalized T1-weighted images were segmented into gray matter, white matter and cerebrospinal fluid in SPM using the optimized segmentation algorithm. Finally, images were smoothed with a Gaussian kernel of 4 mm width to increase signal-to-noise ratio and to reduce residual differences in gyral anatomy between subjects.

Statistical Analyses and Preliminary Results:
Ad 1.) -2.)
Smoothed gray and white matter segments of cystinosis patients were compared to the matched control subjects. Cystinosis patients showed reduced gray matter in the right middle/superior frontal lobe as compared to controls ($p < 0.05$, Family-Wise Error (FWE) corrected for multiple comparisons, T-value = 6). This area of decreased frontal lobe gray matter correlated with an impairment in the *Number-Letter Switching* Condition of the Trail Making test ($p < 0.01$, $r = 0.4$) and the *Category Switching* Condition of the Verbal Fluency test ($p < 0.02$, $r = 0.4$) of the D-KEFS. The volume of other frontal lobe subdivisions did not differ significantly in cystinosis patients as compared to controls. Please see Table 1 for a list of investigated frontal lobe subdivisions.

Conclusions:
So far, our results provide strong evidence for reduced gray matter volume in the superior and middle frontal lobe in cystinosis patients compared to controls. These volume decreases correlate with reduced performance in the Trail Making test and the Verbal Fluency test from the D-KEFS, pointing to the importance of these neural structures for intact executive functioning.

Table 1. List of investigated subdivisions of the frontal lobe

1 Precentral Gyrus L
2 Precentral Gyrus R
3 Superior Frontal Gyrus L
4 Superior Frontal Gyrus R
5 Superior Frontal Gyrus, Pars orbitalis L
6 Superior Frontal Gyrus, Pars orbitalis R
7 Middle Frontal Gyrus L
8 Middle Frontal Gyrus R
9 Middle Frontal Gyrus, Pars orbitalis L
10 Middle Frontal Gyrus, Pars orbitalis R
11 Inferior Frontal Gyrus, Pars opercularis L
12 Inferior Frontal Gyrus, Pars opercularis R
13 Inferior Frontal Gyrus, Pars triangularis L
14 Inferior Frontal Gyrus, Pars triangularis R
15 Inferior Frontal Gyrus, Pars orbitalis L
16 Inferior Frontal Gyrus, Pars orbitalis R
17 Rolandic Operculum L
18 Rolandic Operculum R
19 Supplementary Motor Cortex L
20 Supplementary Motor Cortex R
21 Olfactory Cortex L
22 Olfactory Cortex R
23 Medial Superior Frontal Lobe L
24 Medial Superior Frontal Lobe R
25 Medial Superior Frontal Lobe, Pars orbitalis L
26 Medial Superior Frontal Lobe, Pars orbitalis R
27 Gyrus Rectus L
28 Gyrus Rectus R
29 Insula_L
30 Insula_R
31 Anterior Cingulum L
32 Anterior Cingulum R