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Supplementary Material: Image processing and statistical analysis of brain volume and morphometry analyses between TG6 positive and negative participants

MR Image Processing

The objective of the image processing was to obtain measures of normalised brain volume (NBV) for key grey matter (GM) regions. These were first large regions (cerebellar GM, and the cortical / subcortical cerebral GM), with features of smaller ROIs within these regions also quantified for analysis should initial investigation of their respective larger region prove promising (described further in the statistical analysis).

To obtain cerebellar GM volumes, the cerebellum-dedicated "SUIT" pipeline[1] (http://www.diedrichsenlab.org/imaging/suit.htm) was used. This is an SPM12b[2] (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/) plug-in. Firstly, all T1 images were intensity non-uniformity corrected using "N4ITK"[3]. SUIT then segmented the cerebellum from the rest of the brain via SPM tissue segmentation followed by calculation of the posterior probability of tissue belonging to either the cerebellum or brainstem. The isolated cerebellum was then registered to a template cerebellum via DARTEL, where regional GM volumes were calculated using a probabilistic atlas[4]. For each ROI, left and right hemispheric volume was added together (for ROIs which had a left, right and vermis component, only the left and right were added; the vermis was calculated as a separate structure as the sum of all the individual "vermis" volumes).

To obtain cerebral GM volumes the "recon_all" pipeline from Freesurfer[5] (http://surfer.nmr.mgh.harvard.edu/) was used with the T1 images in their raw DICOM format. Volumes for total cerebral cortical and subcortical GM, as well as the thalamus, caudate, putamen, pallidum, hippocampus and amygdala were extracted. Right and left hemispheric values for the individual subcortical structures were added together to create overall measures.

All volume measures (cerebellar and cerebral) were converted to normalised brain volume (NBV) using the eTIV variable from the Freesurfer output. NBV's are therefore volume as a percentage of total intracranial volume.

Any subcortical ROI found to be significantly different between TG6 groups was followed up with a "vertex" (shape) analysis of that structure using the "FIRST" pipeline (a part of FSL; FMRIB Software Library, https://fsl.fmrib.ox.ac.uk/fsl[6]). Here, subcortical structures were segmented from the N4ITK-corrected T1 images, and surface mesh outputs were generated. These were used to create images which describe the distance which the surface of each participant's structure deviates from the group average. These were generated for each hemisphere, in MNI space (to control for head size), and with a rigid, 6 degrees of freedom registration to additionally control for pose. Groupwise permutation testing via "randomise" was used to examine for differences (described later).

Statistical Analysis

All analyses were performed using IBM SPSS statistics version 23 unless otherwise stated (IBM Corp. Released 2013. IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp, http://www.ibm.com/analytics/us/en/technology/spss/).

The main objective of the analyses was to test for differences in GM brain volume between participants with (TG6+) and without (TG6-) antibodies to TG6.

The distribution of all variables was inspected by the Kolmogorov–Smirnov test and visual inspection; all data were deemed parametric. NBV of **total cerebellar GM** (i.e. the sum of all cerebellar nuclei as an estimate of the cerebellar cortex), **total cortical GM** (i.e. the cerebral cortex), and **total subcortical GM** were compared between groups by independent *t*-test; these will be referred to as the primary ROIs. In the event of any of these comparisons being significant, post-hoc ANOVAs between TG6 groups, which also controlled for age, repeated both the original comparison (e.g. total cerebellar GM), and also tested the NBVs of smaller regions which belong to the primary volume (e.g. individual cerebellar nuclei). This approach was employed as a means of controlling for multiple comparisons. Levene's test of equal variances was used in each ANOVA comparison to ensure that the assumptions of the test had been adequately met.

Similarly, any individual subcortical structure which was found to be significantly different between groups was also investigated via the previously described FIRST analysis. This was so that the spatial pattern of atrophy could be visualised, and also as an attempt to replicate the original finding using a different method in order to increase confidence in it. The statistical model for these was a two group difference implemented by FSLs "randomise" with 10,000 permutations and threshold-free cluster enhancement ("TFCE") correction. This model was run twice; once with no age correction (so that the overall pattern of atrophy can be visualised) and once with age correction (so that atrophy specific to TG6 positivity can be visualised).

The alpha value for all analyses was $p \leq .05$.

Supplementary References

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