Supplementary data

Supplementary methods

Diffusion dataset

Other DTI parameters analysis

As reported in the main text (see Methods), whole-brain maps of voxelwise quantitative DTI metrics were obtained after tensor diagonalization. Fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD) maps were warped to Montreal Neurological Institute (MNI) space using the transform estimated for the registration of T1 image to MNI space, as done with MD maps. FA, AD and RD mean value was determined for each GM cortical, subcortical and cerebellar volume of interest (VOI) in sCJD subjects and controls, with an analogue procedure to that followed for MD. Age-corrected z-scores were used in the Mann–Whitney test to compare non-MD DTI metrics between subjects and controls.

Single-subject analysis of MD

Average MD (± 2 SD) was calculated at lobar and subcortical level in controls; voxel-wise age-corrected z-score MD maps of sCJD subjects were calculated and subsequently binarized by applying a statistical threshold (average MD ± 2 SD of healthy controls, relative to each cortical lobe for cortical voxels and subcortical for subcortical voxels) to create single-subject involvement maps; thus, thus, a voxel was deemed involved if the MD absolute value was > 2 SD from controls average. As the DTI protocol did not always extend down to the base of posterior fossa, we only included the cerebellum in the group-wise analyses, and not in the single-subject maps. We calculated the percentage of the total number of voxels involved for each VOI in each subject and subsequently the average number of voxels involved for each VOI in our cohort. We also analyzed how many subjects had a VOI involved; this was achieved arbitrarily considering a VOI as involved if > 33% of its voxels showed either significantly increased or decreased MD from controls. Additional analyses were also performed using more and less conservative thresholds for decreased and increased MD respectively.
Two spherical seed volume of interest (VOI) with a 5 mm radius were used to derive dorsal and ventral DMN (dDMN and vDMN) (Damoiseaux, 2012; Andrews-Hanna et al., 2010; Jones et al., 2016). Seeds were located within the right posterior cingulate gyrus (PCC), as one of the main hubs of the DMN (Buckner et al., 2008; Leech & Sharp, 2014). In order to focus the analysis on the most involved portion of the PCC, we searched for voxels that had mean diffusivity restriction in the greatest number of sCJD subjects within the same PCC. This was obtained by summing single subject z-score MD restriction maps on the MNI space (see previous diffusion imaging section of Methods) (Damoiseaux et al., 2008; Shirer et al., 2012; Leech et al., 2011). The two identified seeds were located at the following Montreal Neurological Institute coordinates: +16, -48, +34 for the dDMN and +12, -54, +14 for the vDMN. Such coordinates were located within the boundaries of vDMN and dDMN for right PCC (Jones et al., 2016).
Supplementary results

Supplementary Table 1A

Codon 129 Polymorphism demographics of sCJD cohort and comparison with literature data. Codon 129 polymorphism subtypes distribution in our cohort was different than that expected for a sCJD population (Chi-Square, p < 0.001), with MV and VV over-represented and MM under-represented (Collins et al., 2006; Parchi et al., 1999).

| Subgroups        | MM % (n) | MV % (n) | VV % (n) | Total N |
|------------------|----------|----------|----------|---------|
| Paoletti et al.  | 32.4 (12)| 48.6 (18)| 18.9 (7) | 37      |
| Parchi et al. (1999) | 71.6 (215)| 11.7 (35)| 16.7 (50)| 300     |
| Collins et al. (2006) | 66.1 (1061)| 16.9 (272)| 16.9 (271)| 1604    |
| Parchi + Collins | 67 (1276)| 16.1 (307)| 16.9 (321)| 1904    |

Supplementary Table 1B

Molecular classification of sCJD subjects with final pathologic diagnosis and comparison with Collins et al. (2006). In considering molecular classifications we were underrepresented with MM1 and overrepresented with MM2, VV1/2, MV1 and MV1/2 (Kolmogorov-Smirnov, p = 0.001)(Collins et al., 2006).

| Subgroups        | MM1  | MM2  | MM1/2 | MV1  | MV2  | MV1/2 | VV1  | VV2  | VV1/2 |
|------------------|------|------|-------|------|------|-------|------|------|-------|
| Paoletti et al.  | 3.7  | 25.9 | 7.4   | 14.8 | 18.5 | 11.1  | 3.7  | 11.1 | 3.7   |
|                  | (1)  | (7)  | (2)   | (4)  | (5)  | (3)   | (1)  | (3)  | (1)   |
| Collins et. al.  | 59.8 | 4.2  | 3.9   | 5.0  | 9.8  | 0.9   | 1.9  | 13.5 | 1.1   |
|                  | (444)| (31) | (29)  | (37) | (73) | (7)   | (14) | (100)| (8)   |
Analysis of other DTI parameters in GM in sCJD

Fractional anisotropy (FA) was significantly decreased at cortical level (p < 0.001) in sCJD compared to controls, but no significant difference or trend was shown for subcortical and cerebellar GM between sCJD and healthy controls. No significant difference or trend was shown at the cortical level for mean radial (RD) and axial diffusivity (AD). Subcortical GM, however, showed a significantly reduced RD and AD, when compared to controls. Cerebellar RD was significantly reduced (p < 0.001) in sCJD, whereas AD was slightly increased (p < 0.001) when compared to controls (Supplementary Table 2A).

When considering subcortical VOIs individually, in sCJD caudate and putamen both showed restricted FA (p < 0.001), RD (p < 0.001, excepted left caudate p < 0.01) and AD (caudate p < 0.001; left putamen <0.05) whereas pallidum and thalamus showed increased FA (p < 0.001) and generally reduced AD (p values between <0.05 and <0.001) (Supplementary Table 2B).

We are aware that the interpretation of diffusion directionality DTI metrics in the grey matter has to be considered very carefully. Nevertheless, the non-MD DTI metrics results for GM were substantially in line with previous finding (Caverzasi et al. 2014a; Grau-Rivera et al. 2016).

Supplementary Tables 2A, 2B

A) Age-corrected mean z-scores for sCJD for fractional anisotropy (FA), radial diffusivity (RD) and axial diffusivity (AD) per cortical and subcortical areas and cerebellum. In bold are shown statistically significant differences compared to controls, after FDR correction.

| DTI metric | Cortical p | Subcortical p | Cerebellum p |
|------------|------------|---------------|-------------|
|            | Mean ± SD  | Median        | Mean ± SD   | Median        | Mean ± SD   | Median        |
| FA         | -1.22 ± 254 | -1.15         | <0.001      | -0.001 ± 4.55 | -0.08       | 0.8          |
|            |            |               |             |             | -0.11 ± 1.52 | +0.08       |
| RD         | +0.04 ± 0.58 | -0.06         | 0.09        | -0.92 ± 2.17 | -0.95       | <0.001       |
|            |            |               |             |             | +0.43 ± 0.9 | +0.42       |
| AD         | -0.44 ± 3.25 | -0.15         | 0.3         | -2.06 ± 3.52 | -2.15       | <0.001       |
|            |            |               |             |             | -2.76 ± 1.4 | -3.01       |

B) Age-corrected mean z-scores for sCJD for fractional anisotropy (FA), radial diffusivity (RD), and axial diffusivity (AD) of subcortical grey matter VOIs. In bold are shown statistically significant differences with controls, after FDR correction.

| Subcortical VOI | FA | RD | AD |
|-----------------|----|----|----|
|                 | Left | p | Right | p | Left | p | Right | p | Left | p | Right | p |
| Caudate         | -0.76 | <0.001 | -1.15 | <0.001 | -0.35 | <0.01 | -0.82 | <0.001 | -0.32 | <0.001 | -3.83 | <0.001 |
| Putamen         | -5.67 | <0.001 | -5.94 | <0.001 | -1.43 | <0.001 | -1.80 | <0.001 | 1.56 | <0.05 | -2.08 | 0.4 |
| Pallidum        | +1.36 | <0.001 | +1.83 | <0.001 | -0.61 | 0.051 | -0.62 | <0.001 | 3.26 | <0.05 | -6.19 | <0.001 |
| Thalamus        | +5.99 | <0.001 | +5.59 | <0.001 | -0.92 | <0.001 | -1.34 | <0.001 | -2.82 | <0.05 | -3.39 | 0.06 |
Single-subject GM MD analysis

Quantitative supratentorial single-subject maps were created to identify voxels with MD ± 2 SD compared to the controls average in the corresponding lobe or subcortical GM. In Supplementary Figure 1, we compared the DWI images to the single-subject quantitative MD restriction maps for four subjects, chosen as examples of different patterns of involvement. These single-subject maps always revealed more areas of involvement than was seen by visual assessment of DWI images. With a voxel-based approach, we considered a single VOI as involved (or affected) if > 33% of its voxels showed significantly decreased or increased MD. Regarding reduced MD, in average all supratentorial VOIs were involved, with a mean percentage of volume involved for each VOI of 47 ± 13.7% (range 25-81%) (Supplementary Table 3A). Increased diffusivity was much less common, with an average 11. ± 3.2% (range 2-19%) of each VOI showing increased MD; only the temporal pole, caudate, putamen and GP were involved in 3 to 14% of subjects (Supplementary Table 3B). Results obtained through the additional analyses using less (>10% of the voxels) and more conservative (>50% of the voxels) thresholds for increased and decreased MD are reported in Supplementary Tables 3A, B and Supplementary Figure 2.

Cortical areas Although quantitative single-subject maps generally showed similar patterns of involvement as found by visual inspection of conventional DWI images, and also here with predominant involvement of posterior areas mainly part of DMN. They also uniformly documented more areas involved compared to group-wise analysis (Supplementary Figure 1); for example, frontal and insular involvements were often evident in single-subject maps (Supplementary Figure 2 and 3; Supplementary Table 3A), yet, although frequently documented in clinical practice and in visual assessment studies (Tschampa et al., 2007; Vitali et al., 2011; Young et al., 2005), these areas of involvement are usually lost in group-wise analysis, likely due to disease heterogeneity (Grau-Rivera et al., 2017; Caverzasi et al., 2014).

Subcortical areas Most portions of the thalamus, putamen and GP VOIs showed reduced MD (>2/3 of the volume) and this was observed in almost 90% of subjects (Figure 3). The low percent of subjects with caudate involvement, and the low mean percentage of caudate volume with reduced MD is likely due to artifact from the strictly periventricular localization (thus suffering from inter-individual ventricular volume differences) that also affects the DTI post-processing, as already suggested in the literature (Grau-Rivera et al., 2017). We also found that on average about 8-18 % of subcortical VOIs volume showed increased MD, consistent with literature (Grau-Rivera et al., 2017) (Supplementary Table 3B).

Quantification of involvement in sCJD as shown by the present MD analyses generally showed
agreement with visual assessment studies (Vitali et al., 2011; Zerr et al., 2009; Young et al., 2005), but importantly single-subject maps showed more areas as involved than group-wise analyses. It must be underlined, however, that considering as involved voxels (or a percent of voxels in a VOI) below the threshold of MD - 2 S.D. from controls is quite a different approach from a group-wise analysis (which compares all voxels within a certain VOI between sCJD and controls). A significant difference between these two approaches must therefore be taken into account when comparing single-subject and group-wise analyses (especially in such an heterogeneous disease). Single-subject overall showed somewhat symmetric involvement (Supplementary Figure 2 and 3), with parietal and temporal lobes extensively involved, although some cases had asymmetry (not shown), consistent with our clinical experience with visual assessment of DWI (Vitali et al., 2011).

The implementation of quantitative single-subjective analysis, in our opinion, can be a step towards the integration of the standard visual assessment of DWI images and the group-wise quantitative approach already attempted by some authors (Caverzasi et al., 2014; Grau-Rivera et al., 2017) as they may greatly help to assess and quantify change over time (limiting intra and inter-operator reproducibility influencing visual assessment), an element which might be very important for future treatment trials.
Supplementary Figure 1

Single-patient mean diffusivity (MD) reduction maps (on the bottom of each frame) are generally consistent with DWI trace images acquired at \( b = 2000 \text{ s/mm}^2 \) (on the top). Overall, when compared to DWI trace images, single-subject quantitative maps more commonly showed subcortical GM involvement (arrowheads) and more extensive cortical involvement, such as deeper sulcal involvement (arrows). Colors from light blue to blue indicate z-score for each voxel, ranging from \(-2 \text{ SD from controls average MD towards lower values.} \)

Slightly differences in corresponding slice levels are explained by not fully comparable orientation in space of acquisition datasets. A) 67-year-old female, MV N/A, modified Barthel score 100, MMSE 27/30, at 11 months of at least 35 months course (still alive at present); B) 73-year-old female, MV1, modified Barthel score 65, MMSE 20/30, time-ratio = 0.67; C) 66-year-old female, MV2K, modified Barthel score 15, MMSE 9/27, time-ratio = 0.73; D) 58-year-old male, MM1-2, modified Barthel score 100, MMSE 25/30, time-ratio = 0.8. Orientation of image is radiologic (right brain (R) is left side of the image).
A) Group-wise analysis of single-subject data showing percentage of areas and subjects with MD reduction. Table shows two findings: 1) For each volume of interest (VOI), the average percent of voxels with greater than 2 standard deviations reduced MD from controls and 2) For each VOI, the percent of sCJD subjects with thresholded minimum number of voxels (>33% vs. >50%) with greater than 2 standard deviations reduced MD from controls (considered as involved). A VOI is considered involved if either >33% or >50% of its voxels show reduced MD compared to controls. A total of 40 supratentorial cortical and subcortical VOI per hemisphere was considered ((Desikan et al., 2006; Fischl et al., 2002) (did not include cerebellum; see supplementary Methods).

| VOI                              | Left Average % of voxels with MD reduction > 2SD | SD | Right Average % of voxels with MD reduction > 2SD | SD | % of sCJD with VOI involvement with 2 different thresholds | % of voxels with greater than 2 standard deviations reduced MD from controls | SD |
|---------------------------------|-----------------------------------------------|----|-----------------------------------------------|----|------------------------------------------------|---------------------------------------------------------------|----|
| Thalamus                        | 68                                           | 16 | 92                                           | 25 | >33%                                                | >50%                                                            | 69 |
| Putamen                         | 67                                           | 17 | 89                                           | 16 | >33%                                                | >50%                                                            | 69 |
| Pallidium                       | 80                                           | 17 | 92                                           | 17 | >33%                                                | >50%                                                            | 81 |
| Caudate*                        | 26                                           | 17 | 35                                           | 17 | >33%                                                | >50%                                                            | 25 |
| Pericalcarine                   | 57                                           | 17 | 84                                           | 17 | >33%                                                | >50%                                                            | 58 |
| Lingual                         | 43                                           | 17 | 62                                           | 17 | >33%                                                | >50%                                                            | 43 |
| Lateraloccipital                | 63                                           | 17 | 97                                           | 17 | >33%                                                | >50%                                                            | 66 |
| Cuneus                          | 48                                           | 17 | 73                                           | 17 | >33%                                                | >50%                                                            | 46 |
| Banksst                         | 61                                           | 17 | 38                                           | 17 | >33%                                                | >50%                                                            | 38 |
| Parahippocampal                 | 44                                           | 17 | 73                                           | 17 | >33%                                                | >50%                                                            | 45 |
| Transversetemporal              | 48                                           | 17 | 65                                           | 17 | >33%                                                | >50%                                                            | 38 |
| Superior temporal               | 44                                           | 17 | 73                                           | 17 | >33%                                                | >50%                                                            | 43 |
| Middletemporal                  | 58                                           | 17 | 89                                           | 17 | >33%                                                | >50%                                                            | 64 |
| Inferiortemporal                | 69                                           | 17 | 95                                           | 17 | >33%                                                | >50%                                                            | 71 |
| Temporalpole                    | 39                                           | 17 | 54                                           | 17 | >33%                                                | >50%                                                            | 35 |
| Fusiform                        | 58                                           | 17 | 92                                           | 17 | >33%                                                | >50%                                                            | 61 |
| Entorhinal                      | 33                                           | 17 | 35                                           | 17 | >33%                                                | >50%                                                            | 33 |
| Hippocampus                     | 27                                           | 17 | 30                                           | 17 | >33%                                                | >50%                                                            | 26 |
| Amygdala                        | 38                                           | 17 | 59                                           | 17 | >33%                                                | >50%                                                            | 42 |
| Insula                          | 37                                           | 17 | 59                                           | 17 | >33%                                                | >50%                                                            | 39 |
| Rostralantiercingulate          | 40                                           | 17 | 59                                           | 17 | >33%                                                | >50%                                                            | 30 |
| Posteriorcingulate              | 58                                           | 17 | 78                                           | 17 | >33%                                                | >50%                                                            | 48 |
| Isthmusrancingulate             | 57                                           | 17 | 86                                           | 17 | >33%                                                | >50%                                                            | 56 |
| Caudalantertingulate            | 45                                           | 17 | 76                                           | 17 | >33%                                                | >50%                                                            | 41 |
| Supramarginal                   | 39                                           | 17 | 59                                           | 17 | >33%                                                | >50%                                                            | 22 |
| Superior parietal               | 48                                           | 17 | 86                                           | 17 | >33%                                                | >50%                                                            | 44 |
| Precuneus                       | 50                                           | 17 | 81                                           | 17 | >33%                                                | >50%                                                            | 49 |
| Postcentral                     | 38                                           | 17 | 59                                           | 17 | >33%                                                | >50%                                                            | 38 |
| Inferioparietal                 | 57                                           | 17 | 95                                           | 17 | >33%                                                | >50%                                                            | 62 |
| Frontalpole                     | 70                                           | 17 | 92                                           | 17 | >33%                                                | >50%                                                            | 84 |
| Rostralmiddlefrontal            | 34                                           | 17 | 46                                           | 17 | >33%                                                | >50%                                                            | 38 |
| Superior frontal                | 31                                           | 17 | 30                                           | 17 | >33%                                                | >50%                                                            | 28 |
| Precentral                      | 35                                           | 17 | 68                                           | 17 | >33%                                                | >50%                                                            | 36 |
| Pars triangularis               | 36                                           | 17 | 49                                           | 17 | >33%                                                | >50%                                                            | 11 |
| Pars orbitalis                  | 36                                           | 17 | 54                                           | 17 | >33%                                                | >50%                                                            | 16 |
| Pars opercularis                | 35                                           | 17 | 46                                           | 17 | >33%                                                | >50%                                                            | 16 |
| Paracentral                     | 43                                           | 17 | 73                                           | 17 | >33%                                                | >50%                                                            | 32 |
| Medialorbitofrontal             | 46                                           | 17 | 70                                           | 17 | >33%                                                | >50%                                                            | 42 |
| Lateralorbitofrontal            | 50                                           | 17 | 86                                           | 17 | >33%                                                | >50%                                                            | 49 |
| Caudalmiddlefrontal             | 37                                           | 17 | 62                                           | 17 | >33%                                                | >50%                                                            | 14 |
B) Group-wise analysis of single-subject data showing percent of areas and subjects with MD increase.

Table shows two findings: 1. For each volume of interest (VOI), the average percent of voxels with greater than 2 standard deviations increased MD from controls and 2. For each VOI, the percent of sCJD subjects with thresholded minimum number of voxels (>10%, >33% and >50%) with greater than 2 standard deviations increased MD from controls. A VOI is considered involved if either >10%, >33% or >50% of its voxels show increased MD compared to controls. A total of 40 supratentorial cortical and subcortical VOI per hemisphere was considered (Desikan et al., 2006; Fischl et al., 2002) (did not include cerebellum; see supplementary Methods).

| VOI                        | Left Averag e % of voxels with MD increases > 2SD | S D | % of sCJD with VOI involvement with 3 thresholds | Right Averag e % of voxels with MD increases > 2SD | S D | % of sCJD with VOI involvement with 3 thresholds |
|----------------------------|-----------------------------------------------|-----|---------------------------------------------|-----------------------------------------------|-----|---------------------------------------------|
|                            | %                               |      | >10%                                        | %                               |      | >10%                                        |
|                            |                                  |      | >33%                                        |                                  |      | >33%                                        |
|                            |                                  |      | >50%                                        |                                  |      | >50%                                        |
| Thalamus                   | 9                                | 3    | 24                                          | 0                                | 0    | 8                                            | 2    | 19                                          | 0    | 0                                           |
| Putamen                    | 14                               | 1    | 57                                          | 5                                | 0    | 14                                          | 9    | 59                                          | 5    | 0                                           |
| Pallidum                   | 10                               | 8    | 38                                          | 3                                | 0    | 9                                            | 9    | 35                                          | 3    | 0                                           |
| Caudate                    | 18                               | 6    | 95                                          | 3                                | 0    | 11                                          | 7    | 92                                          | 8    | 0                                           |
| Pericalcarine              | 12                               | 5    | 76                                          | 0                                | 0    | 11                                          | 4    | 65                                          | 0    | 0                                           |
| Lingual                    | 12                               | 3    | 73                                          | 0                                | 0    | 11                                          | 3    | 62                                          | 0    | 0                                           |
| Lateraloccipital           | 11                               | 4    | 65                                          | 0                                | 0    | 11                                          | 4    | 62                                          | 0    | 0                                           |
| Cuneus                     | 12                               | 4    | 73                                          | 0                                | 0    | 12                                          | 5    | 62                                          | 0    | 0                                           |
| Bankksst                   | 12                               | 6    | 59                                          | 0                                | 0    | 12                                          | 6    | 62                                          | 0    | 0                                           |
| Parahippocampal            | 13                               | 3    | 89                                          | 0                                | 0    | 11                                          | 4    | 59                                          | 0    | 0                                           |
| Transversetemporal         | 11                               | 5    | 62                                          | 0                                | 0    | 11                                          | 5    | 57                                          | 0    | 0                                           |
| Superior temporal          | 13                               | 4    | 81                                          | 0                                | 0    | 13                                          | 4    | 68                                          | 0    | 0                                           |
| Middle temporal            | 13                               | 5    | 62                                          | 0                                | 0    | 13                                          | 5    | 78                                          | 0    | 0                                           |
| Inferior temporal          | 12                               | 5    | 62                                          | 0                                | 0    | 12                                          | 6    | 59                                          | 0    | 0                                           |
| Temporal pole              | 17                               | 9    | 89                                          | 14                               | 0    | 17                                          | 9    | 76                                          | 8    | 0                                           |
| Fusiform                   | 14                               | 5    | 73                                          | 0                                | 0    | 14                                          | 5    | 78                                          | 0    | 0                                           |
| Entorhinal                 | 13                               | 5    | 70                                          | 0                                | 0    | 15                                          | 5    | 84                                          | 0    | 0                                           |
| Hippocampus                | 2                                | 1    | 0                                           | 0                                | 0    | 3                                            | 1    | 0                                           | 0    | 0                                           |
| Amygdala                   | 5                                | 2    | 0                                           | 0                                | 0    | 9                                            | 4    | 0                                           | 0    | 0                                           |
| Insula                     | 10                               | 4    | 49                                          | 0                                | 0    | 11                                          | 4    | 54                                          | 0    | 0                                           |
| Rostralanteriorcingulate   | 13                               | 7    | 62                                          | 0                                | 0    | 11                                          | 4    | 51                                          | 0    | 0                                           |
| Posteriorcingulate         | 9                                | 4    | 38                                          | 0                                | 0    | 10                                          | 5    | 49                                          | 0    | 0                                           |
| Isthmuscingulate           | 9                                | 4    | 38                                          | 0                                | 0    | 8                                            | 4    | 41                                          | 0    | 0                                           |
| Caudalanteriorcingulate    | 11                               | 5    | 49                                          | 0                                | 0    | 10                                          | 3    | 49                                          | 0    | 0                                           |
| Supramarginal              | 12                               | 3    | 78                                          | 0                                | 0    | 12                                          | 3    | 73                                          | 0    | 0                                           |
| Superior parietal          | 11                               | 2    | 57                                          | 0                                | 0    | 10                                          | 3    | 46                                          | 0    | 0                                           |
| Precuneus                  | 12                               | 4    | 65                                          | 0                                | 0    | 11                                          | 4    | 65                                          | 0    | 0                                           |
| Postcentral                | 10                               | 2    | 54                                          | 0                                | 0    | 11                                          | 3    | 70                                          | 0    | 0                                           |
| Inferior parietal          | 11                               | 4    | 57                                          | 0                                | 0    | 12                                          | 5    | 62                                          | 0    | 0                                           |
| Frontal pole               | 5                                | 4    | 8                                           | 0                                | 0    | 4                                            | 3    | 8                                           | 0    | 0                                           |
| Rostralmiddle frontal      | 11                               | 4    | 62                                          | 0                                | 0    | 11                                          | 3    | 62                                          | 0    | 0                                           |
| Superior frontal           | 10                               | 3    | 46                                          | 0                                | 0    | 9                                            | 3    | 30                                          | 0    | 0                                           |
| Precentral                 | 11                               | 3    | 73                                          | 0                                | 0    | 12                                          | 3    | 81                                          | 0    | 0                                           |
| Pars triangularis          | 14                               | 5    | 81                                          | 0                                | 0    | 15                                          | 5    | 84                                          | 0    | 0                                           |
| Pars orbitalis             | 18                               | 6    | 95                                          | 0                                | 0    | 19                                          | 6    | 97                                          | 0    | 0                                           |
| Pars opercularis           | 12                               | 3    | 70                                          | 0                                | 0    | 13                                          | 3    | 86                                          | 0    | 0                                           |
| Paracentral                | 12                               | 4    | 70                                          | 0                                | 0    | 11                                          | 4    | 59                                          | 0    | 0                                           |
| Medial orbitofrontal       | 17                               | 6    | 89                                          | 0                                | 0    | 16                                          | 5    | 86                                          | 0    | 0                                           |
| Lateral orbitofrontal      | 17                               | 5    | 86                                          | 0                                | 0    | 16                                          | 6    | 89                                          | 0    | 0                                           |
| Caudal middle frontal      | 11                               | 4    | 62                                          | 0                                | 0    | 11                                          | 4    | 65                                          | 0    | 0                                           |
Supplementary Figure 2

A) Percentage of sCJD subjects with involvement of each VOI (>33% of volume)

B) Average percentage of volume involvement for each VOI

Supplementary Figure 2 Legend

Frequency of MD restriction in sporadic Creutzfeldt-Jakob disease (sCJD) at volume of interest (VOI) level. A) Percent of sCJD subjects with restricted MD in 40 supratentorial cortical and subcortical
volumes of interest (VOI) per hemisphere (see Methods, quantified MD restriction greater than 2 SD from controls in > 33% of voxels). B) Average percentage of voxels showing significant MD restriction (greater than 2 SD) from controls in each VOI. Standard deviation (SD) is shown for each bar as bar label. As shown, each VOI had an average of > 25% of voxels involved (range 25-81%, average 47% ± 13.7 S.D.) C) Color-coded brain map overlay of percent of sCJD subjects showing involvement of each supratentorial VOI, as reported in 3A. White represents areas involved in less than 40% (range 24-35%) of subjects, green in 40-59%, light blue in 60-79% (range 62-78%), dark blue in more than 80% of subjects (range 81-97%). Orientation of image is radiologic (right brain (R) is left side of the image).
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