Supplementary Online Content

De Vocht J, Blommaert J, Devrome M, et al. Use of multimodal imaging and clinical biomarkers in presymptomatic carriers of C9orf72 repeat expansion. JAMA Neurol. Published online May 18, 2020. doi:10.1001/jamaneurol.2020.1087

eResults. Supplementary Analysis

eReferences

eFigure 1. Violin Plots of 18F FDG Uptake in Volumes of Interest, After Correcting for GM Atrophy With PVC, in Healthy Controls and PreSxC9

eFigure 2. W-Score Frequency Maps Detailing the Fraction of PreSxC9 With Suprathreshold W-Scores at Voxel Level

eFigure 3. Mean W-Score Images Detailing Tracer Uptake in PreSxC9, Thresholded From 0.5 Standard Deviations From the Norm

eFigure 4. Relative Hypermetabolism in PreSxC9 in relation to healthy controls (pheight < 0.001, at Cluster Level pFWE< 0.05) Following Voxel-Based Partial Volume Corrections, Using SUVr Images

eTable 1. Significant Areas of Decreased Glucose Metabolism in PreSxC9 Compared to HC, Identified Using Voxel-Wise Testing

eTable 2. Significant Areas of Volume Decline in PreSxC9 Compared to HC, Identified Using Voxel-Wise Testing

eTable 3. Significant Areas of Decreased Glucose Metabolism in PreSxC9 Compared to HC, Identified Using Voxel-Wise Testing Following Partial Volume Correction

eTable 4. Significant Areas of Increased Cortical Glucose Metabolism in PreSxC9 Compared to HC, Identified Using Voxel-Wise Testing Following Partial Volume Correction

eTable 5. A Volume-of-Interest (VOI)-Based Analysis After Region-Based Voxel-Wise (RBV) Correction for GM Atrophy, Using the Hammers N30R83 Maximum Probability Atlas

eTable 6. Significant Areas of Increased Cortical Glucose Metabolism in PreSxC9 Compared to HC, Identified Using Voxel-Wise Testing Following Partial Volume Correction

This supplementary material has been provided by the authors to give readers additional information about their work.
eResults. Supplementary analysis

To address the concern that the observed clusters of relative hypermetabolism in PreSxC9 may be induced by global changes (reduced mean of that group), we examined relative hypermetabolism in PreSxC9 relation to healthy controls, by using the ratio over the FDG-PET uptake in the cerebellum, excluding structures reportedly affected by C9orf72 mutation status, predominantly situated in the superior posterior lobe of the cerebellum and vermis in C9orf72 mutation carriers.1-4

We therefore repeated the analysis using SUVR images corrected for the average uptake in cerebellar structures reportedly unaffected by carrying a C9orf72 mutation using the automated anatomical labelling atlas (lobule 3-5, 8 and 10), implemented in PMOD.

We present this data in the following figure (eFigure 4), which should be compared to Figure 1D in the main text, and table (eTable 6), which should be compared to eTable 4. From this analysis we found that the major conclusions were not altered by the different method of analysis.
References

1. Bocchetta M, Cardoso MJ, Cash DM, Ourselin S, Warren JD, Rohrer JD. Patterns of regional cerebellar atrophy in genetic frontotemporal dementia. Neuroimage Clin. 2016;11:287-290.

2. Irwin DJ, McMillan CT, Brettschneider J, et al. Cognitive decline and reduced survival in C9orf72 expansion frontotemporal degeneration and amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2013;84(2):163-169.

3. Mahoney CJ, Beck J, Rohrer JD, et al. Frontotemporal dementia with the C9ORF72 hexanucleotide repeat expansion: clinical, neuroanatomical and neuropathological features. Brain. 2012;135(pt 3):736-750.

4. Whitwell JL, Weigand SD, Boeve BF, et al. Neuroimaging signatures of frontotemporal dementia genetics: C9ORF72, tau, progranulin and sporadics. Brain. 2012;135(pt 3):794-806.
**eFigure 1:** Violin plots of $^{18}$F FDG uptake in volumes of interest, after correcting for GM atrophy with PVC, in healthy controls (in white) and PreSxC9 (in blue). This revealed significant differences in left and right thalamus, insular cortices, the precentral gyrus and right postcentral gyrus.
**eFigure 2:** W-score frequency maps detailing the fraction of PreSxC9 with suprathreshold W-scores at voxel level. Section numbers refer to MNI coordinates. A. represents the fraction of PreSxC9 with W-scores ≤ -1.96, drafted from FDG-PET images uncorrected for PVC. B. reveals the fraction of PreSxC9 with W-scores ≥ 1.96, drafted from FDG-PET images following PVC. C. reveals the fraction of PreSxC9 with W-scores ≤ -1.96, drafted from modulated GM images. Variation in colour depicts the fraction of PreSxC9 with significantly altered metabolism or modulated GM volume exceeding the threshold for abnormality.
**eFigure 3:** Mean W-score images detailing tracer uptake in PreSxC9, thresholded from 0.5 standard deviations from the norm. Section numbers refer to MNI coordinates.  
**A.** Mean W-score image detailing reduced metabolism in PreSxC9 without partial volume correction, **B.** Mean W-score image detailing increased metabolism in the PreSxC9 following partial volume correction.
eFigure 4: Relative hypermetabolism in preSxCS in relation to healthy controls (p<0.001, at cluster level pFWE < 0.05) following voxel-based partial volume corrections, using SUVr images. Slice numbers refer to MNI coordinates.

FDG-PET PVC relative hypermetabolism
| Cluster-level | peak-level | Peak voxel MNI coordinate | Cluster location | Maximu m % change |
|---------------|------------|---------------------------|------------------|-------------------|
| $p_{\text{FWE-corr}}$ | $k_E$ | $p_{\text{FWE-corr}}$ | $T$ | $x$ | $y$ | $z$ | Anatomical Region |
| 0.001 | 8619 | 0.001 | 10.05 | 14 | -34 | 6 | Thalamus (pulvinar) | 42% |
| | | | | -14 | -36 | 6 | Thalamus (pulvinar) | |
| | | | | 4 | -20 | 10 | Thalamus (medial dorsal) | |
| | | | | -26 | 6 | -36 | Temporal pole (BA 36) | |
| | | | | 4 | 6 | -2 | Caudate nucleus | |
| | | | | 8 | 0 | 14 | Caudate nucleus | |
| | | | | 28 | 6 | -38 | Temporal pole (BA 36) | |
| | | | | 22 | -16 | -16 | Parahippocampal gyrus | |
| | | | | 50 | -8 | 10 | Central opercular cortex (BA 48) | |
| | | | | 40 | -12 | 16 | Insular cortex (BA 13) | |
| | | | | 8 | 10 | 10 | Caudate nucleus | |
| | | | | 48 | -8 | -32 | Anterior inferior temporal gyrus (BA 20) | |
| | | | | 44 | -44 | -10 | Inferior temporal gyrus (BA 37) | |
| | | | | 50 | 12 | -14 | Temporal pole (BA 38) | |
| | | | | 50 | -24 | -24 | Inferior temporal gyrus (BA 20) | |
| | | | | 34 | -40 | 0 | n/a | |
| 0.056 | 332 | 0.158 | 4.73 | -22 | 28 | -16 | Orbitofrontal cortex (BA 11) | 30% |
| | | | | -22 | 44 | -12 | Orbitofrontal cortex (BA 11) | |
| | | | | -12 | 34 | -8 | Orbitofrontal cortex (BA 11) | |
| 0.035 | 388 | 0.320 | 4.41 | 24 | 28 | -14 | Orbitofrontal cortex (BA 11) | 30% |
| | | | | 34 | 34 | -10 | Frontal pole (BA 47) | |
| | | | | 24 | 46 | -12 | Frontal pole (BA 11) | |
| | | | | 8 | 20 | -18 | Subcallosal cortex (BA 11) | |

**eTable 1:** Significant areas of decreased cortical glucose metabolism in preSxC9 compared to HC, identified using voxel-wise testing. Table shows all local maxima more than 1 cm apart.
eTable 2: Significant areas of volume decline in presXc9 compared to HC, identified using voxel-wise testing. Table shows all local maxima more than 1 cm apart.

| Cluster-level | peak-level | Peak voxel MNI coordinate | Cluster location | Maximum % change |
|---------------|------------|---------------------------|------------------|-----------------|
| pFWE-corr     | kE         | T            | x y z            | Anatomical Region |                |
| 0.001         | 3951       | 0.006        | 6.23            | -64 -9 7         | Central opercular cortex (BA 22) | 30% |
|               |            |              |                 | -43 -5 1         | Insular cortex (BA 13)             |     |
|               |            |              |                 | -44 10 -5        | Insular cortex (BA 13)             |     |
|               |            |              |                 | -67 -26 19       | Anterior Supramarginal gyrus (BA 22) |     |
| 0.001         | 8522       | 0.016        | 5.89            | 7 -15 11         | Thalamus (medial dorsal)           | 13% |
|               |            |              |                 | -4 -14 11        | Thalamus (medial dorsal)           |     |
|               |            |              |                 | 12 -26 5         | Thalamus (pulvinar)                |     |
|               |            |              |                 | -12 -30 6        | Thalamus (pulvinar)                |     |
**eTable 3:** Significant areas of decreased metabolism in preSxC9 compared to HC, identified using voxel-wise testing following partial volume correction. Table shows all local maxima more than 1 cm apart.

| Cluster-level | peak-level | Peak voxel MNI coordinate | Cluster location | Maximum % change |
|---------------|------------|---------------------------|-----------------|-----------------|
| pFWE-corr     | kE         | pFWE-corr                 | x   | y   | z   | Anatomical Region                     |
| 0.001         | 2925       | 0.001                     | 14  | -32 | 4   | Thalamus (pulvinar)                   | 25%              |
|               |            |                           | -12 | -34 | 4   | Thalamus (medial dorsal)              |                 |
|               |            |                           | 4   | -18 | 10  | Thalamus (medial dorsal)              |                 |
|               |            |                           | 22  | -8  | -16 | Amygdala                               |                 |
|               |            |                           | -22 | 4   | -34 | Temporal pole (BA 28)                 |                 |
|               |            |                           | 44  | -4  | -4  | Insular cortex (BA 13)                |                 |
|               |            |                           | 54  | 10  | -2  | Temporal pole (BA 22)                 |                 |
|               |            |                           | 40  | 4   | -12 | Insular cortex (BA 13)                |                 |
|               |            |                           | -4  | 2   | -8  | n/a                                     |                 |
|               |            |                           | 4   | -6  | 6   | Thalamus                               |                 |
|               |            |                           | -16 | -8  | -20 | Anterior Parahippocampal gyrus (BA 34) |                 |
|               |            |                           | 4   | -6  | -10 | Hypothalamus                           |                 |
|               |            |                           | 26  | 4   | -36 | Temporal pole                          |                 |
|               |            |                           | -6  | 10  | 0   | Caudate nucleus                        |                 |
|               |            |                           | 32  | -38 | -6  | Posterior Parahippocampal gyrus        |                 |
|               |            |                           | 8   | 14  | 0   | Caudate nucleus                        |                 |
| 0.010         | 281        | 0.026                     | 52  | -4  | -34 | Inferior temporal gyrus (BA 20)        | 18%              |
|               |            |                           | 44  | 16  | -34 | Temporal pole (BA 38)                 |                 |
|               |            |                           | 52  | 10  | -28 | Temporal pole (BA 21)                 |                 |
| Cluster-level | peak-level | Peak voxel MNI coordinate | Cluster location | Maximum % change |
|--------------|------------|---------------------------|------------------|-----------------|
| $p_{FWE-corr}$ | $k_E$ | $p_{FWE-corr}$ | $T$ | $x$ | $y$ | $z$ | Anatomical Region | |
| 0.003 | 355 | 0.002 | 6.41 | 12 | -68 | 28 | Precuneous cortex | 6% |
| -4 | -68 | 22 | Precuneous cortex |
| -16 | -64 | 56 | Precuneous cortex (BA 7) |
| -14 | -68 | 26 | Precuneous cortex |
| -6 | -60 | 54 | Precuneous cortex |
| 6 | -62 | 18 | Precuneous cortex |
| -6 | -60 | 12 | Posterior cingulate |
| 0.034 | 204 | 0.072 | 5.15 | -24 | -14 | 58 | Precentral gyrus (BA 6) | 5% |
| -8 | 0 | 66 | Supplementary motor cortex (BA 6) |
| -32 | -6 | 50 | Precentral gyrus (BA 6) |

**eTable 4:** Significant areas of increased cortical glucose metabolism in preSxC9 compared to HC, identified using voxel-wise testing following partial volume correction. Table shows all local maxima more than 1 cm apart.
| Anatomical Region          | Test statistic (Mann-Whitney U) | p_{uncorr-value} | p_{corr-value} (Benjamini-Hochberg) |
|---------------------------|----------------------------------|------------------|-----------------------------------|
| FL_mid fr G_l             | 202                              | 0.79             | 0.83                              |
| FL_mid fr G_r             | 185                              | 0.48             | 0.59                              |
| FL_precen G_l             | 107                              | 0.007            | 0.02                              |
| FL_precen G_r             | 109                              | 0.008            | 0.03                              |
| FL_strai G_l              | 117                              | 0.01             | 0.04                              |
| FL_strai G_r              | 103                              | 0.005            | 0.02                              |
| FL_OFC_AOG_l              | 121                              | 0.02             | 0.04                              |
| FL_OFC_AOG_r              | 125                              | 0.03             | 0.06                              |
| FL_inf fr G_l             | 187                              | 0.51             | 0.61                              |
| FL_inf fr G_r             | 189                              | 0.55             | 0.64                              |
| FL_sup_fr_G_l             | 183                              | 0.45             | 0.56                              |
| FL_sup_fr_G_r             | 169                              | 0.27             | 0.36                              |
| FL_OFC_MOG_l              | 109                              | 0.008            | 0.03                              |
| FL_OFC_MOG_r              | 129                              | 0.03             | 0.06                              |
| FL_OFC_LOG_l              | 154                              | 0.13             | 0.21                              |
| FL_OFC_LOG_r              | 198                              | 0.71             | 0.78                              |
| FL_OFC_POG_l              | 65                               | 0.0002           | 0.01                              |
| FL_OFC_POG_r              | 120                              | 0.02             | 0.04                              |
| Subgen antCing_l          | 80                               | 0.001            | 0.01                              |
| Subgen antCing_r          | 161                              | 0.19             | 0.28                              |
| Subcall area_l            | 212                              | 0.99             | 1                                 |
| Subcall area_r            | 102                              | 0.005            | 0.02                              |
| Presubgen antCing_l       | 142                              | 0.07             | 0.12                              |
| Presubgen antCing_r       | 168                              | 0.25             | 0.35                              |
| Hippocampus_r             | 55                               | 0.00005          | 0.01                              |
| Hippocampus_l             | 66                               | 0.0002           | 0.01                              |
| Amygdala r                | 122                              | 0.02             | 0.04                              |
| Amygdala l                | 133                              | 0.04             | 0.08                              |
| Ant_TL_med_r              | 70                               | 0.0003           | 0.01                              |
| Ant_TL_med_l              | 77                               | 0.001            | 0.01                              |
| Ant_TL_inf_lat_r          | 140                              | 0.06             | 0.11                              |
| Ant_TL_inf_lat_l          | 129                              | 0.03             | 0.06                              |
| G_paraH_amb_r             | 92                               | 0.002            | 0.01                              |
| G_paraH_amb_l             | 150                              | 0.11             | 0.18                              |
| G_sup_temp_post_r         | 212                              | 0.99             | 1                                 |
| G_sup_temp_post_l         | 182                              | 0.43             | 0.55                              |
| G_tem_midin_r             | 102                              | 0.005            | 0.02                              |
| G_tem_midin_l             | 68                               | 0.0002           | 0.01                              |
| G_fus_r                   | 106                              | 0.006            | 0.02                              |
| G_fus_l                   | 95                               | 0.003            | 0.02                              |
| Post_TL_l                 | 113                              | 0.01             | 0.03                              |
| Post_TL_r                 | 103                              | 0.005            | 0.02                              |
| G_sup_temp_ant_l          | 100                              | 0.004            | 0.02                              |
| G_sup_temp_ant_r          | 57                               | 0.00007          | 0.01                              |
| PL_postce_G_l             | 199                              | 0.73             | 0.78                              |
| PL_postce_G_r             | 105                              | 0.006            | 0.02                              |
| PL_sup_pa_G_l             | 150                              | 0.11             | 0.18                              |
| PL_sup_pa_G_r             | 193                              | 0.62             | 0.7                                |
| PL_rest_l                 | 166                              | 0.23             | 0.33                              |
| PL_rest_r                 | 194                              | 0.64             | 0.71                              |
| Insula l                  | 66                               | 0.0002           | 0.01                              |
| Insula r                  | 50                               | 0.00003          | 0.01                              |
| G_cing_ant_l              | 189                              | 0.55             | 0.64                              |
| G_cing_ant_r              | 206                              | 0.87             | 0.91                              |
**eTable 5:** A volume-of-interest (VOI)-based analysis after region-based voxel-wise (RBV) correction for GM atrophy, using the Hammers N30R83 Maximum Probability atlas.

| Region          | Volume | GM Atrophy Left | GM Atrophy Right |
|-----------------|--------|-----------------|------------------|
| G_cing_post_l   | 165    | 0.22            | 0.33             |
| G_cing_post_r   | 170    | 0.28            | 0.37             |
| Cerebellum_r    | 209    | 0.93            | 0.96             |
| Cerebellum_l    | 177    | 0.36            | 0.47             |
| Putamen_l       | 135    | 0.05            | 0.09             |
| Putamen_r       | 113    | 0.011           | 0.03             |
| Thalamus_l      | 98     | 0.003           | 0.02             |
| Thalamus_r      | 66     | 0.0002          | 0.01             |
| CaudateNucl_l   | 76     | 0.0005          | 0.01             |
| CaudateNucl_r   | 99     | 0.004           | 0.02             |
**eTable 6**: Significant areas of increased cortical glucose metabolism in preSxC9 compared to HC, identified using voxel-wise testing following partial volume correction. Table shows all local maxima more than 1 cm apart.

| Cluster-level | peak-level | Peak voxel MNI coordinate | Cluster location | Maximum % change |
|---------------|------------|----------------------------|------------------|------------------|
| pFWE-corr     | kE         | pFWE-corr                  | T | x  | y  | z  | Anatomical Region          |                             |
| 0.054 293     | 0.032 5.26 | -24 12 52                 |    |    |    |    | Precentral gyrus (BA 6)    | 4%                           |
|               |            | -8 2 66                   |    |    |    |    | Superior frontal gyrus (BA 6) |                             |
|               |            | -30 0 52                  |    |    |    |    | Middle frontal gyrus (BA 6) |                             |
|               |            | -18 -4 66                 |    |    |    |    | Superior frontal gyrus (BA 6) |                             |
|               |            | -24 8 48                  |    |    |    |    | Superior frontal gyrus       |                             |
| 0.010 487     | 0.080 4.90 | 12 -68 28                |    |    |    |    | Precuneous cortex           | 5%                           |
|               |            | -16 -62 56                |    |    |    |    | Precuneous cortex (BA 7)    |                             |
|               |            | -6 -68 24                 |    |    |    |    | Precuneous cortex           |                             |
|               |            | -6 -66 34                 |    |    |    |    | Precuneous cortex           |                             |
|               |            | 8 -62 20                  |    |    |    |    | Precuneous cortex           |                             |