Data Supplement

**Appendix e-1**

Inclusion and exclusion criteria of the study participants

*In vivo* brain MRI and [11C](*R*)-PK11195 PET imaging

### Statistical analyses

**Tables**

**Table e-1.** Age-adjusted associations of [11C](*R*)-PK11195 PET, MRI and DTI parameters with clinical characteristics in multiple sclerosis patients.

**Table e-2.** Regional [11C](*R*)-PK11195 binding within healthy controls and relapsing and secondary progressive multiple sclerosis patients.

**Table e-3.** Age-adjusted associations of regional [11C](*R*)-PK11195 binding with DTI parameters in all multiple sclerosis patients.

**Table e-4.** Age-adjusted associations of regional [11C](*R*)-PK11195 binding with MRI parameters in all multiple sclerosis patients.

**e-References**

**Appendix e-1**

### Inclusion and exclusion criteria of the study participants.

### The detailed inclusion and exclusion criteria of the secondary progressive multiple sclerosis (SPMS) patients and healthy controls have been described in detail in our earlier study.1 The inclusion criteria for the relapsing remitting multiple sclerosis (RRMS)/clinically isolated syndrome (CIS) patients were 1) definite diagnosis of RRMS according to the revised McDonald criteria or CIS with no evidence of dissemination in time and 2) typical demyelinating lesions fulfilling the Barkhof criteria in brain MRI.2

### *In vivo* brain MRI and [11C](*R*)-PK11195 PET imaging.

In Turku PET Centre, the brain MRI was performed with Philips Gyroscan Intera 1.5 tesla Nova Dual scanner (Philips, Best, the Netherlands) for the neuroradiological analysis and acquisition of anatomical reference for the PET images. The MRI sequences included axial T1 and T2 weighted, coronal T2 weighted fluid-attenuated inversion recovery (FLAIR), diffusion tensor imaging (DTI) and axial gadolinium-enhanced 3DT1 weighted series.

The co-registration of magnetic resonance images to the PET sum images was performed using statistical parametric mapping (SPM8, version 8; Wellcome Trust Center for Neuroimaging) running on Matlab 2011 (The MathWorks, Natick, MA). The analyses of the DTI data with evaluation of global mean fractional anisotropy (FA) and mean diffusivity (MD) in normal appearing white matter (NAWM) were performed according to methodology reported earlier.3 The MRI for healthy controls at the Wolfson Molecular Imaging Centre (WMIC) was similarly performed with a 1.5 tesla scanner (Philips Achieva; Philips Medical Systems, Best, The Netherlands) as described in earlier studies.4,5 The radiochemical synthesis of [11C](*R*)-PK11195 radioligand was carried out according to the methods described earlier.1 The mean specific radioactivities yielded from the syntheses were 68.2 GBq/µmol [standard deviation (SD) 20.5] in Turku PET Centre (TPC) and 135.0 GBq/µmol (SD 39.0) at the WMIC.

In both centers, the PET imaging was performed using a ECAT HRRT scanner (CTI/Siemens, Knoxville, TN, United States)6 that has an intrinsic spatial resolution of approximately 2.5 mm3.7 After a 6-minute transmission scan for attenuation correction with a 137Cs point source, 60-minute dynamic imaging was initiated simultaneously with an intravenous bolus injection of [11C](*R*)-PK11195. The mean administered radioactivity in the scans in TPC was 474 (SD 29) MBq with no significant differences between the groups [mean (SD) for control, SPMS and RRMS groups were 479 (19), 482 (19) and 463 (42), respectively; *p* = ns]. At the WMIC, the mean administered radioactivity was 590 (SD 108) MBq. The reconstruction of PET image data into 17 time frames was performed according to previously presented methods.1,8 The dynamic PET data was smoothed with Gaussian 2.5 mm post reconstruction filter for TPC data and 2 mm for WMIC data.9 Mutual information realignment in SPM8 was used for the correction of possible displacements between frames.

For the analysis of [11C](*R*)-PK11195 binding within the T1 hypointense lesions and their perilesional areas, a semiautomatic approach was used. At first, the T2 hyperintense white matter (WM) MS lesions were identified utilizing Lesion Segmentation Tool (LST), a toolbox in SPM810 as described in our previous study.1 T1 hypointense lesion mask images were then derived by comparing the segmented WM, gray matter (GM) and cerebrospinal fluid images of the original T1 image to the segmented T1 images obtained from LST’s T1 lesion filling feature. Thereafter, the T1 hypointense lesion masks were visually checked slice by slice and manually corrected if needed. The masks were separated into two groups for gadolinium negative (Gd-) and gadolinium positive (Gd+) T1 hypointense lesions, which were used for the evaluation of the intralesional WM, and as a core for the perilesional WM. For each T1 hypointense lesion mask perilesional mask images with distances of 0-3 mm and 3-6 mm to the lesion mask border were created by dilating the lesion mask image 3 mm and 6 mm and then removing the cores from the resulting images. Overlapping regions surrounding both Gd- and Gd+ masks were removed. An example of the resulting T1 hypointense lesion and perilesional WM masks with a NAWM mask is illustrated in Figure 1. T1 hypointense and T2 hyperintense lesion load volumes were derived from the respective lesion mask volumes.

Region-of-interest (ROI) delineation for cerebellum, striatum, thalamus, WM and combined cortical GM were performed with Freesurfer software (v5.3.0, Laboratory for Computational Neuroimaging, Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA, United States). The thalamus ROI was further eroded 4 mm from its borders to avoid the signal spill over from the choroid plexus and other vasculature in the adjacent ventricles, to match it visually with the specific thalamic [11C](*R*)-PK11195 uptake. The T1-hypointense lesion and perilesional mask images were used as ROIs for the analyses of the dynamic [11C](*R*)-PK11195 images. Finally, NAWM ROI was created by subtracting the T1-hypointense lesion mask image, perilesional mask images, and striatum and thalamus ROIs from the segmented WM. The cortical GM, whole cerebral WM and global parenchymal volumes were derived from the respective Freesurfer segments. For the estimation of normalized lesional, cortical GM, cerebral WM and total brain volumes, a parenchymal fraction was calculated by normalizing the respective absolute volumes with the individual’s total intracranial volume and reported as percentages. For clarity, in presenting the T1 and T2 lesion loads the volumes are reported as absolute volumes in Table 1, whereas for correlational analyses parenchymal fractions were used for the comparability to other parenchymal fractions.

In order to correct for the partial volume effect caused by differences in [11C](*R*)-PK11195 binding between adjacent ROIs and by increased radioligand binding in the meningeal, vascular, bone and soft tissue next to cortical ROIs, partial volume correction using the Geometric Transfer Matrix method11 was performed for all regional time activity curves. Gaussian function with 2.5 mm full width at half maximum was used to approximate the scanner point spread function and for each cortical ROI a corresponding background ROI was used to correct for the background activity.

For the definition of the clustered gray matter reference region using the supervised cluster algorithm (SCA) approach with the SuperPK software, the clustered GM map was further cleaned from contribution of positive blood and high specific binding coefficients. The median reference region k2 value 0.16 minute-1 for Logan’s method wasderived from the parameter estimates of simplified reference tissue model fitted to thalamus time activity curves.

### Statistical analyses.

The groupwise comparisons of the MRI and DTI parameters between MS patients (RRMS and SPMS groups) and age matched controls (younger controls and older controls, respectively) were performed using analysis of variance (ANOVA) with Tukey-Kramer *post hoc* test for multiple comparisons. For the correction of age in RRMS vs. SPMS comparisons, analysis of covariance (ANCOVA, age as covariate) was used.

For the groupwise comparison of [11C](R)PK11195 binding within ROIs, the younger and older controls were pooled as one control group (n=17) after checking there were no statistically significant differences in the radioligand binding between the control groups. This was in order to provide a control group with clearly overlapping ages to the SPMS and RRMS groups to meet the assumptions of age adjustment and to increase statistical power. The statistical testing was then performed with analysis of covariance (ANCOVA) with Tukey-Kramer *post hoc* test for multiple comparisons.

The interaction between regional DVR and clinical and MRI variables (regional volumes, mean FA and MD, Extended Disability Status Scale (EDSS), Multiple Sclerosis Severity Score (MSSS) and disease duration) were examined within the pooled data of all MS patients by using repeated measures analysis of covariance (rm ANCOVA) adjusted for age and where the regional DVRs or volumes were considered as repeated measures using unstructured covariance structure. Models included the main effects of clinical or MRI variables and regional DVR and their interaction. The effect of age on DVR values was compared between the pooled healthy control and MS patient groups using ANCOVA, where the interaction between groups and age was examined yielding an age coefficient for each group. *P* values less than 0.05 were considered as statistically significant.

**Table e-1. Age-adjusted associations**a **of [11C](*R*)-PK11195 PET, MRI and DTI parameters with clinical characteristics in multiple sclerosis patients**b

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **EDSS** | | | **MSSS** | | | **Disease duration** | | | |
|  | Coefficient (SE) | *p* | *p* for interactionf | Coefficient  (SE) | *p* | *p* for interactionf | | Coefficient  (SE) | *p* | *p* for interactionf |
| **[11C](*R*)-PK11195 DVR** |
| *Model 1* |  |  |  |  |  |  | |  |  |  |
| NAWM | 0.018  (0.008) | 0.04c | 0.001c | 0.018  (0.008) | 0.04c | 0.03c | | 0.004  (0.003) | 0.22 | 0.025c |
| Thalamus | 0.024  (0.014) | 0.10 | 0.001c | 0.022  (0.015) | 0.16 | 0.03c | | 0.007  (0.005) | 0.22 | 0.025c |
| Striatum | -0.002  (0.008) | 0.80 | 0.001c | 0.002  (0.007) | 0.78 | 0.03c | | -0.003  (0.003) | 0.33 | 0.025c |
| Neocortex | -0.003  (0.007) | 0.65 | 0.001c | 0.002  (0.007) | 0.78 | 0.03c | | -0.002  (0.003) | 0.41 | 0.025c |
| Cerebellum | -0.004  (0.006) | 0.55 | 0.001c | -0.003  (0.005) | 0.57 | 0.03c | | -0.001  (0.002) | 0.78 | 0.025c |
| *Model 2* |  |  |  |  |  |  | |  |  |  |
| T1 lesionale | -0.011  (0.020) | 0.59 | 0.03 c | -0.016  (0.021) | 0.46 | 0.02 c | | -0.003  (0.008) | 0.66 | 0.22 |
| Perilesional 0-3e | 0.003  (0.008) | 0.68 | 0.03 c | -0.001  (0.009) | 0.90 | 0.02 c | | 0.001  (0.003) | 0.67 | 0.22 |
| Perilesional 3-6e | 0.021  (0.009) | 0.03 c | 0.03 c | 0.019  (0.010) | 0.08 | 0.02 c | | 0.006  (0.004) | 0.15 | 0.22 |
| **DTI and MRI parameters** |  |  |  |
| Mean FA in NAWMd | -36.021  (12.865) | 0.01c | NA | -37.744  (17.279) | 0.04c | NA | | -29.887  (41.532) | 0.48 | NA |
| Mean MD in NAWMd | -0.005  (0.103) | 0.72 | NA | -0.001  (0.016) | 0.94 | NA | | -0.020  (0.035) | 0.58 | NA |
| *Model 3* |  |  |  |  |  |  | |  |  |  |
| T1 lesion loadg | 0.212  (0.070) | 0.008c | 0.12 | 0.154  (0.071) | 0.046c | 0.28 | | 0.051  (0.029) | 0.10 | 0.290 |
| T2 lesion loadg | 0.279  (0.097) | 0.01c | 0.12 | 0.214  (0.102) | 0.05 | 0.28 | | 0.067  (0.040) | 0.11 | 0.0.290 |
| WM parenchymal fractiong | 0.519  (50.276) | 0.08 | 0.12 | 0.191  (0.308) | 0.54 | 0.28 | | 0.207  (0.100) | 0.05 | 0.0.290 |
| GM parenchymal fractiong | -0.255  (0.341) | 0.47 | 0.12 | -0.103  (0.371) | 0.79 | 0.28 | | -0.185  (0.121) | 0.14 | 0.0.290 |
| Brain parenchymal fractiong | -0.038  (0.233) | 0.87 | 0.12 | -0.082  (0.2496) | 0.74 | 0.28 | | -0.036  (0.086) | 0.68 | 0.0.290 |

Abbreviations: DTI = diffusion tensor imaging; EDSS = expanded disability status scale; MSSS = multiple sclerosis severity scale; DVR = distribution volume ratio; SE = standard error; NAWM = normal appearing white matter; FA = fractional anisotropy; MD = mean diffusivity; WM = white matter; GM = gray matter; ROI = region of interest; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

a Regression coefficients for clinical and MRI variables in each ROI were estimated with rm ANCOVA model using contrasts adjusted for age and with different regions of interest regarded as repeated measures. Due to different interactions, three separate models (Model 1, Model 2 and Model 3) were used. These included the main effects of MRI variables or regional DVR values in lesion-associated and non-lesion-associated ROIs, and clinical variables and their interaction. For the interactions of FA and MD to EDSS, MSSS and disease duration, ANCOVA was used.

b n = 20; RRMS n = 10, SPMS n = 10.

c Statistically significant at the level of *p* < 0.05.

d Mean FA and MD data available for 19 patients (10 RRMS and 9 SPMS).

e Gadolinium negative T1 lesional and perilesional regions of interest.

f Statistical significance for global interaction effect between clinical, MRI or DTI variables and regional DVR.g Lesion loads and GM and WM parenchymal fractions calculated as the respective absolute volumes normalized by total intracranial volume

**Table e-2. Regional [11C](*R*)-PK11195 binding within healthy controls and relapsing and secondary progressive multiple sclerosis patients.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Region of interest** | **HC** | **RRMS** | **SPMS** |
| **(n = 17)** | **(n = 10)** | **(n = 10)** |
| NAWM | 1.27 [0.07] | 1.26 [0.05] | 1.38 [0.09] |
| Gd- T1 lesions | NA | 1.22 [0.33] | 1.16 [0.09] |
| Gd- 0–3 mm rima | NA | 1.28 [0.11] | 1.30 [0.08] |
| Gd- 3–6 mm rima | NA | 1.20[0.12] | 1.33 [0.08] |
| Gd+ T1 lesions | NA | 1.41 [0.87] | 1.46 [0.13] |
| Gd+ 0–3 mm rimb | NA | 1.29 [0.31] | 1.53 [0.09] |
| Gd+ 3–6 mm rimb | NA | 1.30 [0.17] | 1.46 [0.09] |
| Thalamus | 1.54 [0.08] | 1.56 [0.08] | 1.75 [0.02] |
| Striatum | 1.26 [0.07] | 1.26 [0.08] | 1.27 [0.08] |
| Neocortex | 1.37 [0.06] | 1.44 [0.09] | 1.43 [0.05] |
| Cerebellum | 1.19 [0.05] | 1.16 [0.05] | 1.15 [0.06] |

The specific [11C](*R*)-PK11195 binding is reported as mean [SD] distribution volume ratios.

Abbreviations: HC = healthy controls; RRMS = relapsing remitting multiple sclerosis;

SPMS = secondary progressive multiple sclerosis; NAWM = normal appearing white matter; Gd- = gadolinium negative; Gd+ = gadolinium positive; NA = not available.

a perilesional circular regions of interest 0-3 mm and 3-6 from the edge of gadolinium negative lesions

b perilesional circular regions of interest 0-3 mm and 3-6 from the edge of gadolinium positive lesions

**Table e-3. Age-adjusted associations**a **of regional [11C](*R*)-PK11195 binding with DTI parameters in all multiple sclerosis patients**b

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Mean FA in NAWMd** | | | **Mean MD in NAWMd** | | |
|  | Coefficient (SE) | *p* | *p* for interactionf | Coefficient  (SE) | *p* for interaction | *p* for interactionf |
| **[11C](*R*)-PK11195 DVR** |  |  |  |  |  |  |
| *Model 1* |  |  |  |  |  |  |
| NAWM | -1.981  (0.470) | 0.001c | <0.001c | 0.002  (0.001) | 0.005c | 0.001c |
| Thalamus | -3.62  (0.727) | <0.001c | <0.001c | 0.004  (0.001) | 0.001c | 0.001c |
| Striatum | -0.674  (0.520) | 0.21 | <0.001c | 0.001  (0.000) | 0.65 | 0.001c |
| Neocortex | 0.150  (0.505) | 0.77 | <0.001c | 0.000  (0.000) | 0.94 | 0.001c |
| Cerebellum | -0.0982  (0.407) | 0.81 | <0.001c | 0.000  (0.000) | 0.23 | 0.001c |
| *Model 2* |  |  |  |  |  |  |
| T1 lesionale | -0.252  (1.626) | 0.88 | 0.01c | 0.001  (0.001) | 0.45 | 0.08 |
| Perilesional 0-3e | -0.713  (0.723) | 0.34 | 0.01c | 0.001  (0.001) | 0.37 | 0.08 |
| Perilesional 3-6e | -2.356  (0.731) | 0.005c | 0.01c | 0.002  (0.001) | 0.01c | 0.08 |

Abbreviations: DTI = diffusion tensor imaging; FA = fractional anisotropy; NAWM = normal appearing white matter; MD = mean diffusivity; SE = standard error; DVR = distribution volume ratio; ROI = region of interest; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

a Regression coefficients for clinical and MRI variables in each ROI were estimated with rm ANCOVA model using contrasts, adjusted for age and with different regions of interest regarded as repeated measures. Due to different interactions, two separate models were used. These included the main effects of DTI variables and regional DVR in lesion-associated and non-lesion-associated ROIs, and their interaction.

b n = 20; RRMS n = 10, SPMS n = 10.

c Statistically significant at the level of *p* < 0.05.

d Mean FA and MD data available for 19 patients (10 RRMS and 9 SPMS).

e Gadolinium negative T1 lesional and perilesional regions of interest.

f Statistical significance for global interaction effect between DTI variables and regional DVR.

**Table e-4. Age-adjusted associations**a **of regional [11C](*R*)-PK11195 binding with MRI volumetric parameters in all multiple sclerosis patients**b

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **T1 lesion load**c | | | **T2 lesion load**c | | | **Cortical GM parenchymal fraction**c | | | **Cerebral WM parenchymal fraction**c | | |
|  | Coefficient  (SE) | *p* | *p* for interactionf | Coefficient  (SE) | *p* | *p* for interactionf | Coefficient  (SE) | *p* | *p* for interactionf | Coefficient  (SE) | *p* | *p* for interactionf |
| **[11C](*R*)-PK11195 DVR** |  |  |  |  |  |  |  |  |  |  |  |  |
| *Model 1* |  |  |  |  |  |  |  |  |  |  |  |  |
| NAWM | 0.102  (0.015) | <0.001d | <0.001d | 0.073  (0.010) | <0.001d | <0.001d | -0.001  (0.000) | 0.06 | 0.008 d | 0.007  (0.007) | 0.35 | 0.56 |
| Thalamus | 0.186  (0.025) | <0.001d | <0.001d | 0.131  (0.016) | <0.001d | <0.001d | -0.001  (0.001) | 0.03d | 0.008d | 0.005  (0.013) | 0.71d | 0.56 |
| Striatum | 0.050  (0.020) | 0.03 d | <0.001d | 0.035  (0.014) | 0.02 d | <0.001d | 0.000  (0.000) | 0.90 | 0.008d | 0.003  (0.006) | 0.64d | 0.56 |
| Neocortex | 0.016  (0.021) | 0.45 | <0.001d | 0.014  (0.014) | 0.33 | <0.001d | 0.000  (0.000) | 0.93 | 0.008d | 0.003  (0.005) | 0.55 | 0.56 |
| Cerebellum | 0.003  (0.017) | 0.86 | <0.001d | 0.005  (0.011) | 0.66 | <0.001d | 0.000  (0.000) | 0.22 | 0.008d | 0.009  (0.003) | 0.03 d | 0.56 |
| *Model 2* |  |  |  |  |  |  |  |  |  |  |  |  |
| T1 lesionale | 0.020  (0.072) | 0.78 | 0.046 d | 0.008  (0.049) | 0.87 | 0.03 d | 0.007  (0.014) | 0.61 | 0.23 | -0.017  (0.017) | 0.34 | 0.62 |
| Perilesional 0-3e | 0.053  (0.027) | 0.07 | 0.046d | 0.038  (0.019) | 0.06 | 0.03d | -0.002  (0.001) | 0.79 | 0.23 | -0.005  (0.008) | 0.54 | 0.62 |
| Perilesional 3-6e | 0.094  (0.074) | 0.005d | 0.046d | 0.068  (0.021) | 0.005d | 0.03d | -0.009  (0.007) | 0.18 | 0.23 | -0.002  (0.008) | 0.78 | 0.62 |

Abbreviations: GM = gray matter; WM = white matter; SE = standard error; DVR = distribution volume ratio; NAWM = normal appearing white matter; ROI = region of interest; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

a Regression coefficients for clinical and MRI variables in each ROI were estimated with rm ANCOVA model using contrasts, adjusted for age and with different regions of interest regarded as repeated measures. Due to different interactions, two separate models were used. These included the main effects of MRI variables and regional DVR in lesion-associated and non-lesion-associated ROIs, and their interaction.

b n = 20; RRMS n = 10, SPMS n = 10.

c Lesion loads and GM and WM parenchymal fractions calculated as the respective absolute volumes normalized by total intracranial volume

d Statistically significant at the level of *p* < 0.05.

e Gadolinium negative T1 lesional and perilesional regions of interest.

f Statistical significance for global interaction effect between MRI variables and regional DVR.

**e-References**

e1. Rissanen E, Tuisku J, Rokka J, et al. In vivo detection of diffuse inflammation in secondary progressive multiple sclerosis using positron emission tomography imaging and radioligand [11C]PK11195. J Nucl Med 2014;55:939-944.

e2. Barkhof F, Filippi M, Miller DH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain 1997;120:2059-2069.

e3. Rissanen E, Virta JR, Paavilainen T, et al. Adenosine A2A receptors in secondary progressive multiple sclerosis: a [11C]TMSX brain PET study. J Cereb Blood Flow Metab 2013;33:1394-1401.

e4. Su Z, Herholz K, Gerhard A, et al. [11C]-(R)PK11195 tracer kinetics in the brain of glioma patients and a comparison of two referencing approaches. Eur J Nucl Med Mol Imaging 2013;40:1406-1419.

e5. Hunter HJ, Hinz R, Gerhard A, et al. Brain inflammation and psoriasis: a [11 C]-(R)-PK11195 positron emission tomography study. Br J Dermatol 2016;175:1082-1084.

e6. Heiss WD, Habedank B, Klein JC, et al. Metabolic rates in small brain nuclei determined by high-resolution PET. J Nucl Med 2004;45:1811-1815.

e7. de Jong HW, van Velden FH, Kloet RW, Buijs FL, Boellaard R, Lammertsma AA. Performance evaluation of the ECAT HRRT: an LSO-LYSO double layer high resolution, high sensitivity scanner. Phys Med Biol 2007;52:1505-1526.

e8. Alakurtti K, Aalto S, Johansson JJ, et al. Reproducibility of striatal and thalamic dopamine D2 receptor binding using [11C]raclopride with high-resolution positron emission tomography. J Cereb Blood Flow Metab 2011;31:155-165.

e9. Hinz R, Jones M, Bloomfield P, Boellaard R, Turkheimer FE, PJ T. Reference tissue kinetics extraction from [11C]-(R)-PK11195 scans on the High Resolution Research Tomograph (HRRT). Neuroimage, 2008: T65.

e10. Schmidt P, Gaser C, Arsic M, et al. An automated tool for detection of FLAIR-hyperintense white-matter lesions in multiple sclerosis. Neuroimage 2012;59:3774-3783.

e11. Rousset OG, Ma Y, Evans AC. Correction for partial volume effects in PET: principle and validation. J Nucl Med 1998;39:904-911.