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Reply

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We read with interest the comments by Raffel et al on our article,[1] which demonstrated increased brain translocator protein (TSPO) expression in multiple sclerosis (MS) by ^{11}C-PBR28. Raffel et al suggested that our study is in conflict with existing ^{11}C-PBR28 literature in MS.[2,3] Our findings, however, are consistent with previous neuropathological and positron emission tomography (PET) imaging observations,[4–7] and recently presented ^{11}C-PBR28 data on MS.[8]

The earlier works cited by Raffel et al were not methodologically designed to investigate neuroinflammation in the gray matter in MS[9]; one study included only 4 stable patients.[3] However, those studies were instrumental in demonstrating the ability of ^{11}C-PBR28 to reveal TSPO expression in white

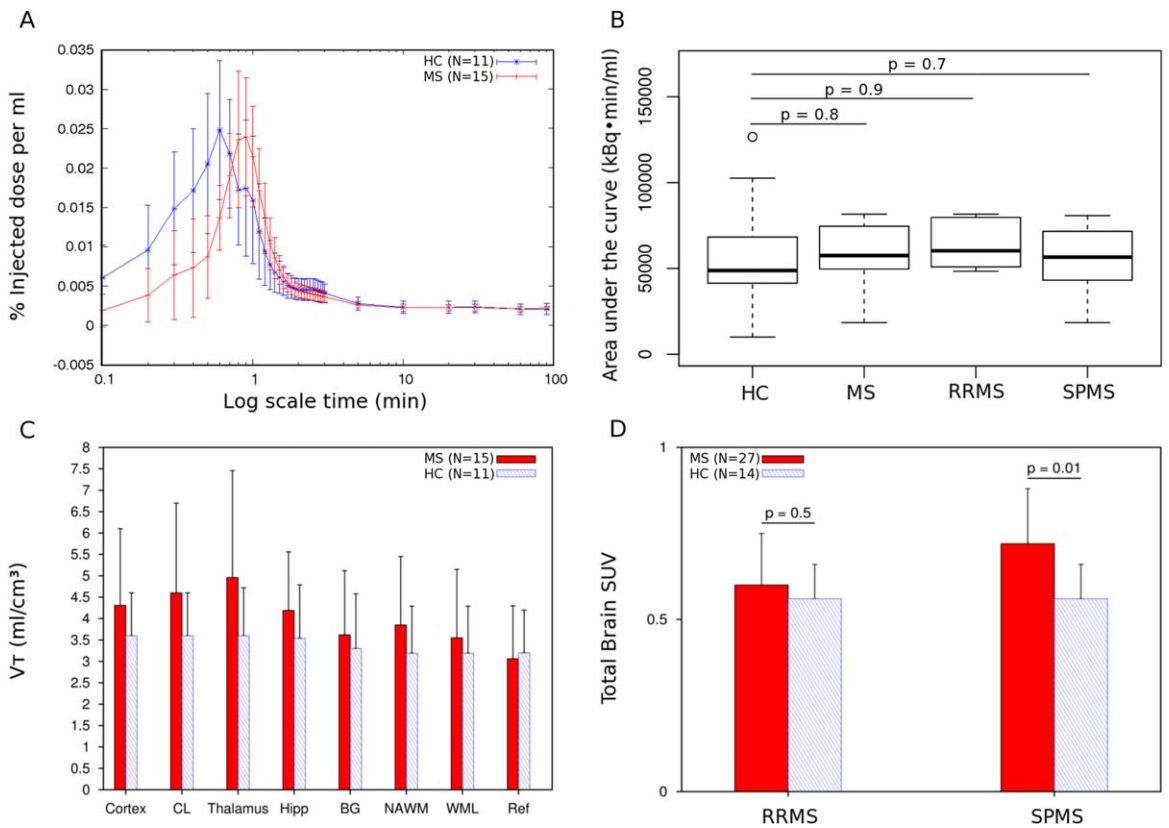

FIGURE: (A) Mean percentage and standard deviation of injected dose per milliliter in plasma over time in multiple sclerosis (MS) subjects (n = 15) and healthy controls (HC; n = 11). (B) Boxplot showing the area under the plasma curves in all MS (n = 15), relapsing–remitting MS (RRMS; n = 5), secondary progressive MS (SPMS; n = 10), and HC (n = 11). No significant differences were found between the groups, by linear regression adjusting for binding affinity. (C) Histogram showing mean ^{11}C-PBR28 volume of distributions (V_T) and standard deviations in different brain regions for both MS subjects (n = 15) and HC (n = 11). No significant differences were found in ^{11}C-PBR28 V_T between the two groups in all regions assessed by using linear regression and adjusting for binding affinity. (D) Histogram showing total brain ^{11}C-PBR28 standardized uptake values (SUV) and standard deviation in subjects with RRMS (n = 12) and SPMS (n = 15), and in HC (n = 14). Significant differences were found in total brain ^{11}C-PBR28 SUV between SPMS and HC, but not in RRMS vs HC (by linear regression adjusting for age and binding affinity). BG = basal ganglia; CL = cortical lesions; Hipp = hippocampus; NAWM = normal-appearing white matter; Ref = reference region; WML = white matter lesions.[Color figure can be viewed at wileyonlinelibrary.com]

matter areas of active inflammation in the disease, and in showing good reproducibility of this radiotracer.

Raffel et al questioned our normalization method that used a pseudoreference clustering approach based on the identification of voxels with similar ^{11}C-PBR28 values in MS and controls, located in the central brain normal-appearing white matter. Because pathological and imaging studies reported frequent and diffuse brain microglia/macrophage activation in MS,[4–7] the use of a consistent anatomical region for normalization would require the assumption that this brain region would be devoid of microglia pathology in all patients. Global brain normalization would also not be optimal in the context of a multifocal disease such as MS. For this reason, a supervised clustering method for normalizing PET data has been previously employed for ^{11}C-PK11195 TSPO imaging.[6,10] Although their algorithm was different from ours, both select as reference regions a set of voxels (in the gray matter in their case) in different anatomical areas, which might differ within each individual.

Raffel et al made the following assumptions: (1) MS subjects show, relative to controls, higher ^{11}C-PBR28 plasma concentrations; and (2) this would translate into a global increase in ^{11}C-PBR28 standardized uptake values (SUV). Hence, the observed increased ^{11}C-PBR28 uptake in MS would not reflect higher TSPO expression in the brain, and thereby neuroinflammation. Currently, we are not aware of any evidence of peripheral blood inflammation associated with increased plasma TSPO binding in MS. MS subjects and controls showed similar levels of ^{11}C-PBR28 plasma concentrations, and no differences in the area under the blood curves (Fig). With the exception of the pseudoreference region, volumes of distribution were generally increased in MS relative to healthy subjects. Finally, relative to controls, only secondary progressive MS subjects had globally increased SUV, but not relapsing–remitting MS cases, which, however, exhibited higher ^{11}C-PBR28 uptake in cortex and cortical lesions.

We conclude that our ^{11}C-PBR28 findings in MS reflect increased brain TSPO expression, suggesting neuroinflammation.

Author Contributions

All authors contributed equally to drafting this reply.

Potential Conflicts of Interest

Nothing to report.

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KCNA2 Mutations Are Rare in Hereditary Spastic Paraplegia

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In a recent study in *Annals of Neurology*, Helbig et al reported that a recurrent *KCNA2* substitution, p.R294H, was identified as the likely cause of complicated hereditary spastic paraplegia (HSP) or ataxia with intellectual disability in 3 families.[1] In 2 families, the pattern of inheritance was autosomal dominant, and in one family with 1 affected individual, the mutation was de novo. Some of the patients had cognitive disability, ataxia, and seizures. *KCNA2* encodes the potassium voltage-gated channel subfamily A member 2, and mutations in this gene were previously associated with epileptic encephalopathy, intellectual disability, and ataxia.[2] Mice with mutations in the *KCNA2* orthologue also presented with cerebellar ataxia.[3]

We extracted and analyzed sequencing data on *KCNA2* from 158 individuals from 65 families with pure or complicated HSP, whose DNA was sequenced using whole exome