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

sequencing. These individuals are of diverse ethnical backgrounds, and most of them were recruited through the CanHSP network,[4] which includes 7 centers across Canada. All patients were diagnosed by neurologists with expertise in motor neuron diseases, and all provided informed consent before participating in the study, which was approved by the respective institutional review boards. The average coverage of *KCNA2* was $\times 217$, with a range of $\times 58$ to $\times 379$ across all samples, and in all samples 100% of the coding nucleotides of *KCNA2* were covered by $> \times 10$.

No nonsynonymous, splice-site, or stop mutations were identified in any of the HSP patients. Only 3 synonymous variants were found. One is a rare variant c.G372A (p.E124E), with allele frequency (AF) of 0.0002 in ExAC (exac.broadinstitute.org), but it was found in only 1 patient of 6 affected with HSP from the same family. Two more common variants were also identified, c.G1185C (p.A395A, AF = 0.05, rs78349687) and c.T1026C (p.D342D, AF = 0.14, rs12407942), which also did not segregate with the HSP phenotype, and the allele frequencies are too common to be considered likely pathogenic.

Our data suggest that HSP patients with *KCNA2* mutations are rare, at least in our ethnically diverse population. We suggest that the various phenotypes reported in individuals with *KCNA2* mutations in the recent studies, including epilepsy, intellectual disability, ataxia, and spasticity, represent a spectrum of the same disorder with the same underlying mechanism, especially because some of the recently reported cases had phenotypes similar to those previously reported. Although novel phenotypes associated with known genes are well recognized, sufficient data that support the proposed genotype–phenotype correlation is necessary. The identification of more families with clinically confirmed HSP and *KCNA2* mutations would support this mutation being a separate entity causing HSP.

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Author Contributions

All authors were responsible for conception and design of the study, and for acquisition and analysis of data. Z.G.-O. and G.A.R. were responsible for drafting a significant portion of the manuscript.

Potential Conflicts of Interest

Nothing to report.

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De Novo *KCNA2* Mutations Cause Hereditary Spastic Paraplegia

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In a recent study, Helbig et al[1] identify a recurrent variant in *KCNA2*, which encodes the voltage-gated potassium channel, Kv1.2, as a novel cause of hereditary spastic paraplegia (HSP) in two unrelated families. Previously, gain-of-function and dominant-negative mutations in *KCNA2* have been implicated in early-onset epileptic encephalopathies, ataxia, or intellectual disability,[2–4] making this an interesting and unexpected finding, where epilepsy and other neurodegenerative disorders overlap.

We therefore examined whole-exome data and performed Sanger sequencing in a cohort of individuals with likely inherited neurological disorders and found 1 heterozygous de novo case with the same mutation described by Helbig et al[1] (Figure). Their third family had a *KCNA2* de novo