Imaging of neuroinflammation in migraine with aura

A [^{11}C]PBR28 PET/MRI study

Daniel S. Albrecht, PhD, Caterina Mainero, MD, PhD, Eri Ichijo, MS, Noreen Ward, MS, Cristina Granziera, MD, PhD, Nicole R. Zürcher, PhD, Oluwaseun Akeju, MD, Guillaume Bonnier, PhD, Julie Price, PhD, Jacob M. Hooker, PhD, Vitaly Napadow, PhD, Marco L. Loggia, PhD,* and Nouchine Hadjikhani, MD, PhD*

Correspondence

Dr. Hadjikhani
nouchine@
nmr.mgh.harvard.edu

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Abstract

Objective

To determine if migraine with aura is associated with neuroinflammation, which has been suggested by preclinical models of cortical spreading depression (CSD) as well as imaging of human pain conditions.

Methods

Thirteen migraineurs with aura and 16 healthy controls received integrated PET/MRI brain scans with [^{11}C]PBR28, a radioligand that binds to the 18 kDa translocator protein, a marker of glial activation. Standardized uptake value ratio (SUVR) was compared between groups, and regressed against clinical variables, using region of interest and whole-brain voxelwise analyses.

Results

Compared to healthy controls, migraineurs demonstrated SUVR elevations in nociceptive processing areas (e.g., thalamus and primary/secondary somatosensory and insular cortices) as well as in areas previously shown to be involved in CSD generation (visual cortex). SUVR levels in frontoinsula cortex, primary/secondary somatosensory cortices, and basal ganglia were correlated with frequency of migraine attacks.

Conclusions

These findings demonstrate that migraine with aura is associated with neuroimmune activation/neuroinflammation, and support a possible link between CSD and glial activation, previously observed in animals.

*These authors contributed equally to this work as co-senior authors.

From the A.A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown.

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Glossary

ACC = anterior cingulate cortex; **CSD** = cortical spreading depression; **IHS** = International Headache Society; **MNI** = Montreal Neurological Institute; **MPRAGE** = magnetization-prepared rapid gradient echo; **NSAID** = nonsteroidal anti-inflammatory drug; **ROI** = region of interest; **SUV** = standardized uptake value; **SUVR** = standardized uptake value ratio; **TSPO** = translocator protein.

Despite being the third most prevalent medical disorder worldwide,¹ the pathophysiology of migraine remains incompletely understood. Cortical spreading depression (CSD) is now well-accepted as the pathophysiologic mechanism underlying migraine aura,² but the mechanisms leading to headache pain in migraine following an episode of aura are still not fully understood in humans (for review, see reference 3). Recent data from rodent models demonstrated that CSD results in a cascade of events leading to the release of proinflammatory mediators, activation of trigeminal nerve fibers, and sterile inflammation.⁴ However, direct evidence of neuroinflammation in migraineurs is lacking.

While the role of neuroinflammation in human pain disorders remains uncertain, our group previously demonstrated that patients with chronic low back pain exhibit elevated levels of the 18 kDa translocator protein (TSPO),⁵ a marker of glial activation.⁶ In the healthy CNS, TSPO is constitutively expressed at low levels by multiple cell types, including glia and neurons.⁷ However, during neuroinflammatory states, TSPO is substantially upregulated, particularly in astrocytes and microglia,⁸ which can be quantified by the PET ligand [¹¹C]PBR28.⁹ In turn, CSD has been shown to induce elevated TSPO-ligand uptake in a rodent model.¹⁰ Despite these results, no study has ever assessed TSPO levels in human migraineurs. Here, we report findings from our investigation in human migraineurs, using combined [¹¹C]PBR28 PET/MRI. We hypothesized that individuals who experience migraine with aura would exhibit increased glial activation, as indicated by elevated TSPO PET signal, in brain regions contributing to aura presentation and in regions involved in nociceptive processing, compared to healthy controls.

Methods

Standard protocol approvals, registrations, and patient consents

This cross-sectional study was conducted at the Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital. The institutional review board and the Radioactive Drug Research Committee approved the protocol, and all participants gave written informed consent.

Participants

Demographic information for study participants is displayed in table 1. Thirteen patients experiencing migraine with aura completed study procedures, and were compared with 16 healthy controls recruited for other studies at our center,

selected for group matching in regard to age, sex, and *TPSO* polymorphism. Demographic and imaging data for a portion of the healthy controls has been reported previously.^{5,11,12} Migraineurs were recruited via neurologists and the Partners clinical trials website from June 2012 to May 2016, and controls were recruited from the general population. Inclusion criteria for migraineurs were diagnosis meeting the 2004 International Headache Society (IHS) criteria for migraine with aura (IHS 1.1), with a minimum of 1 and a maximum of 15 attacks a month, experiencing migraine attacks for at least a year, and willingness to refrain from nonsteroidal anti-inflammatory drug (NSAID) exposure in the 2 weeks prior to scanning. Participants were excluded for recent hospitalization for a major psychiatric disorder, endorsing or testing positive for illicit drug use, any known inflammatory disease (e.g., inflammatory bowel disease), or any contraindications for PET or MRI scanning (e.g., pregnancy, claustrophobia, ferromagnetic implants). During an initial screening visit, a blood sample was obtained to genotype participants for the Ala147Thr *TSPO* polymorphism, which is known to affect binding affinity for [¹¹C]PBR28.¹³ Low-affinity binders (Thr/Thr) were excluded from participation; high-affinity binders (Ala/Ala) and mixed-affinity binders (Ala/Thr) were included and genotype was modeled in the statistical design. Participants were scanned in the interictal period (with the exception of one patient who experienced a migraine attack during the scan session), and required to have at least one migraine attack with aura at least 15 days before the imaging session. This was selected as a threshold based on a previous animal study showing increasing TSPO levels up to 15 days after induction of CSD (no further point was examined).¹⁰ When the data were analyzed excluding the patient who experienced a migraine attack during scanning, the results were unchanged. Therefore, all analyses included this patient.

Twenty-nine participants with migraine were enrolled in the study; out of these, 13 completed scan visits. Causes for attrition were low-affinity binding (*n* = 2), cancelling after first screening for reasons concerning issues with injection of a radioactive tracer (*n* = 8), not wanting to stop taking anti-inflammatories (*n* = 1), lost to follow-up (*n* = 1), and failing to be able to schedule a scanning session following at least 2 episodes of migraine in the last 15 days (*n* = 4).

No migraineurs were taking antimigraine preventive medications. Because there are no previously published studies using TSPO PET to image migraineurs, we had no prior effect sizes on which to base power analyses for sample size determination.

Table 1 Participant characteristics

Variable	Migraineurs (n = 13)	Controls (n = 16)	p Value
Age, y	31.2 ± 13	37.0 ± 11	0.210
Sex, M/F	3/10	6/10	0.404
TSPO genotype, MAB; HAB	6; 7	8; 8	0.837
Weight, kg	68.2 ± 14	66.8 ± 10	0.767
Injected mass, nmol/kg	0.12 ± 0.05	0.11 ± 0.11	0.902
Specific activity, MBq/μmol	66.2 ± 32	87.0 ± 41	0.145
Injected dose, MBq	406 ± 35	428 ± 44	0.163
Migraines/mo	4.22 ± 4.1	NA	
Days between last migraine and PET scan	8.08 ± 5.3	NA	
Total migraines in last 4 weeks	4.00 ± 3.3	NA	

Abbreviations: HAB = high affinity binder; MAB = mixed affinity binder; MBq = megabecquerel. All continuous variables are shown as mean ± SD.

Image acquisition

Dynamic [^{11}C]PBR28 scans were performed with an integrated MRI/PET scanner consisting of a dedicated brain avalanche photodiode-based PET scanner in the bore of a Siemens (Munich, Germany) 3T Tim Trio MRI. Structural T1 images were acquired prior to tracer injection for anatomical localization, spatial normalization of PET data, and generation of attenuation correction maps.¹⁴ Dynamic PET acquisition began with an IV bolus injection of [^{11}C]PBR28. PET data were stored in listmode format.

Data analysis

Standardized uptake value (SUV) images (60–90 minutes post-administration of [^{11}C]PBR28) were generated as described previously.^{5,12} Magnetization-prepared rapid gradient echo (MPRAGE)-based attenuation correction was performed according to published methods.¹⁴ SUV maps were coregistered to the MPRAGE, transformed to Montreal Neurological Institute (MNI) space, and smoothed with an 8 mm (full width at half maximum) Gaussian kernel using FSL tools (FMRIB's Software Library, version 4.1.9, fmrib.ox.ac.uk/fsl/). Reconstruction of cortical surface from the T1 images was performed using Freesurfer 5.3.0 (surfer.nmr.mgh.harvard.edu/). Previously, TSPO PET signal has been shown to exhibit considerable interindividual variability, which may not be linked to neuroinflammation.¹⁵ In order to account for this variability, several groups have reduced variability by normalizing PET signal to uptake in whole brain or whole gray matter.^{5,12,16,17} While this approach may improve the detection of focal effects by robustly reducing between-participant variability, it may be not be appropriate when the condition investigated is characterized by widespread, rather than regional, inflammation, or when the extent of suspected inflammatory response is unknown. For some pathologies, a viable option is the identification of an a priori, anatomically defined pseudo-reference region,

based on knowledge that this region is relatively unaffected in the diseases studied. For instance, our group has recently validated the use of the occipital cortex in patients with chronic low back pain and amyotrophic lateral sclerosis.⁶ However, because the occipital cortex is known to be affected in migraine with aura, for this study we devised a strategy aimed at identifying a data-driven pseudoreference region, based on the absence of statistically significant SUV differences between groups. To this end, whole-brain voxelwise group SUV comparisons were conducted independently for high-affinity and mixed-affinity binders, covarying for age and injected dose (although these variables were not different across groups; p s > 0.16; table 1). The resulting Z-map was thresholded to include only voxels exhibiting values between −0.2 and +0.2 (i.e., p > 0.84), and the thresholded Z-maps for each genotype were combined to create a conjunction mask. The resulting mask was further intersected with an eroded MNI brain mask to ensure that no CSF or extra-brain voxels were included (figure 1). Voxel-wise SUV ratio (SUVR) images were obtained by dividing SUV images by average SUV from this minimal group-difference region. Mean SUVR was extracted from several a priori selected regions of interest (ROIs): thalamus, primary somatosensory (S1) representation of the face, insula, trigeminal nuclei, and primary visual cortex (V1). Thalamus and S1 were chosen because they previously demonstrated elevated [^{11}C]PBR28 SUVR in another pain disorder: chronic back pain.⁵ Specifically, thalamic ROI corresponded to the left thalamic cluster that exhibited statistically significant differences across groups in our previous study.⁵ For the S1 ROI, we selected the somatotopic representation of the face, as our previous study suggested that [^{11}C]PBR28 S1 elevations in pain patients may occur within the somatotopic representations of the body site affected by their pain symptoms (i.e., the representation of the lumbar spine in *chronic*

Figure 1 No group difference in pseudoreference uptake

low back pain patients⁵). Since the face representation is immediately ventral to the representation of the hand, the somatosensory face area ROI was created by having an expert neuroscientist (N.H.) manually draw the portions of the postcentral gyrus and central sulcus ventral to the hand knob¹⁸ on the postcentral gyrus label from the Harvard-Oxford Cortical Structural Atlas, which is distributed with FSL (fmrib.ox.ac.uk/fsl). The insula, trigeminal nuclei, and V1 were included as these regions have been highly implicated in migraine pathology,^{19,20} including aura presentation. Insula and V1 ROIs were obtained using the “insular cortex” and “intracalcarine cortex” labels from the Harvard-Oxford atlas, respectively. All ROI data were extracted from images in standard MNI space. All probabilistic labels were thresholded at the arbitrary threshold of 30. Trigeminal nucleus ROIs were obtained by creating a 3 mm³ sphere around the peak voxel from a previous study employing an air-puff challenge in migraineurs to functionally localize the spinal trigeminal nuclei.²¹

Statistical analysis

Group differences in demographics were assessed with *t* tests for continuous variables and Fisher exact test for categorical variables. ROI SUVR was compared across groups using general linear models, employing Bonferroni correction for multiple comparisons. Associations between ROI SUVR and clinical variables (i.e., number of migraine attacks per month, days between the scanning visit and last attack, and total number of attacks in the last 4 weeks) were assessed with linear regression analyses, with *TSPO* polymorphism as a regressor of no interest. In addition, we assessed group differences and relationships between SUVR and clinical variables on a whole-brain voxelwise level with a mixed-effect analysis using FSL’s FEAT tool, a cluster-forming threshold of $Z = 2.3$, and a cluster size significance threshold of $p = 0.05$ to correct for multiple comparisons. Age and injected dose were included as regressors of no interest in statistical models assessing between-group differences. *TSPO* polymorphism was included as a regressor of no interest in all statistical models.

Data availability statement

Anonymized data will be shared by request from any qualified investigator for purposes of replicating procedures and results.

Results

Participant characteristics

Participant information is displayed in table 1. There were no group differences in distribution of sex or *TSPO* polymorphism, or in average age, weight, or any tracer injection measure ($ps > 0.145$). Clinical details are given in table 2.

A priori ROI group differences

Group comparisons (figure 2) indicated that SUVR in patients was elevated in the left thalamus ($p = 0.002$; corrected), left insula ($p = 0.016$; corrected), and V1 ($p = 0.008$; corrected). SUVR was higher on average for right spinal trigeminal nucleus ($p = 0.056$; corrected), right S1 face area ($p = 0.08$; corrected), and right insula ($p = 0.096$, corrected), though these differences were not statistically significant. Additional brain regions (left S1 face area and left spinal trigeminal nucleus) did not yield group differences ($p > 0.384$, corrected).

Voxelwise group differences

A voxelwise group comparison revealed statistically significant elevations in [¹¹C]PBR28 signal in several regions throughout the brain in migraineurs compared to controls, including posterior insula, frontoinsular cortices, primary and secondary somatosensory cortex, primary motor, visual, and auditory cortices, dorsolateral, ventrolateral, and ventromedial prefrontal cortex, orbitofrontal cortex, and putamen (figure 3, A and C; see table 3 for a complete list of regions). Notably, in addition to primary visual cortex, increased PET signal was observed in motion-processing areas MT and V3A (figure 3B). There were no regions that showed an increase in controls compared to migraineurs.

Associations between imaging and clinical characteristics

In migraineurs, number of migraines per month preceding the scan demonstrated a positive correlation with SUVR in the

Table 2 Clinical characteristics

ID	Sex	Genotype	Age at scan, y	Aura symptoms	Average migraine frequency/ mo	Pain quality	Nausea	Photophobia	Phonophobia	Years with migraine	Days between last attack and scan	Attacks in the 4 weeks preceding scan, n	Quality of the attacks
101	M	Med	48	Motor: difficulty finding words	10–15	Unilateral, alternates between attacks, throbbing pain	No	Yes	Yes	30	4	9	1 Severe, 1 moderate, 7 mild
104	F	High	27	Visual: sparkly zig zag	2–6	Unilateral, throbbing pain, alternates between attacks, allodynia	Yes	Yes	Yes	12	4	3	1 Severe, 2 moderate to severe
				Motor: speech difficulty									
105	F	High	22	Visual: eyes sweeping around, cannot focus, vertigo	10–15	Unilateral, alternates between attacks, throbbing pain, occasionally aura without migraine	No	Yes	Yes	10	4	3	1 Severe, 2 only aura no headache
109	F	Med	30	Visual: spots	2	Unilateral, alternates between attacks, pulsating, mild to severe pain on different attacks	Yes	Yes	Yes	25	14	4	1 Severe, 1 moderate, 2 mild
				Sensory: tingling									
				Motor: speech difficulties									
110	M	Med	32	Visual: gray areas	1–4	Mild to severe, throbbing pain, on both sides, worsened by physical activity, allodynia	Yes	Yes	Yes	23	12	2	1 Mild, 1 moderate
				Motor: speech difficulty									
112	F	High	35	Visual: spots; loss of vision; squiggly lines on the periphery	1–3	Moderate 60% of the time, severe 40% of the time, more often on the left than the right side, pulsating pain, worsened by physical activity, allodynia	No	Yes	No	21	18	3	1 Severe, 2 moderate to severe
				Motor: word retrieval difficulty, slurring									
				Sensory: sensitive head and jaw (like pins and needles)									
113	M	Med	37	Visual: spots	1–3	Unilateral, right-sided 80% of the time, throbbing pain, worsened by physical activity	No	Yes	Yes	20	0	11	1 Severe, 2 moderate, 8 mild

Continued

Table 2 Clinical characteristics (*continued*)

ID	Sex	Genotype	Age at scan, y	Aura symptoms	Average migraine frequency/ mo	Pain quality	Nausea	Photophobia	Phonophobia	Years with migraine	Days between last attack and scan	Attacks in the 4 weeks preceding scan, n	Quality of the attacks
114	F	High	22	Visual: tunnel vision	3–4	Unilateral, throbbing pain, usually on the right side, worsened by physical activity	Yes	Yes	Yes	7	7	2	2 Severe
116	F	Med	23	Visual: left quadrant of visual field is cut out	2–3	Unilateral, mild to moderate pulsating pain, alternating sides, plus also aura without migraine	No	Yes	Yes	7	2	5	2 Mild migraines and 3 auras without migraine
				Motor: speech disturbances									
				Sensory: confusion									
118	F	High	65	Visual: white spots	8	Unilateral, sometimes spreads to the other side, alternates sides, moderate pain	Sometimes	Yes	Yes	50	9	5	2 Mild, 3 moderate
				Sensory: facial numbness; mental confusion									
				Motor: difficulty finding words									
125	F	Med	23	Visual: black spots in upper right visual field	1	Typically starts in the front and spreads backwards, severe throbbing pain, worsened by physical activity, allodynia	Sometimes	Yes	Yes	8	9	3	1 Mild, 1 moderate, 1 severe
				Sensory: extremely sensitive skin; slower cognitive processing; ringing in right ear; muffled/slowed down hearing									
126	F	High	18	Visual: glitters	3	Unilateral, more often on the left, moderate pulsating pain	Yes	Sometimes	Yes	2	8	7	2 Moderate, 5 severe
				Motor: slurred speech									
123	F	High	23	Visual: circle of light or dots; sensory (tingling, numbness)	1	Unilateral, alternates between attacks, moderate to severe throbbing pain, worsened by physical activity	Yes	Yes	Yes	10	14	1	Moderate

Figure 2 Region of interest (ROI) [^{11}C]PBR28 standardized uptake value ratio (SUVR) is elevated in migraineurs with aura

left S1 face area ROI ($r = 0.737$; $p = 0.006$, uncorrected). Monthly migraine frequency also showed a positive association with SUVR in V1 ($r = 0.549$; $p = 0.065$, uncorrected), though it did not meet statistical significance. There were no other significant associations in ROI analyses. Voxelwise regression analyses showed several brain regions where SUVR was positively correlated with number of migraine attacks per month (figure 4, table 3), including the right posterior insula/S2, right S1, left frontoinsula cortex, and pregenual anterior cingulate cortex (ACC). There were no other significant associations between SUVR and clinical variables, whether in ROI or voxelwise analyses.

Discussion

We provide in vivo evidence that migraineurs with one or several attacks of migraine with aura in the preceding 2 weeks exhibit brain elevations in [^{11}C]PBR28 signal, indicative of glial activation. Furthermore, we provide preliminary evidence that neuroinflammation in migraineurs is positively associated with the frequency of migraine attacks.

Previous studies using PET in migraine have examined function/metabolism with H_2O^{15} (e.g., references^{22–26}) or evaluated receptor availability (for example, 5HT_{1A} receptor with [^{18}F]MPFF),²⁷ whereas the present study addressed the question of neuroinflammation with glial uptake of [^{11}C]PBR28 as a proxy for neuroinflammation produced by CSD.

Among the regions displaying elevated [^{11}C]PBR28 signal in the migraineurs were structures involved in pain processing,

including basal ganglia, thalamus, insula, primary and secondary somatosensory cortices, and ACC,²⁸ that our previous work has directly implicated in migraine pathophysiology, by demonstrating increased intrinsic connectivity with the periaqueductal gray matter.²⁹ Interestingly, several of these regions had been shown to have lower 5-HT_{1B} binding in migraineurs, including ACC, sensorimotor cortex, and insula,³⁰ further implicating them in migraine pathology. In addition to pain-relevant regions, we observed elevation in [^{11}C]PBR28 signal in one area previously directly shown to be involved in CSD generation (extrastriate area V3A), as well as in areas affected during episodes of aura (e.g., Broca area).

The present work furthers the interpretation of brain alterations demonstrated in previous studies, particularly in the thalamus and in the basal ganglia. For instance, our group previously showed increased thalamic T1 relaxation time in migraineurs with aura, compared to migraineurs without aura and healthy controls, which could reflect increased glial content, or iron deposition.³¹ In addition, a recent study in migraineurs showed that the thalamus (dorsomedial) demonstrated abnormal oscillatory activity compared to controls.³² The observed oscillations were at a similar frequency to astrocytic Ca^{2+} waves, which could indicate increased astrocytic activation in this region, as has been previously suggested in a study of orofacial pain.³³ By demonstrating that the thalamus is associated with increases in [^{11}C]PBR28 signal, this work suggests that glial activation may underlie the alterations reported in those studies. Interestingly, elevation in dorsomedial thalamic [^{11}C]PBR28 signal was observed in our previous study in low-back pain patients.⁵ Basal ganglia

Figure 3 Voxelwise [^{11}C]PBR28 standardized uptake value ratio (SUVR) is elevated in migraineurs with aura

Regions of elevated PET signal in migraineurs with aura compared to controls are shown in red-yellow color scale. (A) Group differences in cortical [^{11}C]PBR28 SUVR are displayed on surface projections. There were no significant regions for the controls > migraineurs contrast. (B) Flat map of occipital cortex. Left hemisphere is shown on the left, right hemisphere on the right. Lighter gray = gyrus, darker gray = sulcus. (C) An axial slice is shown to display subcortical SUVR differences. CS = calcarine sulcus; M1 = primary motor cortex; MTG = middle temporal gyrus; plns = posterior insula; OFC = orbitofrontal cortex; S1/S2 = primary/secondary somatosensory cortex; SMG = supramarginal gyrus; STG = superior temporal gyrus; TOS = transverse occipital sulcus; V1 = primary visual cortex; vmPFC = ventromedial prefrontal cortex.

disorders have also been associated with migraine,³⁴ and one recent study reported anatomical and functional changes in the basal ganglia of migraineurs that they associated with repeated episodes of pain or the usage of triptans.³⁵ In this article, the authors also hypothesized that differences in the basal ganglia may be related to altered cortico-thalamic inputs and increased excitability state. Interestingly, we found a significant positive correlation between migraine frequency and [^{11}C]PBR28 uptake in the caudate and the putamen, but not in the thalamus. The precise relation between the basal ganglia, the cortico-thalamic activity, and the excitation/inhibition imbalance in migraine should be further examined in future studies.

Numerous preclinical studies provide evidence for a key role of glial cells (such as microglia and astrocytes) in the establishment or maintenance of persistent pain (e.g., reference 36). In the presence of an injury, for instance, these cells respond by undergoing a series of cellular and molecular changes collectively known as glial activation, which include proliferation (e.g., reference 37), upregulation of surface markers and receptors (e.g., P2RX4 and CX3CR1³⁸), and

production of cytokines and mediators and factors that activate or sensitize nociceptive neurons, including interleukin-6, interleukin-1- β , tumor necrosis factor- α , and the brain-derived neurotrophic factor.^{36,39} Since pharmacologic inhibition of microglial^{40,41} or astrocytic activation³⁹ prevents, reverts, or delays the establishment of persistent pain, animal studies provide evidence in support of a key role for glial cells in persistent pain, and may therefore be a promising therapeutic target for human pain as well. While the clinical significance of our observations remains to be evaluated, our data suggest that glial activation does occur in migraine, extending to this disorder our previous observation on a separate group of chronic pain patients.⁵

CSD is now well-established as the substrate of migraine aura² (for review, see reference 3). CSD is a slow propagating depolarization of neurons and glia that translates into neurologic symptoms depending on location. The most commonly reported auras in migraine are visual, but other types of aura exist, indicating involvement of nonvisual cortical regions, for example, somatosensory cortex with the sensation of tingling, or of inferior frontal cortex and Broca area with transitory

Table 3 Coordinates from regions showing significantly larger standardized uptake value ratio (SUVR) in migraineurs compared to controls in the voxelwise analysis ($p < 0.05$, corrected) and coordinates from regions showing significant positive correlations between SUVR and migraines per month in the voxelwise analysis

Region	Z value, corr	MNI coordinates, mm			Cluster size, voxels
		X	Y	Z	
Regions showing significantly larger SUVR in migraineurs compared to controls					
Occipital cortex	5.684	20	−86	−4	11,864
	3.837	−38	−76	6	
	3.734	40	−70	−4	
Middle temporal gyrus	4.686	46	−32	−6	
	4.165	62	−36	−2	
	4.031	66	−20	−16	
Angular gyrus	4.272	40	−62	36	
	3.907	−40	−62	40	
Primary somatosensory cortex	4.184	50	−16	46	
	3.846	60	−16	32	
Ventrolateral PFC	4.097	44	22	10	
Putamen	4.076	30	−4	4	
Lingual gyrus	4.064	18	−66	−4	
	4.059	10	−48	−2	
Fusiform gyrus	3.923	46	−58	−16	
Cerebellum	3.747	0	−56	−28	
	3.463	8	−72	−22	
	3.306	−30	−70	−28	
Primary motor cortex	3.743	50	−2	40	
Dorsolateral PFC	3.741	44	26	28	
Precuneus	3.730	−8	−70	38	
	3.261	−20	−64	−22	
Superior parietal lobule	3.700	−38	−40	52	
Frontal operculo-insular cortex	3.648	42	14	6	
Primary auditory cortex	3.607	40	−26	14	
Primary visual cortex	3.470	−10	−68	12	
Orbitofrontal cortex	3.205	44	34	−10	
Posterior insula	3.119	34	−24	12	
Orbitofrontal cortex	4.878	−36	22	−12	
Superior temporal gyrus	4.443	−52	−32	2	
Middle temporal gyrus	4.119	−50	−16	−18	
Frontal operculo-insular cortex	4.398	−36	20	−2	
	4.284	−42	10	6	
Ventromedial PFC	4.472	−6	38	−16	

Continued

Table 3 Coordinates from regions showing significantly larger standardized uptake value ratio (SUVr) in migraineurs compared to controls in the voxelwise analysis ($p < 0.05$, corrected) and coordinates from regions showing significant positive correlations between SUVr and migraines per month in the voxelwise analysis (*continued*)

Region	Z value, corr	MNI coordinates, mm			Cluster size, voxels
		X	Y	Z	
Ventrolateral PFC	4.253	16	50	-14	
	4.145	-20	56	-6	
	4.097	14	50	-14	
	3.987	26	48	18	
Thalamus	3.914	-4	-16	2	
Dorsolateral PFC	3.861	-18	58	16	
Anterior midcingulate cortex	3.544	-2	36	0	
Premotor cortex	3.340	-2	46	20	
	3.471	-42	2	30	
Caudate	3.414	-14	14	2	
Pallidum	3.007	-20	-6	-4	
B) Regions showing significant positive correlations between SUVr and migraines per month					
Primary motor/primary somatosensory cortex	4.794	-34	-22	44	5,588
Posterior insula/secondary somatosensory cortex	4.076	38	-24	18	
Putamen/internal capsule	4.012	-24	-2	14	
Paracentral lobule	3.475	22	4	10	
	4.034	-14	-28	46	
Premotor cortex	3.892	-42	2	28	
Supplementary motor area	3.607	-8	0	46	
Superior longitudinal fasciculus white matter	3.560	28	-38	34	
Caudate	3.507	-14	2	14	
Perigenual ACC	2.913	12	10	8	
	3.406	4	28	14	
	3.238	-8	38	-2	
Frontoinsular cortex	3.392	-28	24	0	
Primary somatosensory cortex	3.373	42	-14	36	

Abbreviations: ACC = anterior cingulate cortex; PFC = prefrontal cortex.

aphasia.⁴² However, it is possible that some auras originate in brain areas that do not express any specific externalizing symptoms, as could happen in prefrontal cortex, for example. Here, in addition to the primary visual cortex activation, we also observed elevated uptake in 2 visual extrastriate motion processing areas that have been demonstrated to be implicated in migraine pathophysiology, namely V3A and MT,^{2,43} in which we previously reported the originating focus of a visual aura² as well as increased cortical thickness.⁴³ In addition, many patients in the present report also described other aura

symptoms, such as difficulty finding words, speech difficulties, ringing in one ear, or mental confusion. Interestingly, we observed elevated glial activation in the inferior frontal cortex, frontal pole, orbitofrontal cortex, and the primary auditory cortex. The elevation in [¹¹C]PBR28 uptake in these areas could be interpreted as evidence of astrocytic activation following CSD in these cortical areas, which is in line with preclinical data showing a proinflammatory role for CSD-induced astrocytic activation.⁴⁴ However, there is also evidence of microglial activation following CSD,⁴⁵ and the

Figure 4 Migraine frequency is positively associated with [^{11}C]PBR28 standardized uptake value ratio (SUVR)

(A) Regions showing a significant positive correlation between [^{11}C]PBR28 SUVR and migraine attacks per month preceding the scan in migraineurs are shown in red–yellow color scale ($p < 0.05$, cluster-corrected). Regions in green showed significant effects in both voxel-wise analyses, that is, SUVR in these regions was significantly higher in migraineurs compared to controls (see figure 3), and also significantly positively correlated with migraine frequency. No regions displayed significant negative associations between SUVR and migraine frequency. plns = posterior insula; S1/S2 = primary/secondary somatosensory cortex; SMA = supplementary motor area. (B) Average SUVR from several regions showing a positive association between migraine attacks per month and SUVR. SUVR in migraineurs is plotted against migraine frequency; healthy control SUVR is displayed to the left for comparison. Group differences were significant for right posterior insula/S2 ($p = 0.012$), S1 ($p = 0.016$), and frontoinsular cortex ($p = 0.001$). All data are adjusted for age, *TSPO* polymorphism, and injected dose.

specific contributions of these cellular populations to the *TSPO* PET signal cannot be resolved with our current methods.

In addition to the observed group differences in [^{11}C]PBR28 PET signal, we also report a significant positive correlation between SUVR and migraine attack frequency, in the posterior insula, basal ganglia, frontoinsular cortex, S1, and perigenual ACC. Several studies reported associations between the frequency of migraine attacks and functional activity in the insula, both in anterior and posterior subdivisions,^{29,46,47} regions that have been associated with different aspects of migraine pathology,²⁰ emphasizing the importance of insular activity in moderating the probability of migraine attacks. In fact, our recent fMRI study with episodic migraine patients

found cortical amplification and reduced habituation in the insula for trigeminal somatosensory afference.²¹ In addition, one of the aforementioned studies also found increased pain-related activity in S1 of migraineurs experiencing frequent attacks compared to those with less frequent attacks.⁴⁶ This area lies in close proximity to the one in which we found a positive correlation between [^{11}C]PBR28 SUVR and migraine frequency (figure 4), and both are situated in the S1 somatotopic representation of the face. Moreover, migraineurs exhibited significantly elevated *TSPO* PET signal in this area compared to controls, indicating that glial activation in S1 may be increased as a result of more frequent attacks. Interestingly, while few previous studies have reported differential basal ganglia function in migraineurs, one recent study found that noxious stimuli-induced brain activation of the

basal ganglia was significantly different between migraineurs dichotomized by the frequency of migraine attacks.³⁵ This suggests that caudate and putamen may play a role in the transition from episodic to chronic migraine states. Our data indicate that neuroinflammation in several regions, including insula, S1, and basal ganglia, is also associated with the prevalence of migraine attacks. Further research is needed to investigate the putative role of glial activation in both migraine frequency and the transition from episodic to chronic migraine.

There are limitations to the present study. First, the sample size of the current cohort was relatively small, due in part to the stringent criteria requiring a recent migraine attack with aura. Despite this, we were able to detect statistically significant elevations in [¹¹C]PBR28 signal in migraineurs and associations between TSPO PET and the frequency of attacks. These results will need to be replicated in larger samples. Second, in the present study we did not perform kinetic modeling with radiometabolite-corrected arterial input function, which is considered by many to be the gold standard for quantification of TSPO binding. While previous work suggests that semiquantitative ratio metrics may be at least similarly sensitive to detect pathology-related group differences in [¹¹C]PBR28 signal as classic kinetic modeling techniques,^{6,11} future studies will need to validate the use of these metrics in migraine datasets.

Our data demonstrate important in vivo evidence that migraine with aura is accompanied by prolonged signs of neuroinflammation in humans. Our observation may open relatively unexplored therapeutic strategies in migraine, aimed at attenuating glial activation, which have already demonstrated neuroprotective effects in rodent studies.^{48,49} Although a recent study suggested that this approach may be ineffective in chronic migraine,⁵⁰ the lack of therapeutic efficacy for this condition may be due to the fact that chronic migraine pathophysiology is different than that underlying episodic migraine with aura. Future research is needed to better understand the role of neuroinflammation in migraine, and to evaluate whether the patterns of glial activation observed here are specific to migraine with aura, or can also be observed in migraine without aura.

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Disclosure

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Appendix Authors

Name	Location	Role	Contributions (all authors provided critical feedback and helped shape the research, analysis, and manuscript)
Daniel S. Albrecht, PhD	Massachusetts General Hospital, Charlestown	Author	Contributed to data collection, analysis, interpretation of results, and drafting of the manuscript
Caterina Mainero, MD, PhD	Massachusetts General Hospital, Charlestown	Author	Conceived and planned experiments, carried out experiments
Eri Ichijo, MS	Massachusetts General Hospital, Charlestown	Author	Major role in data collection
Noreen Ward, MS	Massachusetts General Hospital, Charlestown	Author	Analyzed data
Cristina Granziera, MD, PhD	Massachusetts General Hospital, Charlestown	Author	Major role in data collection and interpretation of results
Nicole R. Zürcher, PhD	Massachusetts General Hospital, Charlestown	Author	Collected and analyzed data
Oluwaseun Akeju, MD	Massachusetts General Hospital, Charlestown	Author	Major role in data collection
Guillaume Bonnier, PhD	Massachusetts General Hospital, Charlestown	Author	Analyzed data and contributed to interpretation of results

Continued

Appendix (continued)

Name	Location	Role	Contributions (all authors provided critical feedback and helped shape the research, analysis, and manuscript)
Julie Price, PhD	Massachusetts General Hospital, Charlestown	Author	Significantly contributed to interpretation of results
Jacob M. Hooker, PhD	Massachusetts General Hospital, Charlestown	Author	Major role in data collection
Vitaly Napadow, PhD	Massachusetts General Hospital, Charlestown	Author	Contributed significantly to interpretation of results
Marco L. Loggia, PhD	Massachusetts General Hospital, Charlestown	Author	Contributed to data collection, analysis, interpretation of results, and drafting of the manuscript
Nouchine Hadjikhani, MD, PhD	Massachusetts General Hospital, Charlestown	Author	Conceived and planned experiments, carried out experiments, analyzed data, interpreted results, drafted the manuscript

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