Signs of Cortical Inflammation in Migraine Measured with Quantitative Magnetic Resonance Imaging: A Registry for Migraine (REFORM) Study

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Objective: The involvement of cortical inflammation in migraine, particularly migraine with aura, has been a subject of considerable interest, but has proved challenging to demonstrate. We aimed to detect and characterize signs of cortical inflammation in adults with migraine using a novel, multimodal magnetic resonance imaging (MRI) technique.
Methods: We used T2 mapping to measure water content/cellularity, T1 mapping to measure tissue microstructure integrity, and apparent diffusion coefficient (ADC) mapping to measure intra- or extracellular edema. We compared these values between participants with migraine (with and without aura) and healthy controls using general linear models adjusted for age and sex.
Result: Two hundred ninety-six adult participants with migraine and 155 age- and sex-matched healthy controls provided eligible imaging data. Among the participants with migraine, 103 had migraine with aura, 180 chronic migraine, and 88 were ictal during the scan. Participants with migraine had higher quantitative T2 (qT2) in the left occipital cortex than healthy controls ($p < 0.0001$). In migraine with aura, the higher qT2 was more widespread and located bilaterally in the occipital cortices, compared with controls (left, $p < 0.0001$; right $p = 0.004$). Post-hoc analysis revealed overlapping ADC elevations in migraine with aura compared with controls ($p = 0.0069$).
Interpretation: Quantitative MRI changes compatible with cortical inflammation were detected in participants with migraine, and appeared driven by the subgroup with aura. Higher occipital qT2 in migraine with aura might represent either extracellular edema or accumulation of inflammatory microglia or astrocytes. These results support the importance of cortical inflammation in migraine pathophysiology, particularly in migraine with aura.

ANN NEUROL 2025;97:1168–1179

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.27197

Received Sep 24, 2024, and in revised form Dec 3, 2024. Accepted for publication Dec 7, 2024.

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Migraine is a debilitating neurological disorder affecting more than 1 billion people worldwide.[1] The clinical presentation is characterized by recurrent attacks of headache and accompanying symptoms, such as photophobia, phonophobia, and nausea or vomiting.[2] About one-third of those affected experience transient, focal neurological symptoms, also known as aura, which often manifest as visual disturbances preceding the headache phase.[2] The neurobiological substrate of aura is most likely related to cortical spreading depolarization (CSD).[3]

CSD is a self-propagating wave of depolarization that spreads slowly across the cerebral cortex.[4] This event triggers the release of pro-inflammatory mediators,[4] potentially leading to cortical inflammation.[5,6] The complex relationship between CSD, cortical inflammation, and the headache phase of migraine is well-documented in preclinical studies, but remains a contested subject that has been challenging to demonstrate in neuroimaging studies.[7] Of note, results from two magnetic resonance imaging (MRI) studies indicate an intact blood–brain barrier (BBB) during spontaneous attacks, both with and without aura.[8,9] Recent strides have, nonetheless, been made with the combined use of positron emission tomography (PET) and MRI. Notably, two small PET-MRI studies have identified inflammatory signals in the cortical and subcortical regions of 13 adults with migraine with aura. Of interest, in 11 adults with visual aura, these signals were located in the visual cortex and the adjacent parameningeal tissue.[10,11] While this PET tracer is strongly linked to inflammation, it is not entirely specific.[5,12–14] Complementary approaches would therefore be valuable for further assessing the inflammatory nature of the signal in migraine with aura.

Here, we present the results of a novel quantitative, multimodal MRI technique designed to detect cortical inflammation in adults with migraine, both with and without aura. This approach has previously been validated in multiple sclerosis and involves the combination of T2 mapping, T1 mapping, and apparent diffusion coefficient (ADC) mapping to assess microstructural tissue changes that are compatible with inflammatory activity.[15] We compared these measures between adults with migraine and age- and sex-matched healthy controls to explore the role of cortical inflammation in migraine pathophysiology.

Methods

Study Design and Participants

This study used an observational, cross-sectional design with data sourced from the MRI Core of the Registry for Migraine (REFORM) study.[16] The study protocol received approval from the ethics committee of the Capital Region of Denmark (H-20033264) and was preregistered on ClinicTrials.gov (NCT04674020). All participants provided written informed consent before undergoing any study-related procedures.

This study enrolled adult participants with migraine and age- and sex-matched healthy controls. The key inclusion criteria for the participants were: a history of migraine with or without aura for ≥12 months, and an average of ≥4 monthly migraine days within the past 3 months, in accordance with criteria of the International Classification of Headache Disorders, 3rd edition (ICHD-3).[2]

For healthy controls, key exclusion criteria were: a history of any primary or secondary headache disorder (except infrequent episodic tension-type headache), first-degree relatives with a primary headache disorder (except tension-type headache <5 days/month), history of any clinically significant condition, or regular use of medication (full inclusion and exclusion criteria are provided in Supplementary Tables 1 and 2). We matched each healthy control to a participant with migraine who had the same sex and age ± 2 years and balanced the groups according to age, sex, and age distribution.

Clarification of Terms

Although no clear consensus exists on the definition of inflammation, modern definitions commonly describe inflammation as a physiological, regulated, protective response to an underlying process that involves cells of the innate or adaptive immune system and inflammatory mediators.[7,17] Neuroinflammation denotes inflammation within neuronal tissues. In this study, we investigate neuroinflammation through surrogate measures, such as edema and cell accumulation, using quantitative MRI.

Study Procedures

Participants underwent a single MRI scan after a physical and neurological examination, and a semi-structured interview that comprehensively characterized their headache. At the time of the scan, we characterized any headache and its associated symptoms and classified it according to the ICHD-3 criteria for a migraine attack.[2] We also recorded the days since the participant's last headache, migraine attack, aura, and the first day of last menstruation. Participants abstained from acute analgesics, triptans, anti-inflammatory, or anti-histaminergic agents for 48 h and caffeinated beverages or foods for 12 h before the scan.[12]

MRI Procedures

We acquired imaging data on a 3.0 Tesla Siemens MAGNETOM Prisma MRI unit (Siemens Healthineers,

Erlangen, Germany) equipped with a 32-channel head coil. We used foam pads to minimize head motion.

The imaging protocol included magnetization-prepared 2 rapid acquisition gradient echo (MP2RAGE),[18] combined generalized auto-calibrating partially parallel acquisition and model-based accelerated relaxometry by iterative nonlinear inversion (GRAPPATINI),[19] fluid-attenuated inversion recovery (FLAIR), magnetization-prepared rapid acquisition gradient echo (MPRAGE), and diffusion tensor imaging (DTI). The combined scan time was 55 min.[15] Sequence parameters were as follows; GRAPPATINI: 0.7 × 0.7 × 4.0 mm voxel size, TR of 5,000 ms, TE of 10 ms. MP2RAGE: 1.0 × 1.0 × 1.0 mm voxel size, TR of 5,000 ms, and TE of 60 ms. DTI: 2.0 × 2.0 × 2.0 mm voxel size, TR of 11,000 ms, TE of 60 ms. B1 of 0 s/mm^2, B2 of 1,000 s/mm^2, flip angle of 90 degrees, acquired in 30 diffusion directions. For further details of these and auxiliary sequence parameters (MPRAGE and FLAIR), see Supplementary Table 3.

All images were uploaded to a centralized server and reviewed by a certified neuroradiologist (D.T.) who was blinded to group assignments. Any anatomical variations or structural abnormalities were documented, and clinically significant incidental findings were noted in a designated text box, allowing the neuroradiologist to suggest potential clinical or imaging follow-up. The final report was forwarded to the senior author (M.A.), who oversaw the initiation of any necessary follow-up actions.

Preprocessing

We analyzed data in FreeSurfer version 7.2.0 on a single workstation (macOS Big Sur, version 11.6). We reconstructed the structural surface of the brain from the MPRAGE and FLAIR sequences, as previously described for this cohort.[20] This yields pial and white matter surfaces, which delineate the cortical ribbon.

The GRAPPATINI sequence yielded quantitative T2 (qT2) maps, the MP2RAGE sequence produced quantitative T1 (qT1) maps, and the DTI sequence provided ADC maps. Each participant's reconstructions were used as intermediates for more accurate registration of the quantitative maps to the cortical ribbon. Quantitative values were extracted from the cortical ribbon using a volume-to-surface algorithm that measured voxel values between the pial and white matter surfaces, sampled at every 25th percentile. These values were then averaged to yield individual surface maps of cortical qT2, qT1, and ADC values, which were assigned to a common surface template (fsaverage) for comparison between subjects. The surface maps were grouped and smoothed with a full-width at half-maximum kernel of 10 mm for qT2 and ADC maps, and 5 mm for qT1 maps (a 10 mm kernel caused some qT1 map comparisons to fail).[19]

Outcome Measures and Outcomes

This study evaluated cortical qT2, qT1, and ADC values as outcome measures. For histopathological correlates of these measures, see Fig 1.

Participant Classification: Categories and Subgroups

All participants were diagnosed and subgrouped according to the ICHD-3,[2] depending on (1) migraine frequency (either episodic or chronic migraine); (2) presence or absence of aura (migraine with aura or without aura); (3) headache status at MRI, with participants classified as either "ictal," if undergoing a migraine attack, "non-migraine headache," if experiencing a non-migraine headache, and "headache-free," if headache was completely absent (Fig 2). Migraine attacks were defined according to the ICHD-3, with the temporal criterion of 4–72 h exempted, to allow capture of early migraine attacks.[2]

Outcomes

For each outcome measure (ie, cortical qT2, qT1, and ADC), we compared (1) participants with migraine versus healthy controls; (2) between the groups of migraine with aura, migraine without aura, and healthy controls; and (3) between participants with chronic migraine, episodic migraine, and healthy controls. To evaluate the impact of headache status during MRI, we also compared between (4) ictal participants, headache-free participants, and healthy controls (Fig 2).

Furthermore, we explored associations of cortical qT2, qT1, and ADC values with three temporal factors: number of aura days in the past year, and the mean monthly migraine and headache days during the preceding 3 months.

Statistical Analysis

The study's sample size was data-driven, since there are yet no tools to estimate sample sizes for cortical qMRI measures. We compared all participants with migraine (including subgroups) and healthy controls using whole-cortex general linear models (GLM) in FreeSurfer, adjusted for age and sex (cluster-determining threshold of $p < 0.05$ and a clusterwise threshold of $p < 0.05$ based on Monte Carlo simulation). This statistical approach is standard for cortical qMRI.[21,22] For associations with continuous variables, we used a different-onset, different-slope GLM model. Region of interest (ROI) analyses were small volume corrected using new Monte Carlo simulations. We parcellated the cortex based on the Desikan-Killiany Atlas.

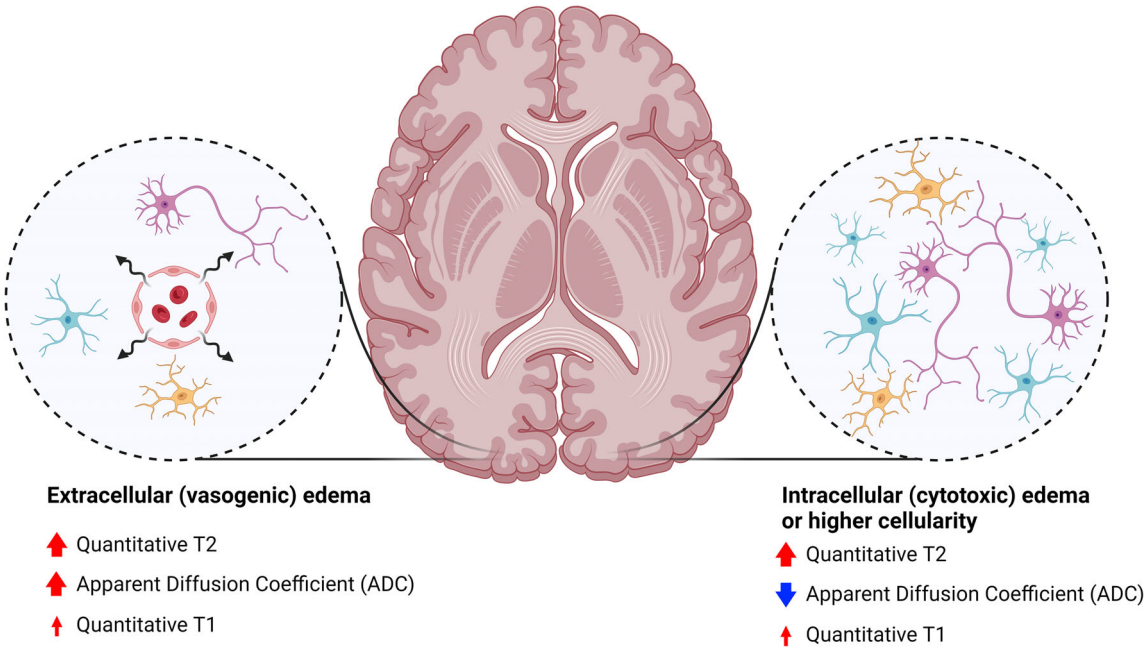

FIGURE 1: Histological interpretation of quantitative magnetic resonance imaging. Transverse relaxation time (qT2) correlates positively with tissue water content, and is increased during conditions of edema or greater cellularity. It correlates negatively with iron accumulation. Longitudinal relaxation time (qT1) correlates positively with tissue water content as well as loss of structural integrity. Like qT2, it also correlates negatively with iron accumulation. The apparent diffusion coefficient (ADC) measures the magnitude of water molecule diffusion and can differentiate extracellular from intracellular water. Extracellular water increases ADC values, whereas intracellular water decreases ADC values. [Color figure can be viewed at www.annalsofneurology.org]

Clinical data are presented as means with standard deviations or medians with interquartile range, depending on normality (assessed with Q-Q plots and histograms). Two-sample Kolmogorov–Smirnov tests compared age distributions of groups, while chi-squared tests assessed differences in categorical variables with all expected cell counts >30. All statistical tests on clinical variables were conducted using R version 4.2.0.

Results

Participants

From November 2020 to June 2022, we enrolled and scanned 306 participants with migraine and 160 healthy controls. After exclusions due to incidental findings, 296 participants and 155 healthy controls were eligible for analysis. The average age of the participants was 41.6 (SD: 12.4) years, and 261 (88.2%) were females. Chronic migraine was diagnosed in 180 (60.8%) and 103 (34.8%) reported a history of migraine with aura (Table 1, Supplementary Table 4 for aura characteristics). There were no significant differences in age ($p = 0.90$), age distribution ($p = 0.96$), or sex ($p = 0.28$) between the participants and healthy controls.

During the scan, 88 (29.7%) participants were experiencing a migraine attack (ictal), 116 (61.7%) a non-migraine headache, and 92 (31.1%) were headache-free (Table 1 and Supplementary Table 5). None experienced an aura attack during this study's paradigm. To examine the ictal phase, we included additional ictal scans from a longitudinal extension of the study. This yielded 132 ictal scans (33 [28.4%] receiving erenumab 140 mg monthly) from unique participants for comparison with controls (Supplementary Table 6 and Supplementary Figs 1 and 2). We also conducted sensitivity analyses excluding participants from the longitudinal extension who received erenumab treatment, which did not significantly alter the reported results.

Imaging Findings

Table 2 summarizes the comparisons of cortical qT2, qT1, and ADC values.

qT2: For qT2, 10 of 16 significant clusters were localized to the occipital cortex. Overall, migraine participants exhibited higher cortical qT2 in the left pericalcarine and lateral occipital cortex ($p < 0.0001$) compared to healthy controls (Fig 3). Those with visual aura (n = 96) displayed higher qT2 within the left lateral occipital ($p < 0.0001$) and bilateral pericalcarine cortices (left; $p = 0.0001$, right; $p = 0.004$) compared to healthy controls (Fig 4 and Supplementary Fig 3). When compared to participants without aura (n = 193), those with aura had higher qT2 in the left pericalcarine cortex ($p = 0.0001$), bilateral precuneus (left; $p = 0.0001$, right;

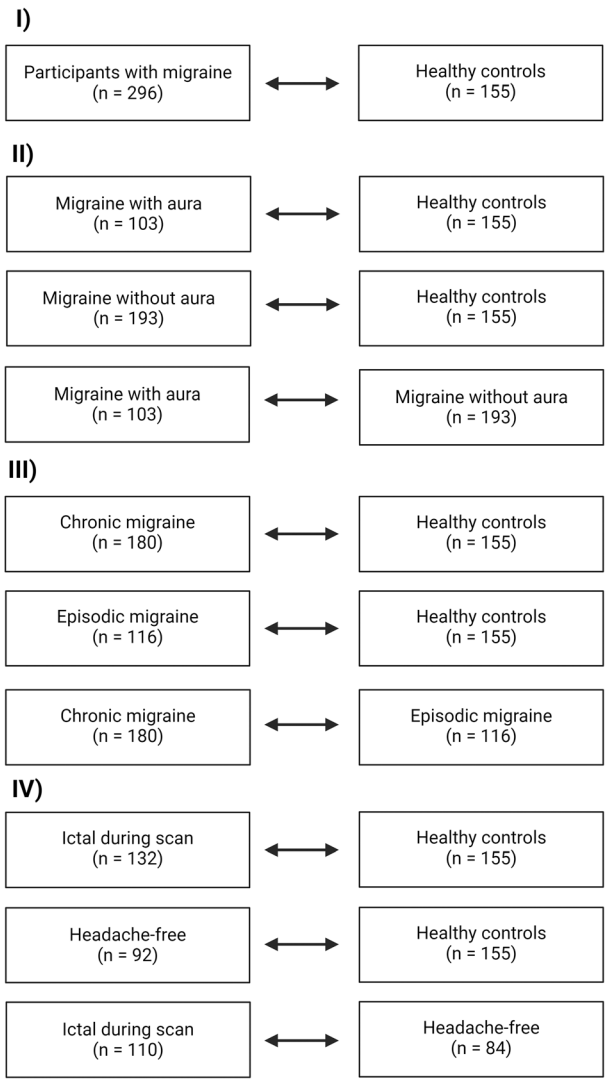

FIGURE 2: Overview of statistical comparisons. Participants could simultaneously belong to multiple non-mutually exclusive subgroups. For instance, a participant could both have episodic migraine subgroup, migraine with aura, and be ictal during the scan. This approach facilitated a detailed investigation of phenotypes across the migraine spectrum.

$p = 0.004$), and right postcentral ($p < 0.0001$) and paracentral cortex ($p < 0.0001$). In post-hoc analysis, participants with aura outside a migraine attack (n = 75) likewise had higher qT2 in bilateral occipital regions, including the bilateral pericalcarine cortex (left; $p = 0.0002$, right; $p = 0.0005$), the left lateral occipital cortex ($p < 0.0001$), and the right superior temporal cortex ($p = 0.006$), compared to healthy controls. Notably, post-hoc analyses showed no significant association between the qT2 signal and aura frequency within the past year or days elapsed since last aura. Likewise, there were no significant differences between participants with aura who were experiencing a migraine attack (without aura) compared to those who were not. For details, see Supplementary Figures 4 and 5.

Participants with chronic migraine displayed higher qT2 in the left pericalcarine ($p = 0.001$) and lateral occipital cortex ($p < 0.0001$) as well as the right cuneus ($p = 0.001$), compared to healthy controls (Supplementary Fig 6). The post-hoc analysis displayed no significant differences in qT2 in those with chronic migraine but without aura (n = 113, 62.8%) compared to healthy controls.

Compared to participants with episodic migraine, those with chronic migraine had lower qT2 in the right precentral ($p = 0.0004$) and middle temporal cortex ($p = 0.001$). Participants with migraine without aura had lower qT2 in bilateral superior frontal cortex compared to healthy controls (right; $p = 0.0001$, left; $p < 0.0001$). There were no significant differences between ictal and interictal participants, between ictal participants and healthy controls, or between interictal participants and healthy controls.

qT1: There were no significant differences in cortical qT1 between participants with migraine, any subgroups of migraine, or healthy controls.

ADC: Participants with chronic migraine had lower ADC in the right anterior cingulate cortex ($p < 0.0001$) and the left rostral middle frontal cortex compared with healthy controls ($p = 0.0004$), and in the right middle temporal cortex compared to participants with episodic migraine ($p = 0.0005$). There were no other significant differences in cortical ADC values between participants with migraine and healthy controls, or between migraine subgroups.

Associations with clinical variables: There were no significant associations between qT2, qT1, or ADC and monthly migraine – or headache days in any regions.

ROI analysis in participants with aura (post-hoc): The cluster of higher qT2 in participants with aura compared to healthy controls overlapped with higher ADC values in the left lateral occipital cortex ($p = 0.0069$, small volume corrected) (Supplementary Table 7 and Supplementary Fig 7). There were no significant differences in the right ROI or in qT1.

Discussion

Our study revealed a significant elevation in quantitative T2 (qT2) within the bilateral visual cortices of participants experiencing migraine with aura, precisely the brain regions associated with the origin of visual aura. From a medical physics standpoint, these higher qT2 values might suggest inflammatory edema and/or increased cellularity, aligning with existing literature correlating qT2 values with tissue water content and cellularity.[23–27] The higher qT2 was further supported by concurrent increases in the apparent diffusion coefficient (ADC) in a

TABLE 1. Demographics and Baseline Characteristics of the Study Population

Characteristics	Healthy Controls	Participants with Migraine	Migraine without Aura[a]	Migraine with Aura[b]	Episodic Migraine	Chronic Migraine
No.	155	296	193	103	116	180
Female, n (%)	133 (85.8)	261 (88.2)	171 (88.6)	90 (87.4)	99 (85.3)	162 (90.0)
Age in years, mean (SD)	41.1 (11.7)	41.6 (12.4)	40.8 (12.4)	43.2 (12.4)	43.3 (12.3)	40.6 (12.3)
Right-handed, n (%)	137 (88.4)	273 (92.2)	176 (91.2)	97 (94.2)	104 (89.7)	169 (93.9)
MMD, mean (SD)	NA	13.3 (6.8)	13.1 (6.5)	13.7 (7.3)	8.2 (2.6)	16.6 (6.6)
MHD, mean (SD)	NA	18.9 (8.1)	18.6 (8.1)	19.8 (8.0)	11.7 (5.6)	23.5 (5.7)
Monthly days with acute medication intake, mean (SD)	NA	11.2 (6.4)	11.3 (5.9)	10.7 (6.9)	9.6 (5.7)	12.2 (6.6)
Using preventive treatment, n (%)	NA	167 (56.4)	107 (55.4)	60 (58.3)	69 (59.5)	98 (54.4)
Medication-overuse headache, n (%)[c]	NA	108 (36.5)	76 (39.4)	32 (31.1)	27 (23.3)	81 (45.0)
Monthly aura days, mean (SD)	NA	NA	NA	4.1 (5.2)	NA	NA
Disease duration, years, mean (SD)	NA	22.9 (12.3)	22.7 (12.2)	24.1 (12.5)	23.7 (12.6)	22.4 (12.1)
History of depression, n (%)	NA	38 (12.8)	27 (14.0)	11 (10.7)	11 (9.5)	27 (15.0)
History of anxiety, n (%)	NA	32 (10.5)	19 (9.8)	13 (12.6)	12 (10.3)	20 (11.1)
Phase during scan						
Ictal, n (%)[d]	NA	88 (29.8)	60 (31.0)	28 (27.2)	23 (19.8)	65 (36.1)
Non-migraine headache, n (%)[e]	NA	116 (61.7)	76 (39.3)	40 (38.8)	40 (34.5)	76 (42.2)
Headache free, n (%)	NA	92 (31.1)	57 (29.5)	35 (34.0)	53 (45.7)	39 (21.6)

Note: Modified from Christensen et al.[18]

ICHD = International Classification of Headache Disorders, 3rd edition; MHD = monthly headache days; MMD = monthly migraine days; NA = not applicable; SD = standard deviation.

[a]Participants diagnosed with migraine without aura (ICHD-3, 1.1) and not migraine with aura (ICHD-3, 1.2).

[b]Participants diagnosed with migraine with aura (ICHD-3, 1.2) who could also have migraine without aura (ICHD-3, 1.1).

[c]Medication-overuse headache fulfilling ICHD-3 criteria (ICHD-3, code 8.2).

[d]Headache fulfilling ICHD-3 criteria for migraine without aura (ICHD-3, code 1.1).

[e]Headache not fulfilling ICHD-3 criteria for migraine without aura (ICHD-3, code 1.1).

localized area in the occipital cortex, indicating the presence of extracellular edema. Importantly, the quantitative T2 clusters in the occipital cortices remained significant when compared to both healthy controls and participants with migraine *without* aura, even when individuals with ongoing migraine headache were excluded, suggesting independence from the ictal phase. Similarly, though less marked, qT2 elevations in the overall migraine group and in those with chronic migraine were driven by individuals with aura.

Aura and Microglial Inflammation

The robust qT2 signal in the visual cortex aligns well with a prevalence of visual symptoms in 93.2% of the participants with aura. In the context of migraine with aura, the most plausible explanation is inflammatory edema or cell accumulation, supported by extensive research linking aura and CSD to inflammation.[4,5,28–32] This relationship between qT2 and inflammation is also evident in other neurologic disorders such as multiple sclerosis and encephalitis.[33–35] Since the qT2 signal was not linked to

TABLE 2. Differences in Cortical qT2, qT1, and ADC Values

Comparison	Cerebral Region	Lobe	*p* Value (−Log(p) at Max Vertex)	Extent of Area (mm^2)	MNI Coordinates X	Y	Z
Cortical qT2							
Participants with migraine versus healthy controls	Left lateral occipital cortex	Occipital	4.2023	2388.77	−29.4	−89.2	−4.6
Migraine with aura versus healthy controls	Left lateral occipital cortex	Occipital	3.4406	4350.81	−11.6	−84.5	10.9
	Left pericalcarine cortex	Occipital	3.8610	1264.92	−28.7	−89.2	−2.3
	Right pericalcarine cortex	Occipital	2.3515	1852.83	15.5	−91.7	−2.7
Migraine with aura versus migraine without aura	Left precuneus	Occipital/parietal	3.6025	1274.54	−14.3	−50.2	38.5
	Left pericalcarine cortex	Occipital	3.5683	1421.24	−13.1	−85.0	9.0
	Right paracentral	Frontal	4.1465	2337.53	18.5	−39.9	45.4
	Right precuneus	Occipital/parietal	2.6735	1255.93	17.8	−67.6	35.7
	Right postcentral	Parietal	4.7404	1228.86	39.8	−24.4	48.4
Participants with chronic migraine versus healthy controls	Left lateral occipital cortex	Occipital	4.2945	1711.73	−28.6	−89.8	−2.6
	Left pericalcarine cortex	Occipital	2.9132	2225.64	−10.6	−91.4	−3.2
	Right cuneus	Occipital	2.9894	2945.79	5.6	−81.8	23.2
Chronic migraine versus episodic migraine	Right precentral	Frontal	−3.3211	1428.44	38.4	4.9	13.5
	Right middletemporal cortex	Temporal	−2.9477	1178.31	63.8	−39.4	−5.3
Migraine without aura compared to healthy controls	Left superior frontal cortex	Frontal	−4.2874	1483.41	−16.5	39.0	41.8
	Right superior frontal cortex	Frontal	−3.8472	1555.73	14.1	58.8	17.0
Cortical qT1							
All specified comparisons	No significant clusters	NA	NA	NA	NA	NA	NA
Cortical ADC							
Chronic migraine versus healthy controls	Left rostral middle frontal gyrus	Frontal	−3.3630	1797.86	−20.3	51.8	24.2
	Right anterior cingulate cortex	Frontal	−4.0750	1474.74	6.2	19.4	30.2
Chronic versus episodic migraine	Right middletemporal cortex	Temporal	−3.2681	2562.65	64.7	−42.0	−5.9

MNI = Montreal Neurological Institute; NA = not applicable.

the time since last aura or aura frequency, this might suggest that occipital inflammation is a trait in people susceptible to migraine aura, and could potentially serve as a biomarker. These changes might result from cortical events associated with aura attacks (eg. CSD), or they might act as a predisposing factor for developing aura

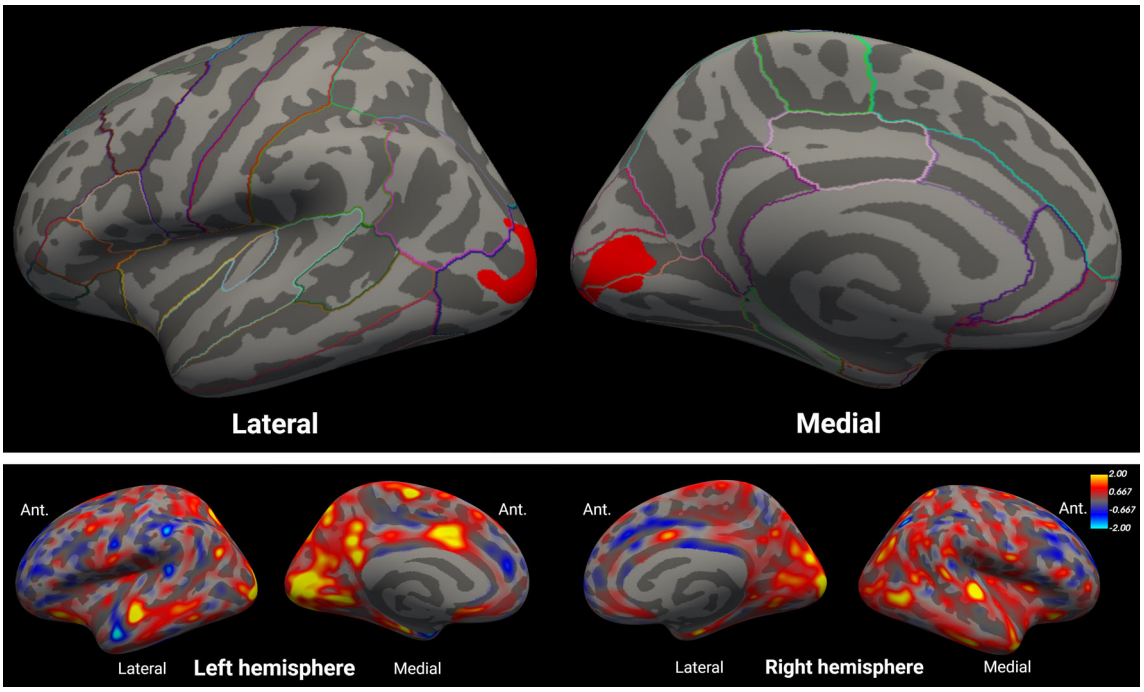

FIGURE 3: Cortical qT2 differences in migraine compared to healthy controls. Upper panel: Higher qT2 (red) in the left pericalcarine and lateral occipital cortex in participants with migraine with and without aura compared to healthy controls ($p < 0.0001$, corrected at clusterwise threshold $p < 0.05$). Lateral and medial view of the left hemisphere. Lower panel: uncorrected transparent significance maps of qT2 values of participants with migraine with and without aura compared to healthy controls, presented as −log of *p*-values and displayed on the lateral and medial aspects of the left and right hemisphere. Warm colors represent higher qT2 values in migraine. [Color figure can be viewed at www.annalsofneurology.org]

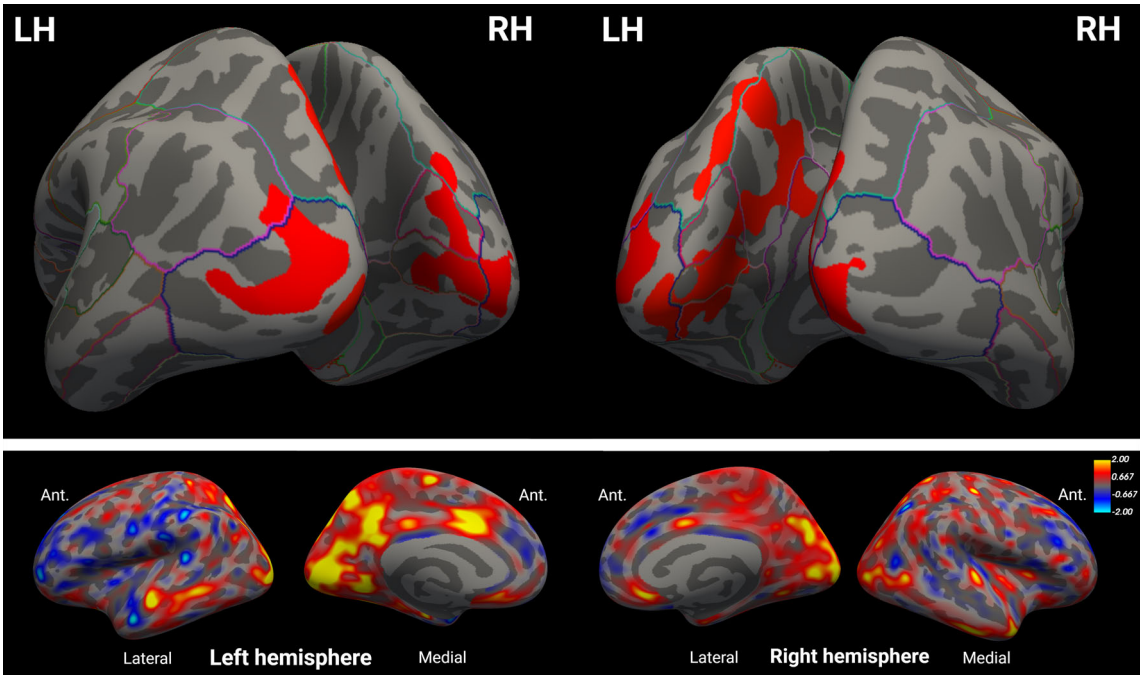

FIGURE 4: Cortical qT2 differences in migraine with aura compared to healthy controls. Upper panel: Higher qT2 (red) in multiple bilateral occipital regions in participants with migraine with visual aura compared to healthy controls (left lateral occipital cluster at $p = 0.0004$, left pericalcarine cluster at $p = 0.0001$, right pericalcarine cluster at $p = 0.004$. All clusters corrected at clusterwise threshold $p < 0.05$). Posterior views of the brain. Lower panel: uncorrected transparent significance maps of qT2 values of participants with migraine with aura compared to healthy controls, presented as −log of *p*-values and displayed on the lateral and medial aspects of the left and right hemispheres. Warm colors represent higher qT2 values in migraine with aura. [Color figure can be viewed at www.annalsofneurology.org]

symptoms. In the latter scenario, the signal could be related to a biologically conferred propensity toward aura. Cortical inflammatory activity could potentially increase local susceptibility to CSD, as suggested by some preclinical models of aura.[36,37] Similar findings of increased qT2 have been instrumental in identifying mesial temporal lobe sclerosis as a nidus in epilepsy cases, a condition often elusive to conventional MRI techniques.[38] Histologically, the qT2 signal in migraine with aura might also reflect increased cellularity, perhaps due to microglial inflammation. This interpretation is supported by previous research utilizing a PET tracer, 11C-PBR28, which binds translocator protein (TSPO), a mitochondrial outer membrane protein upregulated during inflammation due to microglia, astrocytes, and several other cell types.[10] In a study involving participants with frequent aura compared to healthy controls, higher TSPO uptake was observed. A subsequent study similarly revealed higher visual cortex and adjacent parameningeal uptake in participants with visual aura compared to healthy controls and an "active control" group of individuals with chronic low back pain.[11] The authors suggested that this parameningeal uptake might reflect inflammatory activity in macrophages, mast cells, or other cells. It is possible that TSPO upregulation is related to the higher qT2 observed in the current study, with both reflecting microglial or astrocytic proliferation. The sustained TSPO signal, unrelated to the days since the last attack, supports this hypothesis.[10,11] However, it is important to note that glial cells play a crucial role in buffering extracellular potassium and glutamate, and increased buffering capacity would decrease susceptibility to CSD.[39] These considerations suggest that the observed signal may be a consequence of the aura rather than its cause. Finally, the association between TSPO upregulation and qT2 signal in a study on relapsing–remitting multiple sclerosis provides additional evidence for the association between increased microglial cellularity and quantitative T2 alterations.[40] Increased microglial cellularity or edema might likewise manifest as thickening of the visual cortex, as reported in some studies on migraine with aura.[41]

Edema and BBB Integrity

Beyond microglial inflammation, the higher qT2 in migraine with aura could suggest inflammatory edematous changes. This edema is most likely extracellular, given the overlap of elevated ADC and qT2 in a cluster within the lateral occipital cortex. This aligns with animal studies, where CSD induces extravasation and cerebral edema through the release of inflammatory and permeabilizing mediators, such as histamine, prostaglandins, or substance P.[4] While extravasation persists for at least 20 min in these models,[28] our observations appeared more sustained than those in animals. Extravasation and edema can occur following BBB disruption. A prior study did not detect BBB breakdown following aura attacks.[9] The study scanned participants with a median of 7.6 h after aura onset, but found no differences compared to the interictal phase, using dynamic-contrast enhanced MRI with gadolinium. Yet, gadolinium only extravasates with substantial BBB breakdown, and the study was only powered to detect an 11% change in BBB permeability.[42] By comparison, qT2 is more sensitive to subtle changes in tissue water content due to extravasation, as it captures the water increase in the brain tissue.[23,24] Moreover, the approach applied here has limited sensitivity to myelin-water and essentially only captures the increase in extra and intracellular water, rendering it suitable for the study of permeability increases.[19] In contrast, qT1 is less sensitive to water accumulation than qT2, which might explain the absence of differences in cortical qT1 between participants with migraine, healthy controls, or any subgroups of participants with migraine.[15,23] Finally, it is also important to note that the changes appeared sustained in the current and prior studies, which might preclude detection of transient permeability changes within the same individual.[10,11]

Cortical Inflammation and Migraine without Aura

We found no clear involvement of cortical inflammation in migraine without aura. Though participants with chronic migraine displayed a less pronounced increase in qT2 within the bilateral occipital cortex, this was primarily driven by those with concomitant aura. The lack of increase in chronic migraine without aura, compared to healthy controls, episodic migraine, or chronic migraine with aura, suggests a distinct pathophysiological mechanism. Corroborating this observation, we found no changes in quantitative, inflammatory parameters in the cortex during attacks of migraine without aura compared to interictally. This expands upon results from two previous studies, which found that the BBB was intact to gadolinium during migraine attacks with or without aura compared to the interictal state.[8,9] Our findings thus support that increased BBB permeability or focal cortical inflammation are unlikely to be involved in initiating the headache phase of migraine attacks without aura. However, our study was unable to assess the presence of extracortical inflammation during migraine attacks. Animal models of both migraine headache and aura report dural inflammation,[43,44] but cortical inflammation is more consistently reported in models of aura.[5,6] This, together with our findings, suggests that cortical inflammation might be related to aura rather than individual migraine attacks without aura. Whether dural inflammation is present during or in the initiation of migraine attacks without aura

should, in part, be the focus of further neuroimaging research. The development of imaging techniques with sufficient resolution to visualize the dura is a prerequisite for this.

Microstructural Changes Unrelated to Aura

Despite the lack of inflammatory changes in migraine without aura, we observed other quantitative MRI changes. Participants who had migraine without aura displayed lower qT2 within bilateral superior frontal cortices compared to controls. This can be associated with iron deposition, a feature of neurodegeneration.[45] While the prefrontal cortex is thought to be involved in pain modulation,[46] this finding should be interpreted with caution, considering that it was not apparent in participants with chronic migraine compared to controls. Likewise, participants with chronic migraine had reduced qT2 in the precentral and middle temporal cortices, in comparison with participants with episodic migraine but not with controls, underlining the need for confirmatory studies. Participants with chronic migraine also exhibited lower ADC in the right anterior cingulate cortex and left middle frontal cortex, compared to controls, and in the left middle temporal cortex compared to participants with episodic migraine. While some of these areas, such as the anterior cingulate cortex, have clear roles in the processing of pain,[47] others are less obviously involved. Future studies should examine the nature of reduced ADC in chronic migraine further. Importantly, our findings do not indicate accumulating neurodegenerative changes in migraine with or without aura. This aligns with previous studies showing that migraine is not consistently associated with progressive white matter hyperintensities or cognitive decline.[48,49]

Limitations

Our T2 mapping used anisotropic voxels with a high in-plane resolution at the cost of poorer through-plane resolution. While this is the standard approach to acquire qT2 within a reasonable time frame, novel, accelerated T2 mapping approaches might improve the resolution in future studies.[50] Furthermore, within-subject comparisons were not compatible with the current study's design. Consecutive scanning of the same participants with qT2 might provide important information on the origin of the signal in aura, as well as more accurate insights into the ictal phase. Finally, all group comparisons were balanced on age and sex, except for the comparison between chronic and episodic migraine, where there was a significant difference for age. To account for this, all analyses were adjusted for sex and age. Our study included only seven participants who experienced sensory or aphasic aura symptoms without concurrent visual aura, which was insufficient to investigate these aura types in detail. Future studies should be designed to explore the early manifestations of auras occurring outside of the visual cortex.

Conclusions

In this large-scale, cross-sectional study, we demonstrated microstructural alterations consistent with cortical edema and/or increased cellularity within the visual cortices of people with migraine with aura. These findings suggest that cortical inflammation, potentially involving microglial and astrocytic activity, may be an underlying mechanism. Our novel results indicate that microstructural changes compatible with cortical inflammation might be a distinct biological trait of migraine with aura, offering new insights into its pathophysiology.

Acknowledgments

This study was funded by the Lundbeck Foundation Professor Grant (R310-2018-3711). The funding source had no influence on the study design or data interpretation. The authors wish to profoundly thank all participants who took part in this study. Furthermore, we extend our deepest appreciation of colleagues at the Danish Headache Center and Neurobiology Research Unit, whose contributions were key to the success of this study. We wish to extend a special thanks to neuroradiologist Daniel Tolnai, for inspecting the scans. Figure 1 was created with BioRender.com.

Author Contributions

M.A., F.M.A., and H.A. conceptualized and design of the study; R.H.C., H.M.A., M.O.P., R.R., C.G. contributed to acquisition and analysis of data; R.H.C., H.A., H.M.A., M.O.P., R.R., N.H., C.G., F.M.A., and M.A. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

C.G. received advisory board, speaker, consultancy fees, and research support from Novartis outside of the submitted work. F.M.A. has received honoraria for delivering lectures or participated in advisory boards for Novartis. M.A. acts as a consultant, speaker, or scientific advisor Amgen and Novartis and a primary investigator for ongoing Amgen and Novartis trials. Novartis and Amgen hold joint rights to commercialize erenumab. The authors report no other relevant conflicts of interest for the current work. R.C., H.M.A., H.A., M.O.P., R.R., and

N.H. report no relevant conflicts of interest for the current work.

Data Availability

Anonymized data supporting the findings presented in the current study will be shared upon reasonable request from a qualified investigator.

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