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## **Evaluation of a Clustering Approach to Define Distinct Subgroups of Patients With Migraine to Select Electroacupuncture Treatments**

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## Abstract

**Background and Objectives:** To propose a clustering approach to identifying migraine subgroups and test the clinical usefulness of the approach by providing prognostic information for electroacupuncture treatment selection.

**Methods:** Participants with migraine without aura (MWOA) were asked to complete a daily headache diary, self-rating depression and anxiety, and quality-of-life questionnaires.

Whole-brain functional connectivities (FCs) were assessed on resting-state fMRI. By integrating clinical measurements and fMRI data, partial least squares correlation and hierarchical clustering analysis were used to cluster participants with MWOA. Multivariate pattern analysis was applied to validate the proposed subgrouping strategy. Some participants had an 8-week electroacupuncture treatment, and the response rate was compared between different MWOA subgroups.

**Results:** In study 1, 97 participants (age of  $28.2 \pm 1.0$  years, 70 females) with MWOA and 77 healthy controls (HCs) (age of  $26.8 \pm 0.1$  years, 61 females) were enrolled (dataset 1), and two MWOA subgroups were defined. The participants in subgroup 1 had a significantly lower headache frequency (times/month of  $4.4 \pm 1.1$ ) and significantly higher self-ratings of depression (depression score of  $49.5 \pm 2.3$ ) as compared with the participants in subgroup 2 (times/month of  $7.0 \pm 0.6$  and depression score of  $43.4 \pm 1.2$ ). The between-group differences of FCs were predominantly related to the amygdala, thalamus, hippocampus, and parahippocampal area. In study 2, 33 participants with MWOA (age of  $30.9 \pm 2.0$  years, 28 females) and 23 HCs (age of  $29.8 \pm 1.1$  years, 13 females) were enrolled as an independent dataset (dataset 2). The classification analysis validated the effectiveness of the two-cluster

solution of participants with MWOA in datasets 1 and 2. In study 3, 58 participants with MWOA were willing to receive electroacupuncture treatment and were assigned to different subgroups. Participants in different subgroups exhibited different response rates ( $p = 0.03$ , OR CI = 0.086-0.93) to electroacupuncture treatment (18% and 44% for subgroups 1 and 2, respectively).

**Discussion:** Our study proposed a novel clustering approach to define distinct MWOA subgroups, which could be useful for refining the diagnosis of participants with MWOA and guiding individualized strategies for pain prophylaxis and analgesia.

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## Introduction

Migraine is a common neurological disease, and it is characterized by recurring moderate to severe asymmetrical headaches <sup>1</sup>. Researchers have acknowledged that migraine is a complex disease with heterogeneous clinical symptoms<sup>2</sup>, including interindividual variability of disability<sup>3</sup>, attack frequency<sup>4</sup>, and psychological states<sup>5</sup>. What drives such dramatic heterogeneity of migraine across individuals remains elusive.

The definition of homogeneous migraine subgroups that react differently to medications and treatments would have immediate applications in precision medicine. To date, some researchers have attempted to explore the phenotypic or neurological markers of participant subgroups and observed some encouraging evidence <sup>6</sup>. For example, Drysdale et al. defined subgroups by clustering participants with depression according to their brain functional networks and identified individuals most likely to benefit from targeted neurostimulation therapies <sup>7</sup>. The same principle would work for classifying participants with migraine considering that advanced neuroimaging techniques have proven helpful in assessing abnormal brain functional and structural alterations in migraine <sup>8</sup>. Schwedt and colleagues sub-classified migraine into two subgroups and reported between-group differences in their clinical measurements <sup>9</sup>. However, this study did not investigate whether patient subgroups had distinct treatment efficacy in migraine therapy.

Acupuncture reduces migraine attacks and is recommended as an effective alternative to drugs <sup>10</sup>. Functional Magnetic Resonance Imaging (fMRI) studies pointed out that acupuncture might have a therapeutic effect by modulating pain processing pathways on migraine <sup>11</sup>, and variability in acupuncture treatment efficacy might be associated with the

variation of the individual's brain functional network architecture<sup>12</sup>.

Here, we hypothesized that: (1) participants with migraine could be sub-classified into different subgroups based on clinical measurements and brain neuroimaging data; (2) participants in different subgroups have distinct abnormal functional connectivity patterns compared with controls, and (3) participants in different subgroups may have different treatment outcomes. To achieve our aims, we proposed a novel processing pipeline for identifying migraine subgroups, validated the proposed subgrouping strategy using two datasets, and verified the clinical usefulness of the strategy by providing prognostic information for treatment selection. Specifically, in study 1, we adopted the partial least squares correlation (PLSC) analysis and a hierarchical clustering algorithm to divide participants with migraine into different clusters. In study 2, we assessed the external validity of the clustering strategy. In study 3, we evaluated whether the clustering strategy could be clinically valuable in providing prognostic information for an 8-week electroacupuncture treatment.

## **Materials and Methods**

### *Registration, standard protocol approval, and participant consent*

Participants gave written informed consent, and the study aims and experimental procedures were fully explained to all of them. All studies were conducted based on the Declaration of Helsinki where the Institutional Review Board (IRB) of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine and West China Hospital approved the studies. Electroacupuncture treatment was listed in the Chinese Clinical Trial Registry (ChiCTR-IOR-15006648, June 23, 2015). The first participant was enrolled on July 17, 2015.

## *Study 1: Identifying subgroups of migraine without aura (MWOA)*

### *Participants*

We consecutively recruited participants with MWOA from Department of Acupuncture and Neurology at Chengdu University of Traditional Chinese Medicine. The inclusion criteria were based on the 3<sup>rd</sup> edition of the International Classification of Headache Disorders<sup>13</sup>.

When we enrolled a participant with MWOA who met the inclusion criteria, we chose her or his siblings, schoolmates, colleagues, or families as healthy controls (HCs). Potential participants needed to attend a screening appointment so we can check if they are suitable to take part in the project as mentioned in the protocol.

The exclusion criteria included<sup>14</sup>: (1) individuals with any physical disease, including hepatitis, brain tumors, , and epilepsy, based on medical records and clinical evaluations; (2) those with additional comorbid chronic pain disorders (e.g., fibromyalgia and tension-type headache); (3) those with a psychiatric disorder or brain disease; (4) pregnant; (5) those using prescription drugs for migraine in the previous three months; (6) those with nicotine, alcohol, or drug abuse; and (7) those with claustrophobia.

### *Clinical measurements and imaging acquisition*

Participants with MWOA were instructed to report migraine duration during recruitment and complete a daily headache diary, including the headache frequency, Zung self-rating anxiety scale (SAS)<sup>15</sup>, average pain intensity, and Zung self-rating depression scale (SDS)<sup>16</sup>.

The average pain intensity for each migraine attack was assessed using a numerical rating scale (NRS) going from 0 (zero pain) to 10 (the worst pain ever). SAS and SDS are both 20-item surveys rated by respondents with scores ranging from 20 to 80. They evaluate



physiological and psychological symptoms that are assayed by participants based on how each one pertained to them in the past week<sup>15, 16</sup>. Moreover, the quality-of-life questionnaire (MSQ) pertaining to migraine, which is a patient-reported outcome assay with scores ranging from 0 to 100, assessed the quality-of-life of participants with MWoA across three essential aspects in the past four weeks: role function-preventive (RP), role function-restrictive (RR), and emotional function (EF)<sup>17</sup>.

After clinical measurements, participants with MWoA underwent MRI scanning in the migraine's interictal phase (i.e., a minimum of 72 h following a migraine attack). Resting-state brain functional images and high-resolution T1 scans were acquired using a 3.0 Tesla Signa GE scanning machine that had an 8-channel phase head coil located at the MR Research Center of the West China Hospital, Chengdu, China (eMethods).

#### *Whole-brain functional connectivities (FCs) estimation*

Whole-brain FCs were estimated with the GRETNA toolbox (<https://www.nitrc.org/projects/gretna>)<sup>18</sup>. By using the Human Brainnetome Atlas<sup>19</sup>, whole brain was segment into 246 regions of interest (ROIs) (eTable 1). After data preprocessing (eMethods), the blood oxygen level dependent time series of every ROI were extract and averaged, and Pearson correlation coefficients were computed between these time series to form a  $246 \times 246$  correlation matrix containing 30,135 unique FC features. A Fisher Z-transformation was used to convert all correlation coefficients. Linear regression was performed to adjust for the influences of sex and age, resulting in the adjusted FC features<sup>4</sup>.

#### *PLSC analysis*

PLSC was used to investigate the relationship between 30,135 FC features and eight clinical measurements (i.e., migraine duration, headache frequency, NRS, SAS, SDS, MSQ\_EF, MSQ\_RP, and MSQ\_RR)<sup>20</sup>. This resulted in a set of orthogonal latent variables (LVs) that can be used to identify connectivity patterns representing the optimal covariance between functional data and behavioral variables<sup>20</sup> (eMethods and eFigure 1). Every LV is associated with (1) a clinical saliences vector, indicating the contribution weight of every clinical measurement on the FC features–symptom association; and (2) a brain saliences vector, indicating that the contribution weight of each FC feature contributes to the FC features–symptom association. Brain scores, a measure of the projections of FC features on their brain saliences, were calculated for each participant<sup>21</sup>. A large absolute brain score demonstrates a strong contribution of individual FC features to the brain–symptom correlation, and a score close to zero implies a weak contribution<sup>20</sup>.

#### *Hierarchical clustering*

Hierarchical clustering analysis<sup>22</sup> was performed to assign participants with MWoA with similar patterns of brain scores into different subgroups. A dissimilarity matrix was calculated to describe the Euclidean distance between every pair of participants with MWoA. Ward's minimum variance method was then applied to minimize the total within-cluster variance. Clustering analysis was performed using the `pdist.m`, `linkage.m`, and `cluster.m` functions in MATLAB. Without making any a priori assumption of the number of clusters, we repeatedly performed the clustering analysis using 2–8 clusters of participants with MWoA and used the variance ratio criterion to determine the optimal cluster number<sup>23</sup>. The variance ratio criterion represents the ratio of the between-cluster variance to the

within-cluster variance, with a higher variance ratio score (VRS) reflecting better clustering performance. The same hierarchical clustering analysis was also performed on the clinical measurements of participants with MWOA. Permutation tests (5,000 times) were performed to estimate the statistical significance (please see eMethods for technical details).

#### *Comparisons between MWOA subgroups identified by hierarchical clustering*

Between-group comparisons were performed to reveal their possible differences in clinical measurements and whole-brain FC patterns. Two-sample t-tests were used, and the threshold for statistical significance was  $p < 0.001$ . False discovery rate (FDR) was used to correct the multiple comparisons. The matrix of between-subgroup differences of FCs was summed over rows to show the strength of each ROI (please see eMethods for technical details).

#### ***Study 2: Cross-validation of the two-cluster solution of participants with MWOA***

##### *Clinical measurements, imaging acquisition, and data analysis for dataset 2*

An independent dataset in study 2 (dataset 2) were recruited as outpatients from the Department of Acupuncture and Neurology (West China Hospital). The exclusion and inclusion criteria for participants with MWOA and HCs were identical to those in study 1. All clinical measurements and imaging acquisition were identical to study 1 (eMethods).

##### *Cross-validation of the two-cluster solution*

We adopted a multivariate pattern analysis (MVPA) procedure by applying the support vector machine (SVM) on 30,135 FC features to classify MWOA, with and without clustering (eFigure 2 and eFigure 3), and HCs. This analysis could quantify the performance of the

clustering procedure by comparing classification performance with and without clustering in dataset 1, for which the results could be verified in dataset 2 (please see eMethods for technical details).

When the classifier was trained on HCs and MWOA without clustering, the large inter-individual heterogeneity of patients would force the classifier to learn individual-level features rather than class-specific features<sup>24, 25</sup>. Hence, it would lead to poor classification performance in isolating MWOA from HCs. As the hierarchical clustering would minimize migraine heterogeneity within each MWOA subgroup, when the classifier was trained on HCs and MWOA in a subgroup, classification performance (HCs vs. MWOA in a subgroup) could be improved. The area under the receiver operating characteristic curve (AUC), kappa coefficient, and accuracy were calculated. The classification accuracy was defined as the fraction of predictions the model got right and calculated by dividing the number of correct predictions by the total number of predictions (i.e., the total number of participants). Permutation tests (5,000 times) were performed to estimate the statistical significance (please see eMethods for technical details).

### ***Study 3: The diverse responsiveness of MWOA subgroups to electroacupuncture treatment***

#### ***Participants, electroacupuncture treatment, and clinical measurements***

In study 3, some participants with MWOA in datasets 1 and 2 were willing to receive electroacupuncture treatment. After four weeks of baseline clinical evaluation, participants with MWOA received electroacupuncture treatment thrice (30 min each time) per week for eight weeks, i.e., a total of 24 sessions (eFigure 4). The details about the acupoints selected

are described in our previous study<sup>10</sup>. Briefly, four acupoints were used per treatment. All participants with MWoA received electroacupuncture on two compulsory acupoints (GB8 and GB20), and the other two acupoints were selected based on syndrome distinction of meridians in the headache area (included BL60, GB34, GB40, LI4, LR3, SI3, SJ5, and ST44)<sup>10</sup>. Sterile disposable acupunctures, each 25-40 mm long and 0.25 mm wide, were used. Acupuncturists, who were blinded to the classification of participants based on the clustering approach used, received treatment unilaterally by switching from the left and to the right points<sup>10</sup>. Every acupoint was used to achieve the De-qi feeling (such as distention, numbness, soreness, or radiating which shows effectual acupuncture)<sup>10</sup>. We used the HANS acupoint nerve stimulator (model LH 200A; Han Institute, TENS, Nanjing, China) following acupoint insertion. The stimulation frequency was set to 2/100 Hz (changing every 3 seconds), and the stimulus strength ranged from 0.1 to 1.0 mA until the participant was at ease<sup>10</sup>. All participants with MWoA could not ingest prophylactic drugs. However, in instances of excruciating headache, participants with MWoA were given permission to ingest ibuprofen (300-mg sustained-release capsules)<sup>10</sup>. Detailed information about drug intake was noted. After eight weeks of electroacupuncture treatment, clinical measurements for the following four weeks were evaluated again. Participants with MWoA with reduced headache frequency by at least 50% were considered responders.

#### *Responsiveness to electroacupuncture treatment in different MWoA subgroups*

Based on the imaging-based cluster centroid of the two MWoA subgroups in dataset 1, the brain scores of each participant in study 3 were calculated and then used to assign the participant with MWoA to one of the MWoA subgroups. Within each MWoA subgroups, the

electroacupuncture response rate was measured as a ratio between the number of responders and the total number of participants with MWOA. The difference in the electroacupuncture response rate between participants in subgroups 1 and 2 was assessed using the chi-square test.

#### *Electroacupuncture response rate predictions with and without MWOA subgroup labels*

Studies suggested that acupuncture treatment outcomes in MWOA could be predicted from priori brain functional network<sup>12, 26</sup>. To determine whether the MWOA subgroup labels could improve the identification of individuals who are most likely to benefit from electroacupuncture treatment, we performed an MVPA to predict electroacupuncture responders and non-responders based on whole-brain FCs before electroacupuncture treatment or based on the combination of whole-brain FCs and MWOA subgroup labels (eMethods) (eFigure 5). The AUC, kappa coefficient, and accuracy were calculated. Permutation tests (5,000 times) were performed to estimate the statistical significance. The same analysis was also performed to assess whether the differential electroacupuncture response might be explained by simpler measurements.

#### *Data availability*

The MATLAB codes of the MVPA analysis are available on github (<https://github.com/Jixin-Liu/Study-migraine-heterogeneity-using-fMRI.git>). Additional data related to this article will be provided upon reasonable request.

## Results

### *Study 1: MWOA subgroups identified by clustering analysis*

#### *Demographics and clinical measurements*

In total, 110 participants with MWOA and 90 HCs were recruited. Nine participants were excluded for missing data in the headache diary. Four participants and seven HCs were excluded due to incomplete MRI scans. Six HCs were excluded due to schedule conflicts. Thus, ninety-seven participants with MWOA with an average age of  $28.2 \pm 1.0$  years and 77 HCs with an average age of  $26.8 \pm 0.1$  years were selected (dataset 1 and Table 1). There was no significant difference between the two groups with respect to age and sex. The headache frequency was  $6.5 \pm 0.5$  times per month, and the average pain intensity was  $5.6 \pm 0.2$ . The average SAS and SDS scores were  $46.4 \pm 0.9$  and  $44.7 \pm 1.1$ , respectively. The average RR, RP, and EF scores were  $61.3 \pm 1.9$ ,  $71.0 \pm 2.0$ , and  $71.9 \pm 2.0$ , respectively (Table 1).

#### *Hierarchical clustering*

PLSC analysis revealed eight orthogonal latent variables and brain scores in each participant (eTable 2 and eTable 3). When we used the eight clinical measurements to cluster participants with MWOA similarly for all cluster solutions, the VRSs (Figure 1a, black) were much lower than those when the brain scores (Figure 1a, red) were used. The VRS that monotonically decreased with the increase in cluster number was maximal with a two-cluster solution. For the two-cluster solution, VRSs were significantly larger than the chance level when brain scores and clinical measurements were used ( $p = 0.001$ ,  $CI = 0.0-0.002$ , Figure 1b). Moreover, imaging-based clusters had a significantly higher VRS than clinical-based clusters ( $p = 0.005$ ,  $CI = 0.0-0.02-0.008$ , Figure 1b).

### *Comparisons between imaging-based two MWOA subgroups and HCs in dataset 1*

As can be seen from the Table 2, no significant differences in demographics were found. The comparison of clinical measurements between imaging-based two MWOA subgroups in dataset 1 showed that participants in subgroup 1 had a lower headache frequency and MSQ-RP score and higher SDS as compared with the participants in subgroup 2.

The comparison of brain FCs between imaging-based MWOA subgroups in dataset 1 showed that participants in subgroup 1 exhibited higher FCs than those in subgroup 2 (marked in red in Figure 2a), and the between-group differences of FCs were mainly related to the thalamus, amygdala, hippocampus, and the parahippocampal area (the top 12 ROIs, i.e., 5% of 246 ROIs, the same hereinafter, Figure 2a). Additionally, participants in subgroup 1 exhibited higher FCs than HCs (marked in red in Figure 2b), and participants in subgroup 2 showed lower FCs than HCs (marked in blue in Figure 2c).

### ***Study 2: Cross-validation of MWOA subgroups***

#### *Demographics and clinical measurements for dataset 2*

In dataset 2, 37 participants with MWOA and 27 HCs were recruited. Four patients with MWOA were excluded due to an incomplete headache diary. Four HCs were excluded due to discomfort or scheduling issues. Thus, 33 participants with MWOA with an average age of  $30.9 \pm 2.0$  years and 23 HCs with an average age of  $29.8 \pm 1.1$  years were selected from the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (eTable 4). There was no significant difference between the two groups with respect to age and sex. Based on the imaging-based cluster centroid of the two MWOA subgroups in dataset 1, 15 participants



with MWOA in dataset 2 were assigned to subgroup 1, and 18 participants with MWOA were assigned to subgroup 2 (eTable 4). For whole-brain FCs, participants in different MWOA subgroups showed similar between-group differences as those in dataset 1 (eFigure 6).

#### *Classification performance with and without clustering*

In dataset 1 (study 1), when classifiers were trained on all participants without clustering, MWOA and HCs were correctly classified with an AUC of 0.92, a kappa coefficient of 0.84, and an accuracy of 0.92 (all  $p = 0.0008$ , CI = 0-0.0018; 5,000 permutations) (eFigure 7a).

When classifiers were trained on participants after clustering, MWOA in subgroup 1 and HCs were discriminated with an AUC of 0.86, a kappa coefficient of 0.80, and an accuracy of 0.90 (all  $p = 0.0008$ , CI = 0-0.0018; 5,000 permutations) (eFigure 7a). MWOA in subgroup 2 and HCs were more accurately classified with an AUC of 0.94, a kappa coefficient of 0.89, and an accuracy of 0.96 (all  $p = 0.006$ , CI = 0-0.0015; 5,000 permutations) (eFigure 7a).

In dataset 2, when classification models from dataset 1 were used, all MWOA and HCs were classified with an AUC of 0.76, a kappa coefficient of 0.52, and an accuracy of 0.77 (eFigure 7b). However, MWOA in subgroup 1 and HCs were poorly discriminated with an AUC of 0.51, a kappa coefficient of 0.012, and an accuracy of 0.55 (eFigure 7b). MWOA in subgroup 2 and HCs were accurately classified with an AUC of 0.87, a kappa coefficient of 0.74, and an accuracy of 0.91 (eFigure 7b).

No canners' effects were observed between datasets 1 and 2 (please see eResults for details).

### *Study 3: The diverse responsiveness of imaging-based MWOA subgroups to*

#### *electroacupuncture treatment*

Only 58 participants with MWOA in datasets 1 and 2 were willing to receive electroacupuncture treatment (eTables 5-7), and there were 17 electroacupuncture responders (response rate = 29.3%, 17/58, Figure 3a). When the imaging-based cluster centroid was used, 33 participants were assigned to subgroup 1 and 6 electroacupuncture responders were found (response rate = 18%, 6/33, Figure 3b); 25 participants were assigned to subgroup 2 and 11 electroacupuncture responders were found (response rate = 44%, 11/25, Figure 3b). Participants in subgroup 2 had a significantly higher electroacupuncture response rate than those in subgroup 1 ( $p = 0.03$ , OR CI = 0.086-0.93).

To determine whether the differential response might be explained by other measures, the eight clinical measurements and 30,135 brain FCs were also used to cluster participants in study 3 into different subgroups. Unfortunately, all participants with MWOA in study 3 were assigned to the same group (please see eResults for details).

#### *Classification performance*

When the classifiers were trained on whole-brain FCs before electroacupuncture treatment without MWOA subgroup labels, electroacupuncture responders and non-responders were correctly classified with an AUC of 0.63 ( $p = 0.024$ , CI = 0.018-0.03), a kappa coefficient of 0.16 ( $p = 0.16$ , CI = 0.15-0.17), and an accuracy of 0.72 ( $p = 0.0028$ , CI = 0.001-0.0047). However, when MWOA subgroup labels were added as discriminative features, the AUC, kappa coefficient, and accuracy increased to 0.81 ( $p = 0.0012$ , CI = 0-0.0025), 0.62 ( $p = 0.0008$ , CI = 0-0.0018), and 0.83 ( $p = 0.0006$ , CI = 0-0.0015), respectively (Figure 3c).

When the SVM classifier was trained on all eight clinical measurements, electroacupuncture responders and non-responders were not correctly classified with an AUC of 0.54, a kappa coefficient of 0.13, and accuracy of 0.67.

## Discussion

In this study, we identified a preliminary novel approach for identifying MWOA subgroups based on the association between clinical measurements and brain neuroimaging data. We tested the validity of this clustering approach using two independent datasets, and illustrated its clinical values by comparing response rates to the 8-week electroacupuncture treatment of participants in different MWOA subgroups. This demonstrates that personalized treatment approaches to migraine, based on clinical and neuroimaging data, may be attainable.

As one of the most disabling neurological diseases, migraine affects approximately 12% of the population worldwide<sup>27, 28</sup>. Due to its heterogeneity, the treatment outcomes vary tremendously among individuals<sup>29</sup>. This situation could be improved if migraine can be more effectively diagnosed and treated, and a more targeted treatment to disease would improve the treatment outcomes for individuals<sup>30, 31</sup>. Indeed, some studies have defined MWOA subgroups according to clinical categorization schemes based on pain intensity or depression/anxiety levels<sup>4, 32, 33</sup>. However, this strategy cannot fully capture MWOA heterogeneity, as existing classifications based on clinical criteria may not delineate MWOA with neurobiologically distinct characteristics<sup>34</sup>. This shortcoming calls for a novel method to integrate measurements from multiple categories, which would help provide a more comprehensive characterization of individual differences in participants with MWOA. In this study,

combining clinical measurements with brain imaging data, we have obtained a series of latent components that linked a large set of clinical measurements to whole-brain FC patterns in participants with MWoA. The interindividual variability in the expression of these components captured differences among participants with MWoA in their clinical measurements and brain FCs. Clustering on these latent components, all participants with MWoA could be divided into two subgroups. Participants in subgroup 1 had higher self-ratings of depression and abnormal higher brain FCs, and participants in subgroup 2 had higher headache frequencies and abnormal lower brain FCs. These results were largely replicated in an independent dataset. Therefore, our study provided a novel processing pipeline to define distinct MWoA subgroups, which could help improve our understanding of clinically-relevant neurobiological heterogeneity in migraine.

In our findings, the differences of brain FCs between participants in subgroup 1 and 2 were mainly centralized in the thalamus, amygdala, hippocampus, and the parahippocampus. As one of the supraspinal structures that receives projections from the spinal cord, the thalamus plays an important role in pain management by relaying ascending nociceptive information to other brain regions<sup>35</sup>. Although still under investigation, the role of the thalamus in central sensitization, photophobia, and allodynia in migraine has been well documented<sup>36-39</sup>. Considering these studies, the distinct pattern of thalamus-related FCs in our study may be associated with different pain processing and modulation in MWoA subgroup 1 and 2. Subcortical brain structures like the amygdala and hippocampus are key regions for emotional and memory processing. The amygdala plays a crucial role in automatic management of memory and emotional stimuli<sup>40</sup>, while the hippocampus regulates learned

behavior and memory, and pain-related attention and anxiety<sup>41</sup>. These areas are also implicated in migraine<sup>42, 43</sup>. For example, Chen et al. observed abnormal FCs of the amygdala in individuals with chronic and episodic migraine<sup>44</sup>. Schwedt et al. reported greater hippocampus activation while experiencing thermal pain stimuli in participants with MWoA than in HCs, and the strength of activation in the hippocampus had a positive correlation with headache frequency<sup>45</sup>. Therefore, different maladaptive states of neural activity related to the amygdala and hippocampus in different MWoA subgroups may be associated between-group differences in depression level and headache frequencies.

In addition to improving our understanding of clinically-relevant neurobiological heterogeneity in migraine, this study has prognostic potential in elaborating the treatment performance for participants with MWoA. Our results found that electroacupuncture was more effective in relieving pain in participants in subgroup 2 than in subgroup 1. Participants in subgroup 1 had higher depression scores but a lower response rate than participants in subgroup 2. Indeed, several studies pointed out that depression was related to difficulty in revising negative expectations after receiving unexpectedly positive information<sup>46</sup>. Since positive and negative expectations were closely related to participants' analgesic response to subsequent placebo and active medical treatments<sup>47-49</sup>, our results may suggest that depressive symptoms in participants in subgroup 1 could result in negative expectations about pain or treatment outcomes, thus preventing them from experiencing clinical benefits.

Additionally, we found that acupuncture responders and non-responders were more accurately identified when the classifiers were trained on brain FCs before acupuncture treatment with MWoA subgroup labels than those without labels. Hence, MWoA subgroup labels were also a

relevant feature for a priori prediction of electroacupuncture response in participants with MWOA and may help develop more efficient treatment approaches to migraine.

There are several issues that should be discussed in future research. The classification performance improved when the analysis was performed on clustered participants with MWOA in subgroup 2 than un-clustered participants. This result may suggest that brain FC features of MWOA in subgroup 2 were informative for classification and could be generalized into independent datasets. However, poor classification performance was obtained for participants with MWOA in subgroup 1, which could be associated with the fact that there were fewer abnormal brain FCs in MWOA in subgroup 1 than in subgroup 2 (Figure 2b vs. 2c). As the classification analysis was performed based on the differences in FCs between MWOA and HCs, fewer significant between-group differences would make it challenging to maximize the classification margins in SVM, which would result in poor classification performance. Additionally, the number of patients in subgroup 1 was limited. Therefore, caution is warranted in interpreting abnormal FCs in MWOA in subgroup 1.

Additionally, eight clinical measurements in this study could not fully capture the clinical characteristics of participants with MWOA. Standardized procedures to comprehensively collect clinical measurements should be implemented. This will be crucial for refining our clustering method to identify MWOA subgroups with distinct migraine symptoms and behaviors. To avoid studying participants with MWOA during their premonitory phase, the moment elapsed from the time of the MRI until the following migraine attack should be evaluated. For collecting resting state data, future studies should use a static visual stimulus (e.g., an X) to stare at or a boring movie without words or content to prevent participants from

overthinking during the resting state<sup>50</sup>. Given that the numbers of participants are not equal between participants with MWoA and HCs, further studies with a larger sample size are required to have a matched participant design and verify the clinical usefulness of our clustering method.

## **Conclusion**

We proposed a novel processing pipeline to sub-classify participants with MWoA into two different subgroups based on clinical and neuroimaging data, and participants in different subgroups have distinct abnormal functional connectivity patterns and electroacupuncture response rate. Our preliminary study may be useful to identify migraineurs who are most likely to benefit from electroacupuncture therapy.

WNL-2023-000314\_sup --- <http://links.lww.com/WNL/C921>

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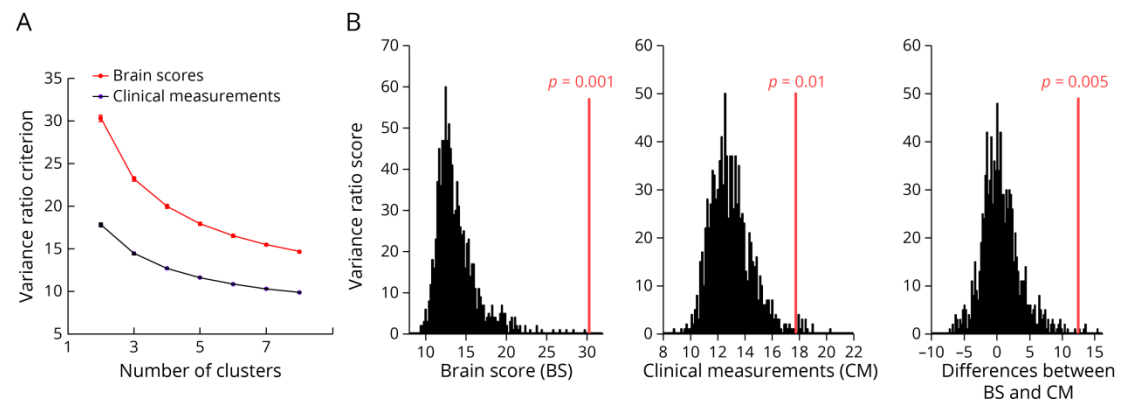


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## Figure legends

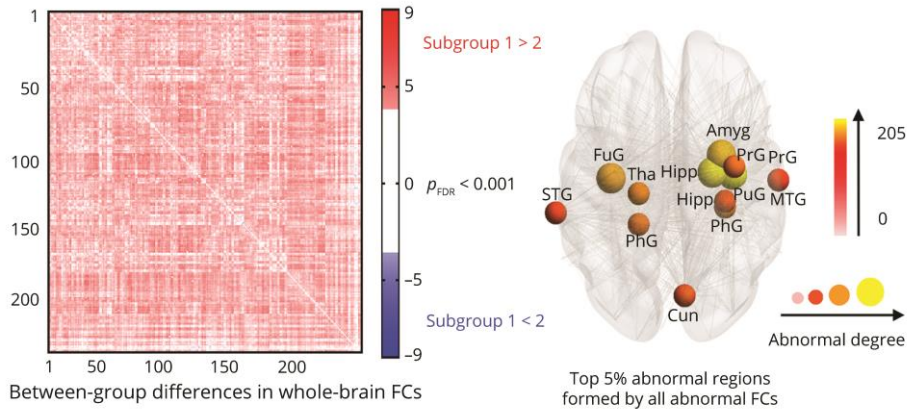
Figure 1. Clustering performance.



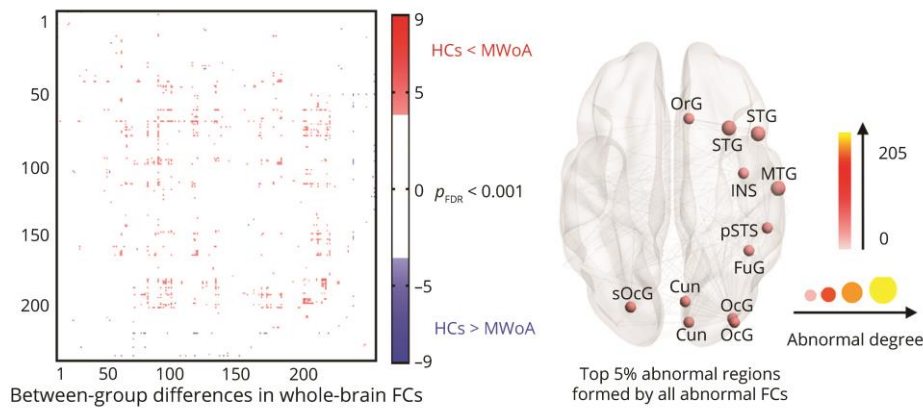
(a) The comparison of hierarchical clustering performance between the use of brain scores and clinical measurements. (b) Statistical comparison of the clustering performance when two clusters were considered.

Figure 2. Abnormal FCs in different MWOA subgroups in dataset 1.

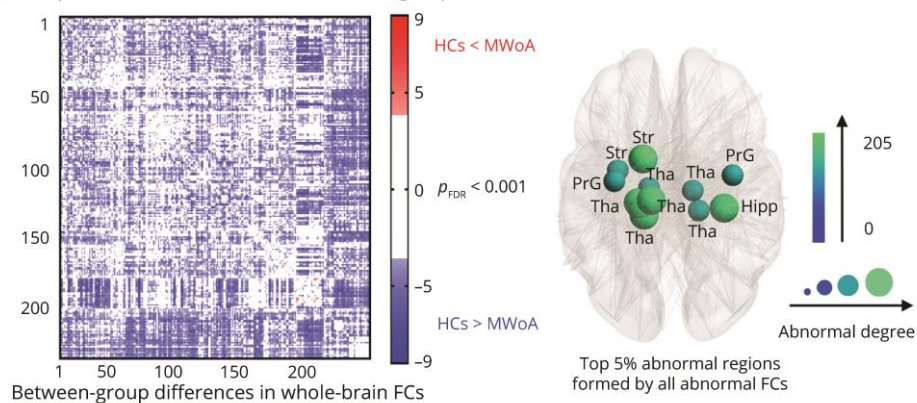
A. Comparisons between MWOA subgroups 1 and 2



B. Comparisons between HCs and MWOA subgroup 1

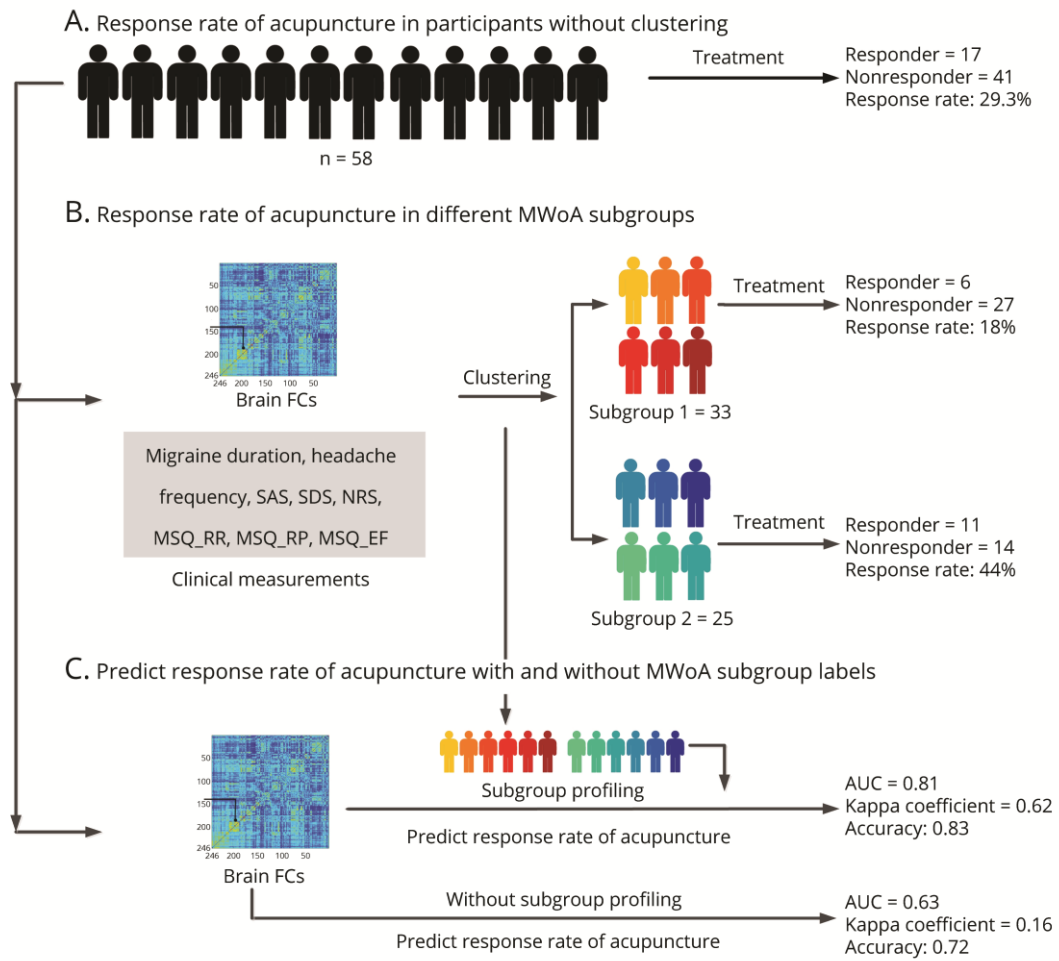


C. Comparisons between HCs and MWOA subgroup 2



Heat maps depicting a pattern of FCs for between-group differences ( $p_{FDR} < 0.001$ ). The size of the node indicates the degree of the between-group differences. The top 5% of abnormal brain regions formed by the abnormal FCs were exhibited. (a) The comparison between participants in the two subgroups. (b) The comparison between participants with MWOA in subgroup 1 and HCs. (c) The comparison between participants with MWOA in subgroup 2 and HCs.

Figure 3. Electroacupuncture response.



The diverse acupuncture responsiveness of an 8-week electroacupuncture treatment in different MWOA subgroups.

Table 1. Demographic characteristics of all participants and clinical measurements of participants with MWoA in dataset 1.

Characteristics	participants with MWoA (n=97)	HCs (n=77)
Age (years)	28.2 ± 1.0	26.8 ± 0.1
Sex (female/male)	70/27	61/16
Migraine duration (months)	87.7 ± 6.9	(24, 240)
<i>Migraine attacks during the past four weeks</i>		
Headache frequency (times/month)	6.5 ± 0.5	(2, 15)
NRS	5.6 ± 0.2	(4, 8)
SAS	46.4 ± 0.9	(33, 61)
SDS	44.7 ± 1.1	(29, 64)
<i>Migraine-specific quality-of-life questionnaire</i>		
RR	61.3 ± 1.9	(27, 88)
RP	71.0 ± 2.0	(34, 100)
EF	71.9 ± 2.0	(33, 100)

Data were reported as mean ± standard error unless otherwise indicated. The range from the 5<sup>th</sup> to the 95<sup>th</sup> percentile of clinical measurements was also provided. MWoA, migraine without aura; HCs, healthy controls; NRS, numerical rating scale; SAS, self-rating anxiety scale; SDS, self-rating depression scale; RR, role function-restrictive; RP, role function-preventive; EF, emotional function.

Table 2. The comparison of demographic characteristics and clinical measurements between different MWoA subgroups in dataset 1.

Characteristics	MWoA subgroup 1 (n = 20)	MWoA subgroup 2 (n = 77)	p	CI	effect size
Age (years)	25.1 ± 1.9	29.1 ± 1.1	0.099	(-8.66, 0.75)	0.42
Sex (female/male)	16/4	54/23	0.58	(0.51, 5.65)	0.11
Migraine duration	73.9 ± 11.7	91.2 ± 8.2	0.31	(-51.45, 16.69)	0.25
<i>Migraine attacks during the past four weeks</i>					
Headache frequency (times/month)	4.4 ± 1.1	7.0 ± 0.6	0.032	(-5.09, -0.24)	0.55
NRS	5.3 ± 0.4	5.7 ± 0.2	0.37	(-1.19, 0.44)	0.23
SAS	47.1 ± 1.9	46.3 ± 1.0	0.71	(-3.53, 5.19)	0.10
SDS		43.4 ± 1.2	0.019	(1.03, 11.21)	0.60
<i>Migraine-specific quality-of-life questionnaire</i>					
RR	57.6 ± 4.0	62.3 ± 2.1	0.31	(-13.91, 4.41)	0.26
RP	63.2 ± 2.0	73.0 ± 2.3	0.04	(-19.67, -0.03)	0.50
EF	71.0 ± 4.2	72.2 ± 2.3	0.82	(-11.05, 8.71)	0.06

Data were reported as mean ± standard error unless otherwise indicated. MWoA, migraine without aura; CI, confidence interval; NRS, numerical rating scale; SAS, self-rating anxiety scale; SDS, self-rating depression scale; RR, role function-restrictive; RP, role function-preventive; EF, emotional function. The comparisons were performed using two-sample t-tests, except sex, which was assessed using the chi-square test.  $p < 0.05$  was considered significant.

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## Evaluation of a Clustering Approach to Define Distinct Subgroups of Patients With Migraine to Select Electroacupuncture Treatments

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