Discovery of the mechanisms of acupuncture in the treatment of migraine based on functional magnetic resonance imaging and omics

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Abstract

Migraine is one of the most prevalent and disabling neurological disease. The current treatments are limited. Acupuncture is a promising complementary and replacement therapy, but further clinical evidence is needed. Besides, the influence of acupuncture on migraine is not an immediate effect, the mechanism of this effect is not clear yet. This study is to provide further clinical evidence for the anti-migraine effects of acupuncture and explore the mechanism. Herein, a randomized controlled trial (48 participants in total; 10 normal controls and 38 migraineurs) was performed. The 38 migraine patients were divided into blank control, sham acupuncture, and acupuncture. The baseline characteristics were assessed for 2 weeks before the study. After that, patients were subjected to two courses of treatment, each of which lasted 5 days, with an interval of 1 day between the two courses (11 days in total). Outcome data collection, fMRI measurement, and blood sample collection were performed before and at the end of the treatment period. Effectiveness of treatment was evaluated using pain questionnaire. The fMRI data was analysed. Blood plasma was collected for metabolomics and proteomics studies. Results showed that acupuncture effectively relieves migraine symptoms, and not merely via the placebo effect. The anti-migraine mechanism involves a complex network which is related to regulating the blood oxygen levels, reversing brain energy imbalance and regulating inflammation. Brain regions of migraineurs affected by acupuncture include the limbic system and default mode network, and regions involved in cognitive and emotional processing of pain, sleep, and attention.

Introduction

Migraine is one of the most prevalent and disabling neurological disease that is characterized by a pulsating, severe, and often unilateral headache, mostly accompanied by loss of appetite, weakness, nausea, and photophobia.[1] It has a high impact on quality of life with an estimated lifetime prevalence of 35.3%.[2] Despite the obvious severity, migraine is largely neglected and was described as “underestimated, under-recognized and under-treated” by the World Health Organization (WHO) in 2016.

The current migraine management focus largely on pharmacological therapy using drugs like nonsteroidal anti-inflammatory drugs, antiepileptic drugs, ergotamines, and triptans.[3,4] These pharmacotherapies may alleviate symptoms, but often accompanied by adverse effects such as progressing from episodic migraine to chronic migraine and exacerbation of the chronic patient's condition caused by the medication overuse. Therefore, an increasing number of patients are making use of effective, non-pharmacological alternative therapies.

Acupuncture is used worldwide as an effective treatment for pain, especially headache and migraine pain, but further investigation is required.[5,6] According to previous reports, the underlying mechanism involves stimulating the body to release endogenous analgesic substances, promotes the body’s healing and blood flow responses by micro-injury from the needles.[7–9] Functional magnetic resonance imaging (fMRI) data provided evidence of a direct link between acupuncture points and specific brain activity.[10,11] Despite many studies suggest that acupuncture represent a safe and effective form of migraine therapy, further clinical evidence is needed to provide support for its clinical value. What's more, according to our clinical observation, the influence of acupuncture on migraine is not an immediate effect, but a cumulative effect. This phenomenon has also been confirmed by others, Patel, et al.[12] found that acupuncture therapy is only effective after a certain number of sessions in the treatment of migraine, but the mechanism of this effect is not clear yet.

The purpose of this study is to explore the anti-migraine effects and mechanisms of acupuncture. Herein, a randomized controlled trial was conducted to investigate the effectiveness of acupuncture in combating migraine. A sham acupuncture group was included to examine the placebo effect of acupuncture. Imaging and omics methods were used to clarify the mechanism underlying the anti-migraine effects of acupuncture. The similarities and differences in the data from acupuncture and sham acupuncture groups were compared to elucidate the acupuncture-specific anti-migraine effects.

Materials And Methods

Patient consent and ethics approvals

Written informed consent was obtained from all participants, and the study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the Beijing University of Chinese Medicine (DZMEC-KY-2018-44).

Study population

Inclusion criteria: ≥ 18 and ≤ 55 years of age; a diagnosis of migraine with or without aura, fulfilling the diagnostic criteria in the third edition of the International Classification of Headache Disorders (ICHD-3); a history of migraine for at least one year; a history of at least two migraine attacks per month; a VAS score of ≥ 4.

Exclusion criteria: headache secondary to other diseases; rare type of migraine; migraine as a concomitant symptom of severe primary disease; contraindications to fMRI; and participated in other acupuncture or prophylactic medication treatment research in the past three months.

Study Design

This randomized controlled single blind study last from September 1st, 2018 to May 26, 2019. Participants were enrolled by study site personnel at Dongzhimen Hospital (Beijing, China).

Migraine patients were divided into blank control (B), sham acupuncture (S), and acupuncture (A) using a randomisation table. Healthy volunteers were recruited into the normal control (N). The baseline characteristics were assessed for 2 weeks before the study and the general condition of the participants was recorded. After that, patients were subjected to two courses of treatment, each of which lasted 5 days, with an interval of 1 day between the two courses. Outcome data was collected before and at the end of the treatment (Supplementary Fig.S1).

Acupoints for the acupuncture treatment (Supplementary Fig.S2A): **1.** Gallbladder meridian 20 (GB20); **2.** Liver Meridian 3 (LR3); **3.** Extra Points of Head and Neck 5 (EX-HN5); **4.** Governing vessel 20 (GV20); **5.** Extra Points of Head and Neck 1 (EX-HN1). The disposable sterile acupuncture needle (0.30 mm × 40 mm) penetrated the skin vertically; the needle angle was subsequently adjusted (transverse insertion for GV20 and EX-HN1; oblique insertion for EX-HN5). The needle was inserted until it elicited the patient's feeling of qi, i.e., a feeling of soreness, numbness, distension, and heaviness. The treatment was applied once a day for 30 min (amplitude of lifting-thrusting: 0.3–0.5 cm; frequency: 60–90 times/min; twirling angle: 90–180°).

Acupoints for sham acupuncture treatment (Supplementary Fig.S2B): **1.** Midpoint of the line between the elbow tip and the armpit. **2.** Midpoint of the line between the medial epicondyle of the humerus and the ulnar wrist. **3.** Junction of the deltoid muscle and biceps brachii on the anterior edge of the upper arm. **4.** One to two centimetres beside the Zusanli acupoint (ST36). The disposable sterile acupuncture needle entered the skin horizontally to a puncture depth of 4 mm. No feeling of qi was elicited and no therapeutic manipulation was applied.

The blank control group received no treatment during the study; compensated acupuncture treatment was provided after the study.

Measures of clinical effectiveness

VAS, PSQI, GAD-7, MSQ, attack frequency, and adverse events data were collected before and at the end of the treatment period.

fMRI

All images were acquired using a 3T scanner (Siemens Verio, Munich, Germany) within 30 min after treatment. Participants lay supine and were asked to be awake during the acquisition and close their eyes. The BOLD signal and T1 were recorded. Gradient echo-echo planar imaging sequence was applied for resting-state RS-fMRI image acquisition. Magnetization-prepared rapid gradient echo T1-weighted sequence mode was applied for high-resolution structural image acquisition.

Metabolomics

Blood samples were processed in routine steps. The supernatant was then collected for metabolomics analysis. In addition, 10 μL of each sample was pipetted into a centrifuge tube to prepare quality control samples for ultra-performance liquid chromatography–tandem mass spectrometry. The same method was used to investigate instrument precision, method repeatability, and sample stability.

Proteomics

Blood samples were processed in routine steps. Quality control and proteolysis were subsequently performed. A multi-iTRAQ experimental protocol including peptide labelling was designed and performed to compare the plasma protein profile between groups. The LC-20AB liquid phase system was used for peptide fractionation. Separation was performed using a Thermo UltiMate 3000 ultra-high-performance liquid chromatography system. The peptides separated by liquid phase chromatography were ionised using a nanoESI source and then passed to a tandem mass spectrometer Q-Exactive HF X for data-dependent acquisition mode detection.

Data analysis

Clinical data

The Chi-square test was used for gender data analysis. A two-sample t-test was used for age data analysis. The Wilcoxon rank-sum test was used to analyse other data on clinical effectiveness. IBM SPSS Statistics 25.0 was used for statistical analysis. $P < 0.05$ was considered statistically significant.

fMRI

DPABI v2.0 was used to perform fractional ALFF (fALFF) and ReHo analyses.[13–15] Statistical analyses were performed using SPM12. To eliminate the effect of individual diversification, the fALFF or ReHo value of each voxel was converted into a z-score by subtracting the mean fALFF or ReHo value and dividing the standard deviation of the whole-brain fALFF or ReHo map. Finally, z-standardized fALFF or ReHo were spatially smoothed with a 6 mm FWHM Gaussian kernel. A two-sample t-test was used to compare the differences between normal control and before-treatment groups, with head motion, age, and gender as covariates. A paired t-test was used to compare the differences before and after treatment, with head motion as the covariate. $P < 0.001$ (uncorrected) was considered statistically significant, and the cluster size was set at ≥ 5 voxels.

Metabolomics

Compound discoverer 3.0 was used for liquid chromatography–tandem mass spectrometry data processing. The metaX R was used for data pre-processing and statistical analysis. Principal component analysis was performed to determine the similarity between the groups and eliminate outlier samples. The variable importance in projection (VIP) values of the first two principal components of the partial least squares method-discriminant analysis model, the fold change, and Student's t-test analysis were used to screen for differential metabolites (*VIP ≥ 1; fold change ≥ 1.2 or ≤ 0.83; P* < 0.05).

Proteomics

IQuant was applied to the quantification of proteins.[16] All the proteins with a false discovery rate of less than 1% was selected for downstream analysis. Proteins with *fold change ≥ 1.2 or ≤ 0.83* and *Q value ≤ 0.05* were determined as differential protein.

Omics data integrated analysis

Migraine related targets were collected from DrugBank, the Therapeutic Target Database, the Online Mendelian Inheritance in Man database, and the DisGeNET database. A protein-metabolite interaction network was constructed based on the Stitch, IntAct, and Reactome databases. The network was visualized using Cytoscape 3.8.1. Metabolomic and proteomic integrated pathway enrichment analysis was performed by MetaboAnalyst 5.0 using the default parameters. Pathways with $P < 0.05$ and three or more hits were regarded as enriched.

Results

Participants

48 participants were enrolled, including 10 normal controls and 38 migraineurs. The 38 patients were randomly assigned into blank control, sham acupuncture grou, and acupuncture group. Among them, 7 patients withdrew (1 patient was pregnant), and 3 patients were lost during the study. Thus, 28 patients received treatment and were included in the safety and full analysis populations.

The baseline characteristics of the study participants are summarized in Table 1, data was well balanced across groups. The mean age was 35.2 years. The majority of patients were female (57.1%). The mean age at migraine diagnosis was 22.7 years, and the mean duration of migraine diagnosis was 12.5 years. A total of 20 patients (71.4%) reported using ≥ 1 analgesic medication during the study. Visual Analogue Scale, (VAS), 7-item Generalised Anxiety Disorder scale (GAD-7), and Migraine-Specific Quality-of-Life Questionnaire (MSQ) scores were well balanced across treatment groups.

Clinical effectiveness and safety

Clinical effectiveness outcomes are summarized in Table 1. The patients' VAS scores were significantly reduced after acupuncture treatment, as were the PSQI and MSQ score; the GAD-7 score was not significantly affected. Interestingly, sham acupuncture treatment reduced the patients' VAS scores, but the PSQI, MSQ score, and GAD-7 score were not significantly affected. Impressively, acupuncture treatment showed a superior effect over sham acupuncture treatment ($P < 0.05$) in reducing the MSQ score. There was no significant difference in any of the outcomes in the blank control group. No local hematoma or aggravation of headache was observed during the study, and no adverse events were observed in the treatment groups.

fMRI imaging data

In order to investigate changes in resting-state fMRI induced by treatments, fractional amplitude of low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) analysis were performed. Compared with normal controls, migraineurs showed significantly differential ALFF values in the left middle frontal gyrus, left inferior parietal lobe, right middle temporal gyrus and left superior temporal gyrus; showed significantly differential ReHo values in the left medial frontal gyrus, left middle frontal gyrus, left frontal lobe, right superior frontal gyrus, left middle temporal gyrus, right inferior temporal gyrus, right precuneus and right anterior cingulate (Supplementary Table S1 and S2). Differential regions were more extensive in the acupuncture group after treatment than in the sham acupuncture and blank control groups (Fig. 1). Regions with differential ALFF values in acupuncture group before and after treatment included the cerebellum, right lingual gyrus, left fusiform, left middle occipital gyrus, thalamus, right superior temporal gyrus, temporal lobe, precuneus, left insula, right extra-nuclear region, right supramarginal gyrus and cingulate gyrus; regions with differential ReHo values in acupuncture group before and after treatment included the left angular gyrus, left cerebellum, right middle frontal gyrus, left Inferior frontal gyrus orbital part, left Insula, left superior temporal gyrus, left ventral posterior lateral nucleus of the thalamus and left postcentral gyrus (Supplementary Table S1 and S2).

Changes in metabolite abundances

Twenty-eight migraine patients and ten normal controls were included. Principal component analysis score plots in both positive and negative modes are shown in Supplementary Fig.S3. Partial least squares-discriminant analysis score plots in both positive and negative modes (Fig. 2A-H) show that there was a clear distinction among groups. We identified 178 differentially expressed metabolites between the normal group and acupuncture group at day 0 (metabolites of migraine induced changes), 166 between the acupuncture day 0 and day 10 (metabolites of acupuncture induced changes); 231 between the normal group and sham acupuncture group at day 0, 29 between the sham acupuncture day 0 and day 10; 158 between the normal group and blank group at day 0, and 150 between the blank day 0 and day 10. Details about the differentially expressed metabolites see Supplementary Table S3. Among metabolites of migraine induced changes, 25 were significantly regulated by acupuncture (Fig. 2I). A hierarchical clustering analysis was performed to analyse changes in the 25 differential metabolites. A heat map was generated to show the average changes in the content of these metabolites (Fig. 2J).

Changes in protein abundances

Twenty-eight migraine patients and ten normal controls were included. In total, 2,730,225 spectra were generated; 5.860 peptides and 1353 proteins were identified with a 1% false discovery rate. Furthermore, migraine and treatment induced changes were investigated, 11 differentially expressed plasma proteins were recognized in the A 0d compared with the N 0d, 14 in the A 10d compared with the A 0d, 10 in the S 0d compared with the N 0d, 5 in the S 10d compared with the S 0d, 38 in the B 0d compared with the N 0d, and 19 in the B 10d compared with the B 0d (volcano plots see Fig. 3A-D; details about the differentially expressed proteins see Supplementary Table S4). Among proteins of migraine induced changes, two were significantly regulated by acupuncture, including platelet factor 4 (PLF4) and Tudor domain-containing protein (TDRD3), details see Fig. 3Eand F.

Integrated omics analysis

A total of 195 migraine-related targets were collected from public databases (details about the targets see Supplementary Table S5). To investigate the relationship between migraine related targets and acupuncture related targets, a protein-metabolite interaction network was constructed (Supplementary Fig.S4, Supplementary Table S6). 184 of 195 migraine targets, 30 of 165 differential metabolites, and 23 differential proteins were mapped to the network. The results of the pathway enrichment analysis of differential metabolites and proteins are shown in Fig. 4. The regulating effect of acupuncture on migraine was related to hypoxia-inducible factor (HIF)-1 signalling pathway, linoleic acid metabolism, glycerophospholipid metabolism, amino acid metabolism, etc (Fig. 4A, Supplementary Table S7). Figure 4B shows that mainly those pathways related to focal adhesion and regulation of the actin cytoskeleton were affected by sham acupuncture treatment (details about the enriched pathways see Supplementary Table S8).

Analysis of therapeutic mechanisms, based on the Kyoto Encyclopedia of Genes and Genomes pathway database and related literature, revealed that the anti-migraine effects of acupuncture and sham acupuncture involve complex networks in the body (Fig. 4C). The significant changes were mainly related to energy metabolism, inflammation and hypoxia-related pathways. Compared with normal controls, migraineurs showed a significant decrease in purine metabolism, linoleic acid metabolism, actin cytoskeleton, and an increase in amino acid metabolism and pyruvate content. Both acupuncture and sham acupuncture affected glycerophospholipid metabolism; however, they had opposite regulatory effects on choline levels. Patients treated with acupuncture showed a remarkably increased glycolysis and obvious decreases in tryptophan metabolism and steroid hormone biosynthesis. Also, acupuncture tended to upregulate purine metabolism, which significantly decreased in migraineurs. Fibronectin 1 in the actin cytoskeleton regulation pathway was significantly upregulated by sham acupuncture.

Discussion

The major findings in the present study are as follows: (1) Further clinical evidence for the effect of acupuncture on migraine was provided to consolidate previous findings; (2) We propose a hypothesis of the mechanism of acupuncture in the treatment of migraine; (3) Sham acupuncture is very different from acupuncture in terms of curative effect, affected brain regions and signalling pathways.

Our clinical study revealed that acupuncture can effectively alleviate migraine symptoms, the modes of action and underlying mechanisms of acupuncture therapy may be different from sham acupuncture therapy. Results showed that both acupuncture and sham acupuncture therapy can effectively alleviate migraine symptoms. Based on VAS scores, the effect was more pronounced in the acupuncture group than in the sham acupuncture group, although differences were not statistically significant. This is consistent with the results of previous studies, that is, placebo response is widely observed in clinical trials of pain treatment.[17] Furthermore, acupuncture could improve the sleep quality and especially the migraine-specific quality of life. However, our study showed that the GAD-7 index of migraineurs was not significantly affected by acupuncture. We speculate that the psychological state of disease and the pain state interact with each other, but the current psychological assessment scale lacks assessment of pain status, thus making it inadequate for accurate assessment of psychological changes during pain relief.

fMRI data showed that changes in the acupuncture group involved more brain regions than in the sham acupuncture group, indicate that the two treatments have different effects on brain activity. After acupuncture treatment, the ReHo and ALFF values increased in brain regions involved in the processing of pain, including sensory conduction of pain (thalamus), cognitive processing of pain (middle frontal gyrus), emotional processing of pain (cerebellum), and the default mode network (precuneus); In contrast, the ReHo and ALFF values decreased after acupuncture therapy in brain regions involved in the processing of pain (inferior frontal gyrus), emotional processing of pain (insula, cerebellum), default mode network (inferior parietal lobule) and limbic system (cingulate gyrus). Moreover, the ALFF value decreased in the fusiform gyrus but increased in both the lingual gyrus and superior temporal gyrus after acupuncture therapy. Notably, the lingual gyrus in the occipital lobe has been identified as an important structure in cortical spreading depolarization, which is involved in the onset and persistence of migraine.[18] Furthermore, the function of thalamus is related to regulating sleep and attention, and the ALFF value in the thalamus was increased after treatment, this phenomenon may explain the effects of acupuncture on sleep quality and migraine-specific quality of life. On the other hand, the brain regions affected by sham acupuncture were limited. Sham acupuncture therapy increased the ALFF value in a part of the brain region that is involved in the default mode network (inferior parietal lobule), but decreased the ALFF value in parts of brain regions that involve emotional processing of pain (insula). This may explain why sham acupuncture has an analgesic effect to some extent.

Migraine is a multifactorial disorder. Initially, researchers focused mainly on the neurovascular system and neurotransmission, until Willem Amery put forward the hypothesis that the pathogenesis of migraine is related to metabolism in 1982.[19] Since then, a growing body of evidence shows that migraine is at least partly an energy deficiency syndrome marked by mitochondrial dysfunction.[20] Inflammation also plays an important role in migraines. It has been reported that the increase in migraine frequency leading to chronic migraine involves neurogenic neuroinflammation, possibly entailing increased expression of cytokines via activation of protein kinases in neurons and glial cells of the trigeminal neurovascular system.[21] Integrated analysis showed that the mechanism of acupuncture involves complex networks in the body which were mainly related to regulating oxygen supply, energy metabolism, and inflammation. We found that migraineurs' bodies were in a state opposite to hypoxia, compared with the normal control, the hypoxic markers, hypoxanthine and xanthine, were significantly reduced in the migraineur group. Besides, the levels of the upstream and downstream metabolites, inosine and 5-hydroxy acid, were also significantly decreased. Additionally, the pyruvate levels were significantly increased. Furthermore, glyceraldehyde 3-phosphate dehydrogenase, a key enzyme of glycolysis, and lactate increased significantly after acupuncture treatment. Compared with normal controls, there was a downtrend in the levels of GAPDH and lactate in migraineurs. Hypoxanthine and xanthine levels also increased after acupuncture therapy. According to the astrocyte-neuron lactate shuttle theory, neurons activated and release the excitatory neurotransmitter glutamate, stimulate astrocytes to absorb glucose, trigger glycolysis, and produce lactate to provide energy for neurons, which may explain the phenomena that acupuncture can regulate the metabolism of brain energy and alleviate the brain energy imbalance of migraine patients by promote the glycolysis process of astrocytes. Acupuncture treatment resulted in the enrichment of pathways related to inflammation, including the PPAR signalling pathway and linoleic acid metabolism. The levels of (9S)-hydroxyoctadecadienoic acid were upregulated while APO A-II levels were downregulated by acupuncture. We also compared the pathways affected by sham acupuncture with those affected by acupuncture treatment, and found that the two main pathways affected by sham acupuncture involved glycerophospholipid metabolism and regulation of the actin cytoskeleton. Acupuncture exerts complex effects in the human body, and further in-depth research is needed to explore the underlying mechanisms.

In summary, our study is the first to elucidate the anti-migraine mechanisms of acupuncture from the perspectives of clinical effectiveness indices, fMRI, proteomics, and metabolomics. Our findings verified that acupuncture can effectively relieve migraine symptoms, and not merely via the placebo effect. The anti-migraine mechanism of acupuncture involves a complex network which is related to regulating the blood oxygen levels, reversing brain energy imbalance and regulating inflammation. Brain regions of migraineurs affected by acupuncture include the limbic system and default mode network, and regions involved in cognitive and emotional processing of pain, sleep, and attention.

Abbreviations

ALFF = amplitude of low-frequency fluctuation; BOLD = blood oxygenation level-dependent; fALFF = fractional ALFF; fMRI = functional magnetic resonance imaging; GAD-7 = 7-item Generalised Anxiety Disorder scale; HIF-1 = hypoxia-inducible factor 1; MSQ = Migraine-Specific Quality-of-Life Questionnaire; PSQI = Pittsburgh Sleep Quality Index; ReHo = regional homogeneity; VAS = Visual Analogue Scale

Declarations

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Data are available from the corresponding authors upon reasonable request: Dr. Tian guihua. Email: rosetgh@163.com. Supplementary material including Fig.S1- S4, Table S1-S8.

Competing interests

The authors report no competing interests.

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Authors' contributions

KH, LC and XYL contributed to the design of the study protocol. LC is responsible for the conception and design and writing the initial form of the manuscript and its editing. XYL is responsible for the conception and design and manuscript editing. KH and LC are responsible for the conception and design, financial support, manuscript editing, and final approval of the manuscript. All authors declare contribution to this paper. All authors approved the final version of the manuscript.

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Tables

Table 1 Baseline clinical characteristics and efficacy outcomes

Characteristics of study participants at baseline

	Normal control (n=10)	Blank control (n=9)	Sham acupuncture (n=9)	Acupuncture (n=10)
Age, mean (SD), y	30.4 (7.2)	35.7 (6.6)	33.0 (7.5)	37.4 (11.0)
Female, n (%)	7 (70)	3 (33)	5 (56)	8 (80)
Male, n (%)	3 (30)	6 (67)	4 (44)	2 (20)
Age at migraine diagnosis, mean (SD), y	null	20.6 (8.1)	19.9 (7.9)	27.4 (12.0)
Duration of migraine diagnosis, mean (SD), y	null	14.6 (9.8)	13.1 (6.9)	10 (7.4)
Health status, mean (SD)	null	3 (0.8)	2.4 (0.8)	3.1 (0.7)
Concomitant medications using during study, n (%)	null	7 (70)	5 (55.6)	8 (80)
VAS, mean (SD)	null	7.2 (0.6)	6.6 (1.6)	7.1 (1.5)
Attack frequency, mean (SD), w	null	1.9 (1.0)	0.83 (0.3)	2.5 (1.7)
PSQI, mean (SD)	null	8.2 (4.6)	5.8 (2.1)	9.6 (1.4)
GAD, mean (SD)	null	6.3 (6.8)	4.2 (2.7)	4.4 (3.6)
MSQ total, mean (SD)	null	28.9 (20.8)	29.6 (18.5)	26.4 (19.4)
Role function-restrictive, mean (SD)	null	18.3 (10.2)	19.3 (12.2)	16.2 (8.3)
Role function-preventive, mean (SD)	null	6.1 (6.5)	5.1 (6.4)	4.8 (6.9)
Emotional function, mean (SD)	null	4.4 (5.9)	4.5 (3.2)	5.4 (5.3)

Efficacy outcomes

	Blank control (n=9)	Sham acupuncture (n=9)	Acupuncture (n=10)	S0d vs A0d	S0d vs S10d	A0d vs A10d	S10d vs A10d	B0d vs B10d
VAS, mean (SD)	6.4 (1.1)	3.4 (2.3)	2.4 (2.1)	—	*	*	—	—
Attack frequency, mean (SD), w	1.9 (1.4)	1.2 (0.8)	1.5 (21)	*	—	—	—	—

Table 1 Baseline clinical characteristics and efficacy outcomes (continued)

Efficacy outcomes

	Blank control (n=9)	Sham acupuncture (n=9)	Acupuncture (n=10)	S0d vs A0d	S0d vs S10d	A0d vs A10d	S10d vs A10d	B0d vs B10d
PSQI, mean (SD)	7.9 (4.2)	4.3 (2.5)	6.3(1.7)	*	—	*	—	—
GAD, mean (SD)	4.3 (3.6)	1.8 (2.4)	1.7 (2.1)	—	—	—	—	—
MSQ total, mean (SD)	22. 8 (21.1)	11.1 (13.4)	5.5 (6.7)	—	—	*	*	—
Role function-restrictive, mean (SD)	14.3 (11.2)	5.9 (6.4)	3.9 (5.2)	—	—	*	*	—
Role function-preventive, mean (SD)	3.8 (6.1)	3.1 (4.9)	0.6 (1.3)	—	—	*	*	—
Emotional function, mean (SD)	4.7 (5.2)	2.1 (3.0)	1.0 (1.6)	—	—	*	*	—
Adverse events	none	none	none	null	null	null	null	null

Note:

“*” statistical significant difference（P < 0.05), “—” no statistical significant difference; Visual Analogue Scale (VAS, Pittsburgh Sleep Quality Index (PSQI), Generalized Anxiety Disorder (GAD-7), Migraine Specific Quality-of-Life Questionnaire (MSQ); Sham acupuncture day 0 (S 0d), Sham acupuncture day 10 (S 10d), Acupuncture day 0 (A 0d), Acupuncture day 10 (A 10d), Blank control day 0 (B 0d), Blank control day 10 (B 10d).

Figures

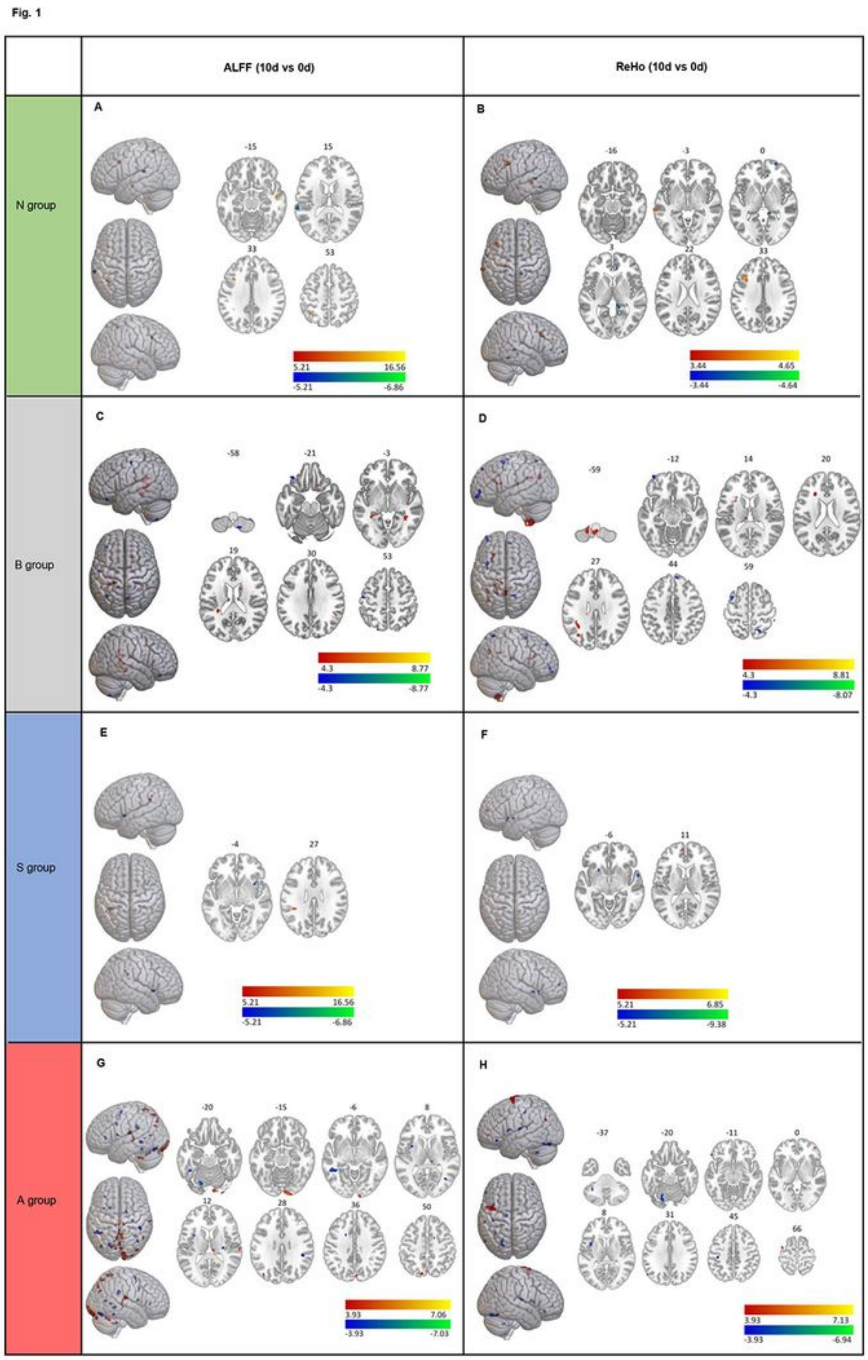

Figure 1

Significant differences in amplitude of low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) between groups. Regions with increased and decreased ALFF (**A**) and ReHo values (**B**) in migraineurs compared with normal controls (N group). Regions with increased and decreased ALFF (**C**) and ReHo values (**D**) in blank control group (B group) before and after treatment. Regions with increased and decreased ALFF (**E**) and ReHo values (**F**) in sham acupuncture group (S group) before and after treatment. Regions with increased and decreased ALFF (**G**) and ReHo values (**H**) in acupuncture group (A group) before and after treatment.

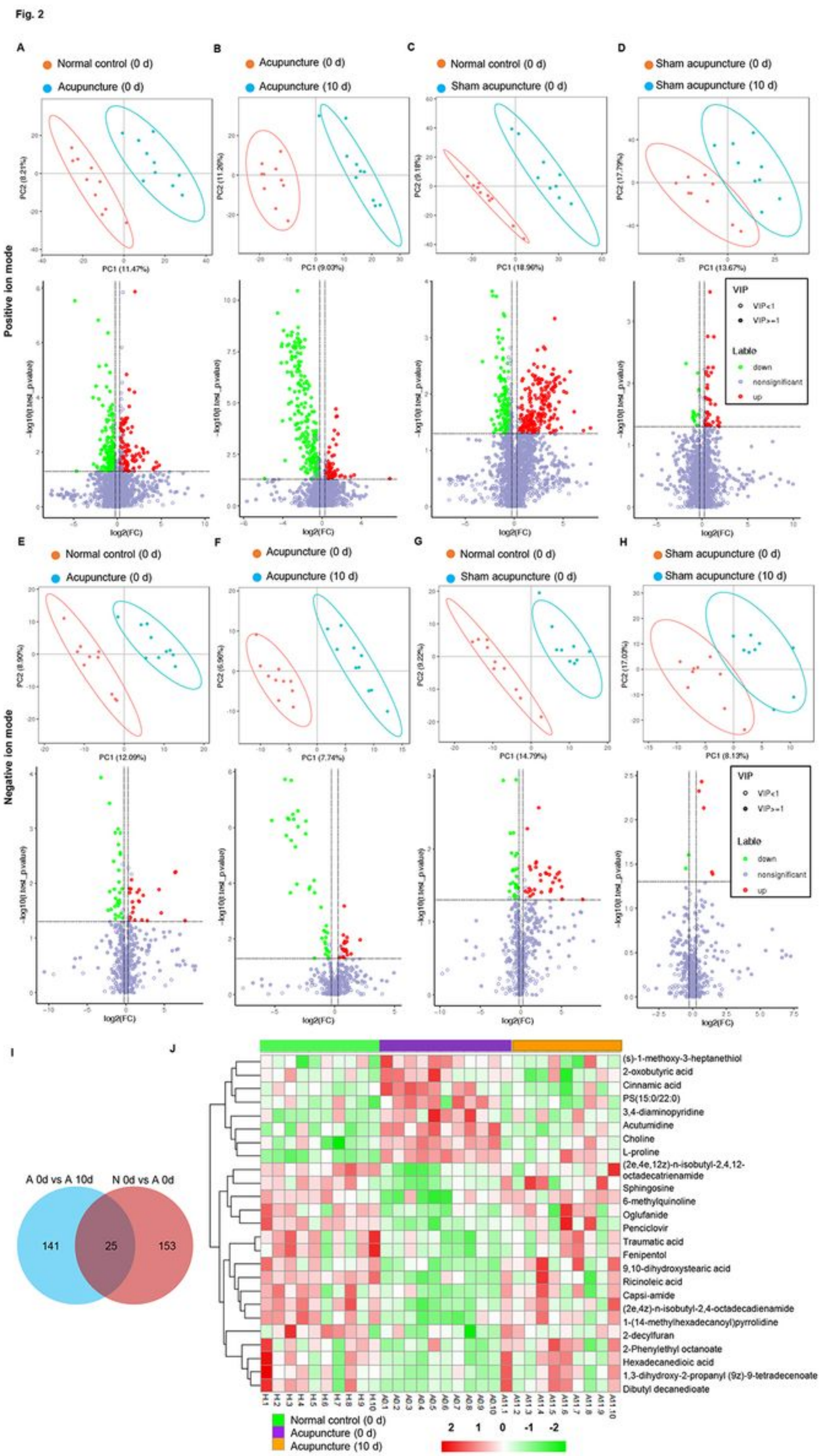

Figure 2

Differential metabolites analysis between groups. (**A-D**) PLS-DA scores and volcano plots in positive ion mode. (**E-H**) PLS-DA scores and volcano plots in negative ion mode. VIP≥1, fold change ≥1.2 or ≤0.83, p-value<0.05 are set as the significant threshold for differentially expression. (**I**) Venn diagram of differential metabolites identified in normal control (0d) vs acupuncture (0 d) and acupuncture (0 d) vs acupuncture (10 d), indicating 25 overlaps between sample groups. Results from positive and negative mode analyses were combined. (**J**) Heatmap of the 25 overlapping metabolites.

Fig. 3

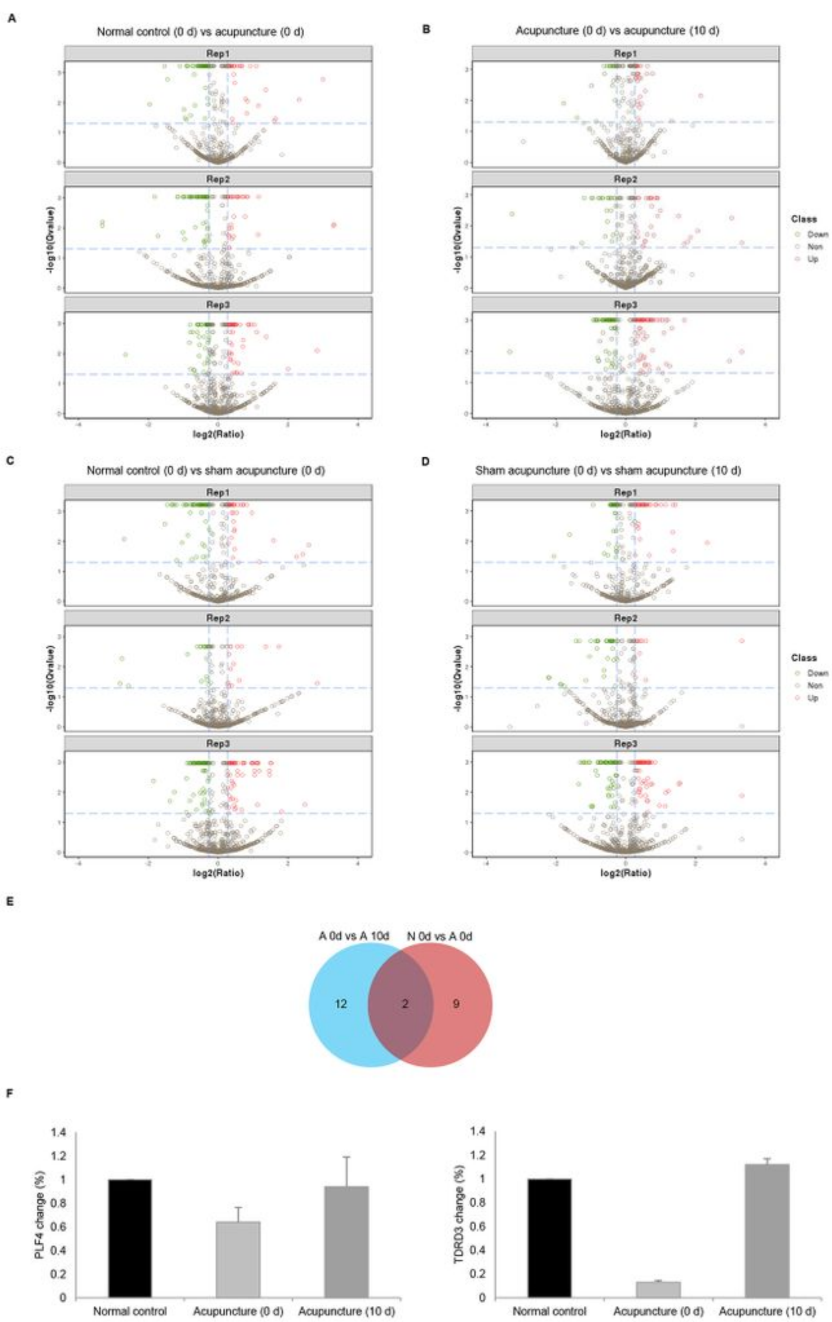

Figure 3

Differential proteins analysis between groups. Volcano plots of proteins in (**A**) normal control (0 d) vs acupuncture (0 d), (**B**) acupuncture (0 d) vs acupuncture (10 d), (**C**) normal control (0 d) vs sham acupuncture (0 d) and (**D**) sham acupuncture (0 d) vs sham acupuncture (10 d). Qvalue<0.05 and fold change > 1.2 or ≤0.83 are set as the significant threshold for differentially expression. (**E**) Venn diagram of differential proteins identified in normal control (0 d) vs acupuncture (0 d) and acupuncture (0 d) vs acupuncture (10 d), indicating 2 overlaps between sample groups. (**F**) Expression level of the 2 overlap proteins. TDRD3: Tudor domain-containing protein 3, PLF4: Platelet factor 4.

Fig. 4

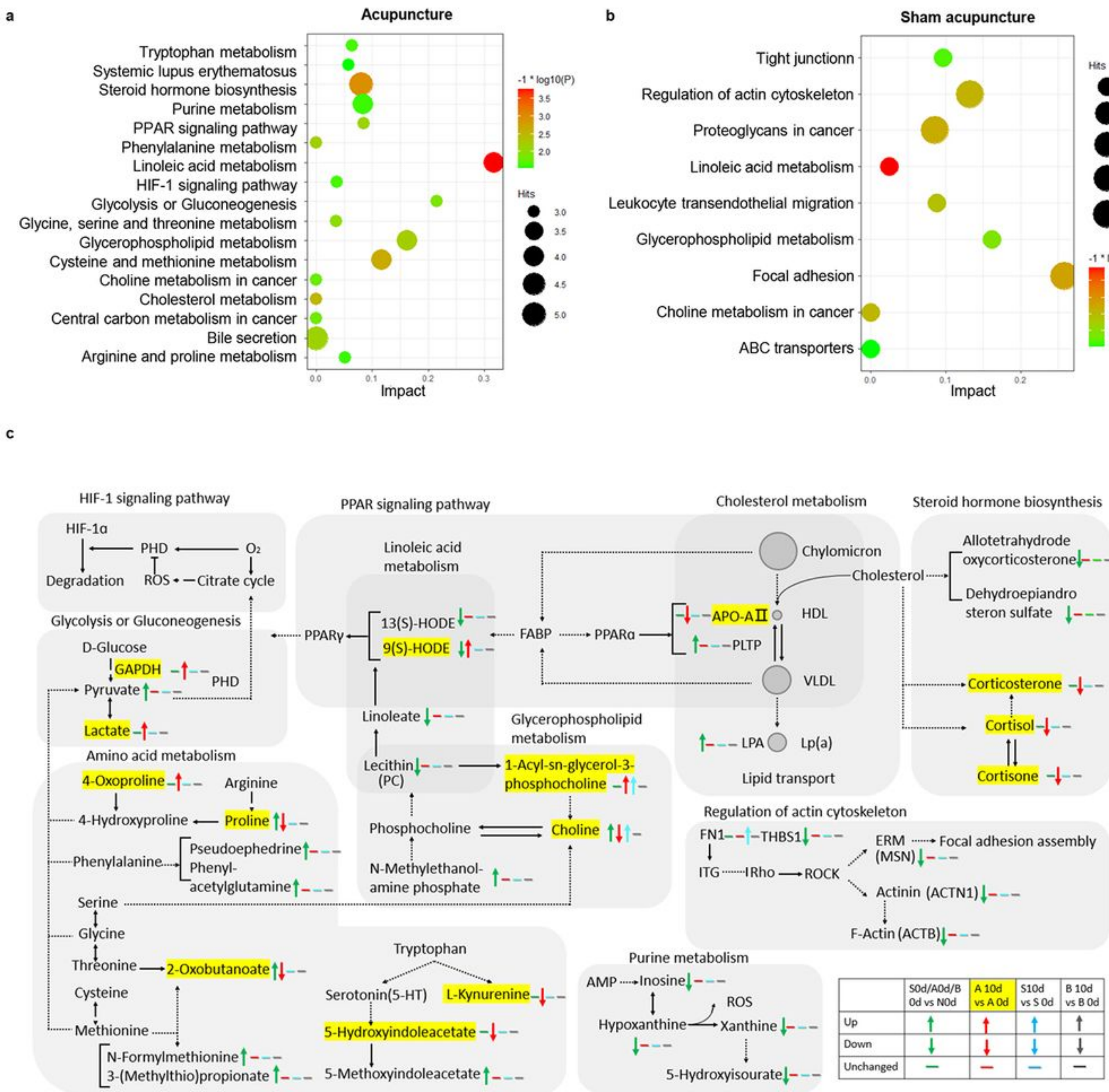

Figure 4

Integrated pathway analysis. Integrated pathway enrichment of acupuncture (**A**) and sham acupuncture (**B**) related targets based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Network map of enriched pathways (**C**). Targets regulated by acupuncture were marked in yellow.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation.pdf