BMJ Open

Association of nociceptive, neurocognitive, psychological and genetic profile on conditioned pain modulation in women with migraine: protocol for a case–control study

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To cite: Fernández de las Peñas C, Cigarán-Méndez M, Ambite-Quesada S, *et al*. Association of nociceptive, neurocognitive, psychological and genetic profile on conditioned pain modulation in women with migraine: protocol for a case–control study. *BMJ Open* 2025;**15**:e103305. doi:10.1136/bmjopen-2025-103305

► Prepublication history for this paper is available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2025-103305).

Received 07 April 2025
Accepted 28 July 2025

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ABSTRACT

Introduction Migraine is a primary headache showing a multifactorial component that includes altered pain processing, psychological/emotional problems, neurocognitive and executive function deficits, all with a possible genetic association. The aim of the current study will be to evaluate the association between sensitisation, psychological/emotional, neurocognitive and genetic profile on conditioned pain modulation (CPM) in women with migraine from a multidisciplinary perspective.

Methods and analysis A cross-sectional observational case–control study including 90 women with chronic migraine, 90 women with episodic migraine and 90 women without migraine (as controls) will be conducted. Clinical variables (disability, pain), processing (sensitisation-associated, neuropathic-like symptoms), psychological/emotional (anxiety, depression, sleep quality, catastrophising), neurocognitive (attention), executive functions (memory, mental inhibition, speed processing) and genetics (Val158Met polymorphism rs4680 gene) will be assessed in all subjects by healthcare professionals. Subsequently, CPM will be evaluated with the cold-pressor test paradigm by assessing changes obtained in mechanical and thermal stimuli. The association of each group of variables on CPM will be analysed with multivariate analyses (OMNIBUS analysis of variance). A network model will also be created to identify those variables showing the greatest key measure of centrality with the rest of the severity indicators (strength, intermediation and closeness) to establish the potentially therapeutic targets in patients with migraine from a multidisciplinary point of view.

Ethics and dissemination The protocol of the current study has been approved by the Ethics Committee of all involved institutions (Hospital Universitario Fundación Alcorcón 24–117, Universidad Rey Juan Carlos 010220240912024). All procedures will be conducted following the Declaration of Helsinki. Participants will be informed of the aims and procedures of the study and will receive the informed written consent which should be signed before their inclusion. Study results will be disseminated through peer-reviewed publications and presentations at scientific meetings.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The main strength of the current study will be the comprehensive analysis of conditioned pain modulation activation in migraine sufferers.
⇒ The main limitation of this protocol will be the cross-sectional nature of the study.
⇒ The second limitation will be the topic of medication intake which can be slightly different among the patients.
⇒ The third limitation will be that only the rs4680Val-158Met polymorphism of the catechol-O-methyltransferase is investigated.

INTRODUCTION

Migraine is a primary headache disorder showing a prevalence of 10% in the general population in both adults[1] and adolescents/children.[2] Migraine causes high disability with consequent social, work, economic and health impact to those who suffer from it.[3] These data support the need for a biopsychosocial approach[4] from a multidisciplinary viewpoint of migraine.[3]

Current theories contemplate migraine as a multifactorial condition where multiple hypotheses, for example, nociceptive (sensitisation processes), vascular (activation of trigemino-vascular system), cortical (spread cortical depression) and cognitive (emotional/cognitive alterations) interact at

the same time and under a potential genetic influence.[5] In fact, all these mechanisms have been proposed as hallmarks to be integrated into multifactorial models of migraine.[6]

There is evidence about the presence of an altered pain processing in patients with migraine manifested as an exaggerated response to different stimuli (ie, hyperalgesia or allodynia) and/or the presence of deficits in conditioned pain modulation (CPM).[7] However, current data are inconclusive for both. For instance, there is evidence supporting the presence of hyperalgesia/allodynia to pressure, thermal or electrical stimuli within the symptomatic area (trigeminal) in people with migraine; but the presence of hyperalgesia in extratrigeminal pain-free areas is inconclusive.[8 9] Likewise, evidence supporting the presence of alteration in CPM in people with migraines is heterogeneous, since some studies have shown an inefficiency of these mechanisms while others did not find such deficits.[10] It is important to note that most studies did not control for sex (the activation of CPM seems to be lower in females than in males), the frequency of migraines (episodic or chronic) or potential factors related to pain processing (central sensitisation-associated symptoms, presence of neuropathic-like symptoms), which also have an effect on CPM.

Individuals with migraine usually report cognitive symptoms including difficulty concentrating, memory problems and decreased mental processing speed both during and between migraine attacks.[11] One of the aetiological theories of migraine that would explain the presence of cognitive alterations would be the 'spread cortical depression' accounting during a migraine attack.[12] These changes at brain level cause sensitivity to external stimuli and problems focusing attention and making decisions.[12] In fact, attention and executive function are those cognitive processes most affected during the migraine attacks.[13] Nevertheless, previous studies had provided heterogeneous results, because they did not differentiate between episodic and chronic migraine, they included men and women, and the presence of mood disorders (eg, depressive or anxiety levels), sleep quality or pain catastrophising was not controlled.[11 13] In fact, there is evidence confirming that approximately 25% of migraine sufferers also exhibit symptomatology of depression or anxiety[14] and poor quality and structure of sleep.[15] The relevance of these aspects in the development of migraine lies in the fact that these processes share an anatomical substrate within the central nervous system; thus, the presence of emotional alterations can precipitate migraine attacks and enhance sensitisation. This hypothesis has been recently confirmed in a study that found an association between migraine-induced disability, sleep problems and impaired pain processing.[16] Accordingly, any study investigating altered processing in patients with migraine must control the association and effect of these emotional/cognitive aspects.

One cognitive aspect that has received particular attention in patients with migraines is pain catastrophe and the attitude towards pain.[17] Pain catastrophising is a maladaptive cognitive-affective response to pain and a tendency to exaggerate pain symptoms with a feeling of helplessness. This maladaptive cognitive-affective response could increase the perception of pain by enhancing sensitisation mechanisms. In fact, it has recently been observed that a catastrophic attitude towards pain is associated with a lower quality of life in patients with migraine.[18] Most studies investigating CPM have not controlled for the presence of pain catastrophising thought, a variable that can affect pain processing.[19]

Finally, the presence of a genetic component in migraines has always been considered.[20] Advances in the field of genetics have led to several studies trying to identify the presence of polymorphisms associated with a higher (or not) risk of suffering from a pain condition. The catechol-O-methyltransferase (COMT) gene, an enzyme involved in the metabolic degradation of neurotransmitters such as dopamine, norepinephrine or epinephrine, is one of the most investigated polymorphisms in individuals with chronic pain, particularly the rs4680 Val158Met single nucleotide polymorphism.[21] However, evidence on the role of this polymorphism in migraine is inconsistent. The only meta-analysis conducted to date did not find significant association between rs4680 Val158Met polymorphism and an increased risk of developing migraine.[22] Although current evidence suggests that the rs4680 Val158Met polymorphism does not appear to be associated with an increased risk of migraine, it does not exclude an association with clinical phenotype. In fact, it has been found that individuals with chronic migraine carrying the Met/Met genotype (with the lowest COMT activity) exhibit higher pressure pain hypersensitivity than those with a Val/Val genotype (with the highest COMT activity).[23] This research study opened a door to the possibility of a genetic association with the clinical severity of chronic pain such as migraine, and not only on the risk of suffering or not from that pain condition. In fact, a recent meta-analysis confirmed that the association of the Val158Met polymorphism on pain processing is conditioned by a proper (or not) functioning of CPM.[24] Again, no previous study has investigated the association of rs4680 Val158Met polymorphism on CPM in migraine.

Because migraine is more prevalent in females than in males[1] and the underlying mechanisms are associated with biological sex differences,[25] clinical research should be conducted with a 'sex perspective'.[26] Therefore, this study will be conducted just in women since nociceptive, psychological and neurocognitive mechanisms are different between men and women. Accordingly, we plan to conduct a case–control study including variables of pain sensitisation, psychological/emotional and neurocognitive variables (including executive function), as well as genetic predisposition, all these manifestations of nociplastic pain, evaluating endogenous pain modulation in women with migraine. The primary aim of this study will be to determine the potential association of sensitisation, psychological/emotional and neurocognitive variables

(including executive functions) in CPM in women with migraine. The secondary aim will be to identify the association of the rs4680 Val158Met polymorphism of the COMT gene on endogenous pain modulation in women with migraine.

METHODS AND ANALYSIS

Study design

A cross-sectional observational case–control study following the Strengthening the Reporting of Observational studies in Epidemiology guidelines[27] will be conducted. All patients will be recruited from the headache unit at the Neurology Department of Hospital Universitario Fundación Alcorcón (HUFA), an urban hospital in Madrid (Spain). All appointments will be performed at the Department of Physical Therapy, Occupational Therapy, Rehabilitation and Physical Medicine of Universidad Rey Juan Carlos (URJC). The study recruitment is planned to start in May 2025.

Patient and public involvement

There was no patient and public involvement in this study design.

Participants

A non-probabilistic sampling of consecutive cases attending the headache unit of the hospital will be used. Thus, consecutive women with a diagnosis of migraine according to the third edition of International Headache Society[28] by an experienced neurologist will be included. Migraine history will include location, pain quality, years with headache attacks, the frequency and intensity of headaches, family history and medication intake. They will be excluded if they present: (1) other primary or secondary headache, including medication overuse headache[28]; (2) a history of cervical trauma (ie, whiplash) or cervical herniated disc; (3) presence of comorbid medical diseases (eg, fibromyalgia); (4) a current psychiatric diagnosis based on Diagnostic and Statistical Manual of Mental Disorders (DSM-V) (eg, major or mild neurocognitive disorders, schizophrenia); (5) taking any medication (eg, antipsychotics, anticonvulsants or anticholinergics) able to affect cognition; (6) pregnancy or (7) had received any medical intervention, including aesthetic blocks, in the past 3 months.

A control (non-migraine) group of women without a history of migraine (headache) diagnosis and without reporting a headache attack the previous year, matched by age to the migraine group, will be recruited from local announcements. Participants will receive a clinical medical examination by a neurologist to determine their inclusion in the study. The exclusion criteria of the migraine group will also be applied to the control group.

Patient-Related Outcome Measures related to migraine

Clinical data will be collected in a headache diary for 4 weeks including the frequency of migraine attacks (days/month), the duration of each migraine attack (hours) and the intensity of the attack (Numerical Pain Rate Scale, 0–10 points).[29]

Migraine-related disability will be assessed with the Migraine Disability Assessment Scale (MIDAS) and the sex-item Headache Impact Test (HIT-6). The MIDAS evaluates disability caused by migraine by asking the days of partial or total loss within the previous 3 months regarding 3 daily life activities such as (1) paid work (or school); (2) household chores and (3) family, social, leisure activities.[30] The score is calculated from the sum of missed days and classifies migraine-related disability as grade 1 or little/no disability (from 0 to 5); grade 2 or mild disability (from 6 to 10); grade 3 or moderate disability (from 11 to 20); and grade 4 or severe disability (21 or greater points).[31] The Spanish version of the MIDAS questionnaire is a valid and reliable tool to measure migraine-related disability in university subjects.[32]

The HIT-6 items questionnaire measures the adverse impact of headaches by asking five questions related to social functioning, role functioning, vitality, cognitive functioning and psychological distress. Each question is answered on a 5-point Likert scale, where higher scores are associated with higher impact of migraine (score ranges from 36 to 78). The HIT-6 has shown good internal consistency, test–retest reliability, construct validity and responsiveness in migraine patients[33] and different languages, including Spanish.[34]

Patient-Related Outcome Measures related to pain processing

The following Patient-Related Outcome Measures (PROMs) assessing symptoms associated with pain processing will be assessed: the Central Sensitisation Inventory (CSI) and the Self-administered Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS).

The Spanish version of the CSI will be used in this study to identify the presence of sensitisation-associated symptoms.[35] Each symptom is scored on a 5-point Likert scale providing a score ranging from 0 to 100 points where a cut-off score of 40 points suggests the presence of sensitisation-associated symptoms.[36] The CSI has shown psychometric strength for assessing sensitisation-associated symptoms in people with persistent pain.[37] Further, the CSI has exhibited a sensitivity of 83% and a specificity of 55% to identify the presence of sensitisation, but it may also have a high false-positive rate in patients with complex pain and comorbid medical conditions.[38]

The Spanish version of S-LANSS will be used to identify if women with migraine experience neuropathic-like associated pain symptoms.[39] The S-LANSS assesses 7 questions scored from 0 to 24 points, where a cut-off score of ≥12 points suggests the presence of neuropathic-like pain symptoms.[40]

Psychological and cognitive PROMs

The variables within the psychological/cognitive domain will be anxiety/depressive levels, hypervigilance, pain catastrophising, kinesiophobia and sleep quality.

Anxiety and depressive symptoms will be assessed with the Spanish version of the Hospital Anxiety and Depression Scale (HADS).[41 42] The HADS is a 14-items PROM including 7 items related to anxiety (HADS-A) and another 7 items related to depressive (HADS-D) symptomatology.[43] The total score of each scale ranges from 0 to 21 points, higher values represent more anxiety or depressive levels. In individuals with headache, the HADS has shown good internal consistency and reliability.[44]

Pain catastrophising will be assessed with the validated Spanish version of the Pain Catastrophising Scale (PCS).[45] The PCS is a 13-item PROM evaluating the following three dimensions (rumination, magnification and helplessness) by describing different thoughts and feelings that individuals may experience when they are in pain. The total score ranges from 0 to 52 points, where a cut-off score of 30 is associated with significant levels of pain catastrophising.[46] The PCS has been previously used in individuals with migraine.[17 47 48]

Kinesiophobia levels, that is, defined as excessive, irrational and debilitating fear of physical movement, will be assessed by the Spanish version of the shortened version of the Tampa Kinesiophobia Scale (TKS-11).[49] The TSK-11 is a PROM composed of 11 items related to fear of movement to be answered on a 4-point Likert scale from 1 (totally disagree) to 4 (totally agree). The total score can range from 11 to 44 points. The TSK-11 has shown good internal consistency, test–retest reliability, responsiveness, concurrent validity and predictive validity.[50] Thus, the TKS-11 has shown appropriate structure for assessing fear of motion in migraine patients.[51]

The Spanish version of the Pittsburgh Sleep Quality Index will be used to assess the quality of sleep.[52 53] This PROM provides a score on sleep quality (from 0 to 21 points) based on 19 questions evaluating different aspects of sleep eg, usual bedtime, wake-up time, number of hours slept and time needed to fall asleep.[54]

The Spanish version of the short-form 9-items Pain Vigilance and Awareness Questionnaire (score 0–45 points) will be used to evaluate pain hypervigilance, for example, ideas of observing, monitoring and focusing on pain.[55]

Neurocognitive PROMs

Visuospatial memory

Visual perception, visual construction ability and spontaneous memory retention will be assessed using the Rey-Osterrieth Complex Figure (ROCF).[56] Participants are first asked to reproduce (copy) a geometric figure (composed of 18 black lines) from memory onto a sheet of paper both immediately (immediate recall) and after 20–30 min (delayed recall). No instructions are provided to memorise the figure, as the aim is to measure what is spontaneously retained by the participants. The following scores will be obtained: ROCF_Copy, immediate recall and delayed recall points (calculated by dividing copy points by maximum points); ROCF_Recall, which is the percentage of the recall (delayed recall points divided by immediate recall points); and ROCF_TimeCopy, the time taken to copy the figure.

Selective attention

Selective attention/concentration will be measured using the Spanish version of the d2 Attention test (d2).[57 58] The d2 test consists of 14 lines of 47 characters (totalling 658 items) where participants must identify and mark every letter 'd' with two small dashes (either both above, both below or one above and one below). These 'd' letters are the target items, while other combinations, such as 'p' or 'd' with one or no dashes, are those irrelevant. Each line must be completed on 20 s (total test usually takes 8–10 min). The following scores will be provided: d2_TR (total items attempted across the 14 lines), d2_TA (correctly identified relevant items), d2_O (relevant items missed and/or omitted), d2_C (irrelevant items mistakenly marked), d2_TOT (total test effectiveness, calculated as TR− (O+C)), d2_CON (concentration index, calculated as TA - C), d2_TR+ (the line with the most attempted items), d2_TR− (the line with the fewest attempted items) and d2_VAR (variation index, calculated as the difference between TR+ and TR).

Executive functions

Processing speed will be assessed by using the 'symbol search' (SS) subtest of the Wechsler Adult Intelligence Scale (WAIS-IV) battery.[59] The SS is a paper-and-pencil test including two sections: a key area with nine nonsensical pairs of digits and symbols, and a response area where digits are randomly placed alongside blank spaces. Participants are required to fill in the blanks with the corresponding symbols from the key as quickly as possible within 120 s.

Working memory will be assessed using the 'Digits D/R/I' subtest of the WAIS-IV battery.[60] It consists of the following three tasks: digit span forward, participants are asked to repeat a series of orally presented digits in the same order they are given; digit span backward, participants are required to repeat the digits in reverse order; and digit span sequencing, participants must repeat the digits read by the examiner in ascending numerical order.

Mental inhibition will be evaluated through the 'response inhibition index' of the 5-Digit Test (FDT).[61] The FDT is a Stroop-like task including the following four parts of 50 items each: Reading, Counting, Election and Alternation. The Reading and Counting tasks assess automatic and simple cognitive processes, while the Election and Alternation tasks assess more complex processes. The score is calculated by multiplying the number of errors by the time taken to complete each part. The following scores will be obtained: Decoding_FDT, time (s) needed to read all numeric items; Retrieving_ FDT, time (s) taken to read all non-numeric items (eg, asterisks); Inhibiting_FDT, time needed to read the same numeric item repeatedly and Shifting_FDT, time required to read a set of mixed numeric items within a box.

Planning and decision-making will be evaluated using the Zoo Map Test.[62] The Zoo Map test includes two parts: one evaluating the ability of planning in a context where no predefined pattern is required; and a second part evaluating the individual ability to apply a concrete external strategy. On each part, errors made are subtracted from the sequence score on the test sheet. The scores obtained in both parts are summed to generate a total sequence-error score from 0 to 16 points.

Genetic variable: Val158Met polymorphism of the COMT

Biological sample collection

Unstimulated whole saliva samples will be collected into collection tubes for 5 min by using the passive drooling technique according to standardised procedures. Participants will be seated and relaxed during saliva sample collection and will be asked not to eat, drink or chew gum for 1 hour before sample collection. The volume of saliva secreted will be measured using a 200–1000 µL micropipette. All saliva samples will be centrifuged at 3000 rpm for 15 min to obtain the cell sediment self-collection procedure immediately after the collection. The supernatant and the precipitate (cells for DNA extraction) will be aliquoted into Eppendorf tubes. The supernatant will be stored at −80°C and the pellet at −20°C until the analysis. Saliva samples will be collected instead of blood because it is a non-invasive and stress-free assessment method and considering that salivary DNA is equivalent in quantity and purity to blood DNA.[63]

Genome DNA extraction

The genomic DNA will be extracted using a MagMAX DNA Multi-Sample Ultra 2.0 Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. The resulting DNA will be assessed for concentration and purity with Quant-iT PicoGreen dsDNA reagent (Thermo Fisher). DNA will be diluted to 5 ng/µL, using 1×Tris-EDTA (TE) buffer (Sigma-Aldrich, Dorset, UK). The qPCR reaction mixtures of 10 µL contained a total of 10 ng gDNA as a PCR template, 1x TaqMan Gene Expression PCR Master Mix and 0.6x Genotyping TaqMan-probe assay.

Val158Met polymorphism genotyping

The Val158Met polymorphism genotyping will be done with TaqMan Predesigned SNP Genotyping Assays (Thermo Fisher Scientific). Real-time PCR plates will be run in the Quantstudio 12K Flex System (Thermo Fisher) of Genomics Unit (Madrid Science Park Foundation, Spain) under standard conditions (95° for 10 min and 40 two-step cycles consisting of 95°C for 15 s and 60°C for 1 min) and analysed with Genotyping App of Thermo Fisher Cloud. Each genotype will be identified using specific fluorescent dyes. The Val158Met polymorphism will be determined by the analysis of an adenine to guanine change in the sequence:

CCAGCGGATGGTGGATTTCGCTGGC **[A/G]** TGAA GGACAAGGTGTGCATGCCTGA

Psychophysical outcomes: pressure and thermal pain thresholds

Pressure pain thresholds (PPT) will be assessed bilaterally at a trigeminal area (the temporalis muscle) and two remote pain-free areas (lateral epicondyle, tibialis anterior) with an electronic algometer (Somedic AB, Farsta, Sweden). The pressure will be applied perpendicularly to the point at a rate of approximately 30 kPa/s with a random order in the point. Three repetitions will be made at each point with an interval of 30 s to avoid temporal summation. The mean of the three repetitions will be calculated and used for statistical analysis.

Pain thresholds to heat (HPT) and cold (CPT) will be calculated following the method of limits[64] with an advanced thermal stimulator (ATS System, Medoc Pathways System, Israel). A 30×30 cm thermode will be placed on a trigeminal branch (V1) area (frontalis muscle). Three repetitions will be obtained with an interval of 30 s. The mean of the three repetitions will be calculated and used for analyses.

Conditioned pain modulation

CPM assesses the functioning of endogenous pain modulation pathways, that is, descending pain inhibitory mechanisms.[65] To do this, first, two stimuli (mechanical and thermal) will be applied, then the patient will be exposed to the conditioned stimulus and finally, the two stimuli will be applied again.

In the present study, the cold-pressor test will be used as a conditioning paradigm following previous guidelines.[66 67] Participants will immerse one hand (the hand of the side of migraine in women with unilateral migraine or the dominant hand in women with bilateral migraine or control women) for 1 min in water at a temperature of 10°C. A circulating water container will be used (Huber K20-cc NR, Offenburg, Germany), so that the temperature will not vary around the immersion site. Every 30 s the patient will have to verbally indicate the perceived pain intensity on a 10-point numerical scale (0: no pain; 10: maximum possible pain). This measurement will be carried out at 30 and 60 s after the hand has been immersed in water. Patients will be asked to remove their hands from water and both mechanical and thermal stimuli will be assessed again following the same procedure described above. The cold-pressor test has shown good to excellent intrasession reliability in healthy volunteers and chronic pain patients.[68]

Subsequently, CPM will be evaluated with the cold-pressor immersion paradigm on changes obtained in mechanical and thermal stimuli. The absolute (kPa or °C) and the percentage (%) differences between PPT, HPT and CPT values before/after cold-pressor test will be calculated. Impaired CPM is operationally defined as no change or a negative change in measures, taken directly following termination of the conditioning stimulus (cold-pressor paradigm).[69] Figure 1 summarises the study design.

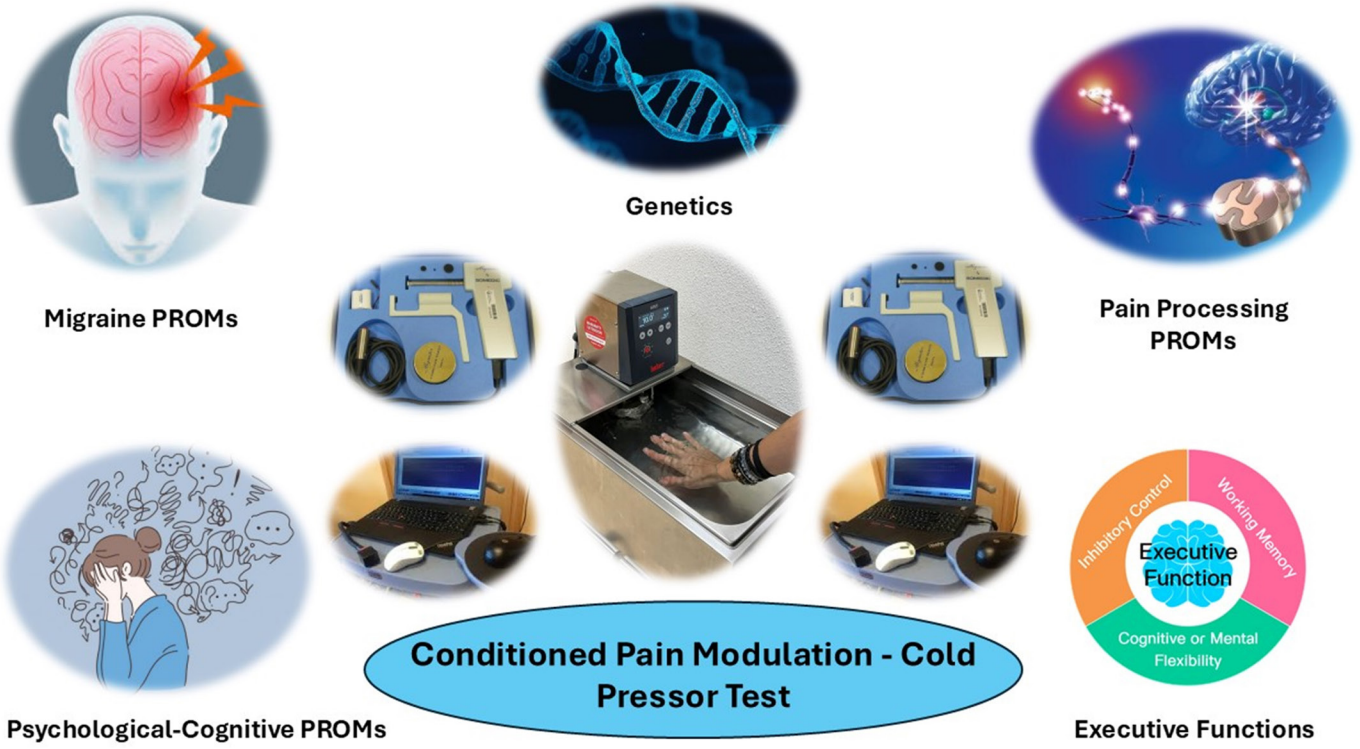

Figure 1 Summary of the study design showing clinical, psychological/cognitive, pain processing, psychophysical, neurocognitive (executive functions) and genetic variables assessed throughout the evaluation of conditioned pain modulation in women with migraine. PROMs, Patient-Related Outcome Measures.

Sample size calculation

The sample size required for the study was calculated using the G*Power V.3.1.9.7 computer program. The following statistical parameters were entered into the 'F tests' family of tests for a repeated-measures MANOVA statistical test on the main outcome of the study, CPM: effect size (f2): 0.25; α: 0.05; statistical power: 0.90; number of groups: 3; number of measures: 2. Once these parameters were entered, the necessary sample size identified was 206 participants. The study will include 210 subjects who will be divided into the following three groups: a group composed of 70 women with chronic migraine; a group composed of 70 women with episodic migraine and a group composed of 70 control women.

Statistical analysis proposal

All statistical analyses will be conducted using the Statistical Package for the Social Sciences (SPSS) Software. Outliers will be identified through boxplots, considering values below the first quartile minus 1.5 times the IQR and above the third quartile plus 1.5 times the IQR as potential outliers. Assumptions of normality and sphericity will be verified.

Descriptive analyses will be used for description of the sample. Frequencies and percentages will be provided for categorical variables and mean and SD for continuous variables. χ^2 (for categorical variables) and one-way analysis of variances (for continuous variables) will be applied to calculate intergroup differences (episodic migraine, chronic migraine, controls) in all variables (clinical variables, pain processing, psychological, cognitive, neurocognitive, executive functions, distribution of Val158Met genotype) to identify potential covariates to be included in posterior analyses.

Separated multivariate repeated-measures analysis of covariance (OMNIBUS RM-ANCOVA) will be performed to identify the effect of migraine over CPM (changes in PPT, HPT, CPT) by controlling the confounders (clinical, pain processing, psychological, cognitive, neurocognitive, executive function, Val158Met genotype). For these analyses, psychophysical (PPT, HPT, CPT) outcomes (before and after the conditioning stimulus) will be the within-subject factor, group (episodic migraine, chronic migraine, controls) will be the independent variable and the remaining variables (clinical, pain processing, psychological, cognitive, neurocognitive, executive function, Val158Met genotype) will be the covariates. Thus, due to the inclusion of three groups in the analyses, we will apply a correction for multiple comparisons, where p values <0.015 (0.05/3) will be considered as statistically significant. Finally, partial eta squared (n^2p) will be used to calculate effect sizes, with values of 0.01 indicating a small effect, 0.06 indicating a medium effect and above 0.14 indicating a large effect.[70] Bonferroni post hoc tests will be applied to assess intergroup differences.

ETHICS AND DISSEMINATION

The study has been approved by local ethics committees (Hospital Universitario Fundación Alcorcón HUFA

24_117; Universidad Rey Juan Carlos URJC_01022024 0912024). All participants will receive informed consent in accordance with Spanish legislation and should sign before their enrolment in the study. All procedures of this study will follow the ethical standards of the Declaration of Helsinki. The confidentiality of the data of participants will be guaranteed in accordance with current legislation. Study findings will be submitted to be potentially published in peer-reviewed scientific journals and will be presented at international meetings.

Limitations of the study

The main limitation of the current protocol is the potential cross-sectional nature of the study. Although this would be the most comprehensive analysis of CPM activation in migraine sufferers, the cross-sectional nature of the study will not permit determining a cause-and-effect direction of the findings. Second, since medication intake will not be changed and can be slightly different among the patients, this factor may introduce some bias in the outcomes. Finally, we will investigate the rs4680Val158Met polymorphism of the COMT which may be somewhat limiting given the complex genetic underpinnings of migraine. Future studies should include a greater number of polymorphisms and other genes to further clarify their potential association in CPM in migraine sufferers.

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Contributors All the authors cited in the manuscript had substantial contributions to the concept and design, the execution of the work or the analysis and interpretation of data; drafting or revising the manuscript and have read and approved the final version of the paper. CFdlP: conceptualisation, visualisation, methodology, validation, data curation, writing-original, writing-review and editing. MC-M: methodology, validation, data curation, writing-original draft, writing-review and editing. SA-Q: conceptualisation, methodology, validation, data curation, writing-original draft, writing-review, editing. CG-C: conceptualisation, methodology, data curation validation, writing-original draft, writing-review, editing. FGF-P: conceptualisation, methodology, data curation validation, writing-original draft, writing-review, editing. JCP-H: methodology, validation, data curation, writing-original draft, writing-review, editing. AT-A: methodology, validation, data curation, writing-original draft, writing-review and editing. GP-M: validation, writing-original draft, writing-review and editing. JAV-C: validation, data curation, writing-original draft, writing-review, editing. BA-M: validation, data curation, writing-original draft writing-review, editing. CO-B: validation, data curation, writing-original draft, writing-review and editing. LA-N: validation, data curation, writing-original draft, writing-review and editing. Ald-I-L-R: methodology, validation, data curation, writing-original draft, writing-review and editing. CFdlP is the guarantor.

Funding The project has been funded by the el Ilustre Colegio Profesional de Fisioterapeutas de la Comunidad de Madrid y el Colegio Oficial de Farmacéuticos de Madrid in a competitive call, through the Call for Multidisciplinary Research Grants in Pain and Physiotherapy, on the occasion of the Pain and Physiotherapy 2024 Congress.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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