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A novel cortical biomarker signature for predicting pain sensitivity: protocol for the PREDICT longitudinal analytical validation study

David A. Seminowicz[a,b,c], Katarzyna Bilska[c,f], Nahian S. Chowdhury[c], Patrick Skippen[c], Samantha K. Millard[c,f], Alan K.I. Chiang[c], Shuo Chen[d], Andrew J. Furman[a,b,e], Siobhan M. Schabrun[c,*]

Abstract

Introduction: Temporomandibular disorder is a common musculoskeletal pain condition with development of chronic symptoms in 49% of patients. Although a number of biological factors have shown an association with chronic temporomandibular disorder in cross-sectional and case control studies, there are currently no biomarkers that can predict the development of chronic symptoms. The PREDICT study aims to undertake analytical validation of a novel peak alpha frequency (PAF) and corticomotor excitability (CME) biomarker signature using a human model of the transition to sustained myofascial temporomandibular pain (masseter intramuscular injection of nerve growth factor [NGF]). This article describes, a priori, the methods and analysis plan.

Methods: This study uses a multisite longitudinal, experimental study to follow individuals for a period of 30 days as they progressively develop and experience complete resolution of NGF-induced muscle pain. One hundred fifty healthy participants will be recruited. Participants will complete twice daily electronic pain diaries from day 0 to day 30 and undergo assessment of pressure pain thresholds, and recording of PAF and CME on days 0, 2, and 5. Intramuscular injection of NGF will be given into the right masseter muscle on days 0 and 2. The primary outcome is pain sensitivity.

Perspective: PREDICT is the first study to undertake analytical validation of a PAF and CME biomarker signature. The study will determine the sensitivity, specificity, and accuracy of the biomarker signature to predict an individual's sensitivity to pain.

Registration details: ClinicalTrials.gov: NCT04241562 (prospective).

Keywords: Biomarkers, Electroencephalography, Nerve growth factor, Orofacial pain, Susceptibility, Transcranial magnetic stimulation

1. Introduction

Temporomandibular disorder (TMD) is the second most common musculoskeletal pain condition after back pain, with an annual incidence of 4% and development of chronic symptoms in 49% of patients.[36,38] Although a number of biological factors have shown an association with chronic TMD in cross-sectional and case control studies including sensitivity to mechanical stimuli,[39] upregulated central nociceptive processing,[30,32] increased heart rate and reduced heart rate variability,[22] single-nucleotide polymorphisms,[37,40] elevated levels of proinflammatory cytokines,[37] elevated interstitial glutamate concentration,[2] and altered brain structure and function,[21] these have either failed to yield clinically meaningful predictive power or have not undergone comprehensive validation in prospective trials. Consequently, there are no biomarkers available that can predict the development of chronic TMD. In fact, there are no biomarkers qualified (considered valid and psychometrically sound) by the Food and Drug Administration for use in clinical trials or clinical practice for any musculoskeletal pain condition.[41]

In most patients with chronic musculoskeletal pain, a peripheral anatomical cause for pain cannot be identified. For example, myofascial TMD is more commonly associated with stress and anxiety than anatomical pathology,[42] whereas 90% of all chronic low back pain is diagnosed as "nonspecific."[18] In conditions where a structural impairment can be detected (ie, articular cartilage damage in osteoarthritis), the magnitude of pain fails to correlate with the extent of tissue damage.[11] These observations suggest a role for the brain in the development and

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[a] Department of Neural and Pain Sciences, University of Maryland School of Dentistry, Baltimore, MD, United States, [b] Center to Advance Chronic Pain Research, University of Maryland Baltimore, Baltimore, MD, United States, [c] Neuroscience Research Australia, Sydney, New South Wales, Australia, [d] Division of Biostatistics and Bioinformatics, Department of Public Health and Epidemiology, University of Maryland School of Medicine, Baltimore, MD, United States, [e] Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD, United States, [f] University of New South Wales, Sydney, New South Wales, Australia

**Corresponding author. Address: Neuroscience Research Australia, Sydney, 2031, New South Wales, Australia. Tel.: +61 2 9399 1075. E-mail address: s.schabrun@neura.edu.au (S.M. Schabrun).*

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maintenance of chronic pain. Indeed, early investigations suggest that variability in brain connectivity circuits can predict sensitivity to a transient pain stimulus in healthy individuals.[31] Although these data have not yet been expanded and the relevance to clinical pain is unknown, brain imaging methods are widely considered to have potential as diagnostic, prognostic, and predictive biomarkers of chronic pain.[6]

Using brain imaging methods of electroencephalography (EEG) and transcranial magnetic stimulation (TMS), preliminary evidence for a unique biomarker signature—combined resting-state peak alpha frequency (PAF; the frequency band within the 8–12 Hz range displaying maximal power) and corticomotor excitability (CME; excitability of the corticomotor representation of a target muscle)—has recently been demonstrated. In studies using long-lasting human pain models, slow PAF and low CME are associated with high pain severity and longer pain duration.[13,34,35] Consistent with this, low CME in the acute stage of clinical pain is associated with high pain severity and the presence of pain at 6-month follow-up.[3] These data suggest the combination of slow PAF and low CME may be a plausible predictive biomarker for the development of chronic TMD.

Here, we outline the experimental protocol and statistical analysis plan to undertake analytical validation of the PAF/CME biomarker signature using a standardized human model of the transition to sustained myofascial temporomandibular pain (masseter intramuscular injection of nerve growth factor [NGF]). We hypothesise that the PAF/CME biomarker signature will predict pain sensitivity (primary) and pain severity and duration (secondary) with at least 75% accuracy in a human transitional pain model of TMD. In addition, we aim to (1) determine the sensitivity, specificity, and accuracy of the PAF/CME biomarker at predicting pain sensitivity, severity, and duration, (2) determine the reportable range of test results and reference intervals for fast vs slow PAF and high vs low CME, and (3) establish optimization of the model and automation and simplification of methods for biomarker detection.

2. Methods

2.1. Design

A multisite longitudinal, experimental study will be used to follow healthy individuals for a period of 30 days as they progressively develop and experience complete resolution of NGF-induced muscle pain. All data collection will be performed at the Australian site (Neuroscience Research Australia; NeuRA), and blinded data processing and analyses will be performed at the U.S. site (the University of Maryland Baltimore; UMB). The UMB site will also be responsible for standardization and automation of analytical methods. A data and safety monitoring plan has been established, and an independent monitoring committee will conduct annual reviews of study progress and safety. Ethical approval has been obtained from the University of New South Wales (HC190206) and the University of Maryland Baltimore (HP-00085371). All procedures will be conducted in accordance with the Declaration of Helsinki. Written informed consent will be obtained and participants will be free to withdraw from the study at any time. The study is prospectively registered on ClinicalTrials.gov (NCT04241562).

2.2. Participants

2.2.1. Inclusion and exclusion criteria

Healthy men and women with no medical complaints, no history of chronic pain, and no current acute pain between the ages of 18 and 44 years will be included. These inclusion criteria are justified based on data from the OPPERA prospective cohort study that demonstrates only marginally greater TMD incidence in females than males and an incidence rate of first-onset TMD of 2.5% per annum among 18–24-year-olds and 4.5% per annum among 35–44-year-olds.[36] Exclusion criteria are: (1) inability or refusal to provide written consent, (2) presence of an acute pain disorder, (3) history or presence of any chronic pain disorder including migraine, (4) history or presence of any other medical or psychiatric complaint, (5) use of opioids or illicit drugs in the past 3 months, (6) current smoker or using nicotine replacements, (7) pregnant or lactating women, and (8) contraindicated for TMS (metal implants, epilepsy).[16] Participants will be recruited through notices placed on community notice boards at UNSW and NeuRA, flyers, mailings, and social media platforms (such as Facebook) as well as the use of a volunteer healthy participant database held by NeuRA.

2.2.2. Sample size

One hundred fifty healthy subjects will be included. Our preliminary data[13,34,35] indicate consistent associations between PAF and future pain severity, as well as strong relationships between CME and pain severity. The design of the current discovery-based study is not amenable to traditional power calculations because the outcomes are not *P*-value-based inference but rather predictive. Larger sample sizes in the training set give better classification, whereas larger sample sizes in the testing set give higher accuracy. We have chosen a sample size that provides good classification and accuracy. Allowing for a 10% dropout rate, we will enrol 165 subjects.

2.3. Data collection procedures

2.3.1. Overview

Participants will first complete a phone screen and if eligible, a time will be made for the day 0 visit. At the day 0 visit, after reviewing eligibility criteria, participants will complete informed consent (considered enrolment in the study) and questionnaires. Participants will complete twice daily electronic pain dairies from day 0 to day 30 and attend 3 laboratory visits of ~2 hours duration on days 0, 2, and 5. Each laboratory visit will include assessment of pressure pain thresholds (PPTs), and recording of PAF and CME. Intramuscular injection of NGF will be given into the right masseter muscle at the end of each test session on days 0 and 2 (**Fig. 1**). These procedures are detailed below.

2.3.2. Electronic diaries

Diaries will be completed using a computer, tablet, or phone at 10 AM and 7 PM each day from day 0 to day 30. Electronic diary completion will take 2 minutes. Participants will rate their pain intensity on an 11-point numerical rating scale anchored with "no pain" at zero and "worst pain imaginable" at 10 at rest, and during activities of daily living such as chewing, swallowing, drinking, talking, yawning, and smiling.[9] Participants will be prompted to complete the pain diary twice per day (10 AM and 7 PM) each day. If the diary is not completed for 2 consecutive days, participants will be followed-up by phone.

2.3.3. Questionnaires

At Day 0 only, participants complete a health history form assessing medical history. We will use the following National

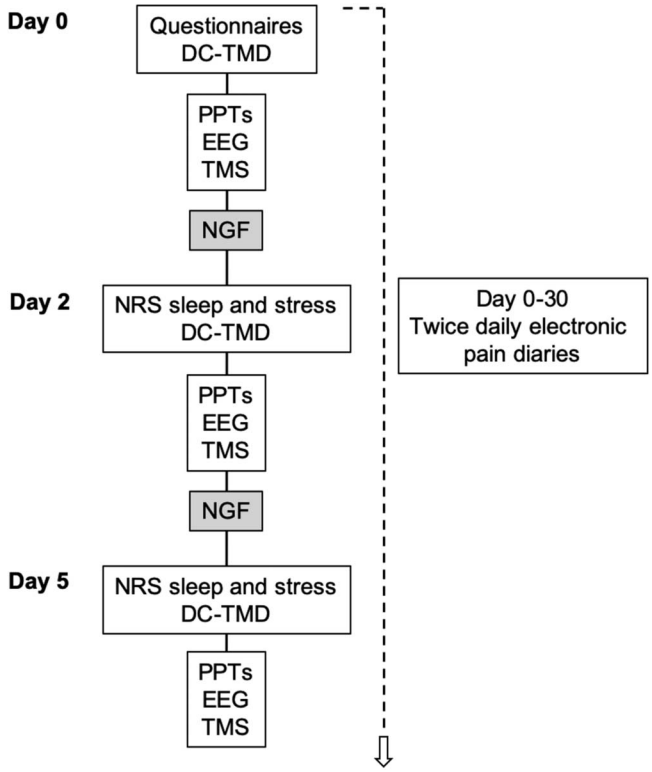

Figure 1. Experimental protocol. Questionnaires—Health history form, Pain Catastrophising Scale, Brief Pain Inventory Pain Severity and 7-item Interference subscales; SF-8 Health Survey; Sleep Scale; Patient Health Questionnaire-2 item; Generalised Anxiety Disorder 2 item Questionnaire; Tobacco, Alcohol, Prescription Medications, and other Substances Questionnaire; Perceived Stress Scale; and Pennebaker Inventory of Limbic Languidness (PILL) questionnaire. DC-TMD, Diagnostic Criteria for Temporomandibular Disorder; EEG, electroencephalography; NGF, nerve growth factor; NRS, numerical rating scale; PPTs, pressure pain thresholds; TMS, transcranial magnetic stimulation.

Institutes of Health common data elements (CDE) for pain biomarkers including: Pain Catastrophizing Scale[43]; Brief Pain Inventory Pain Severity and 7-item Interference subscales[17,45]; SF-8 to assess general health[50]; Sleep Scale; PHQ-2 to assess depression[19]; GAD-2 to assess anxiety[20]; and Tobacco, Alcohol, Prescription medications, and other Substances. Participants will also complete the Perceived Stress Scale[46] and the Pennebaker Inventory of Limbic Languidness questionnaire.[29] These questionnaires assess factors that have been associated with first-onset and/or chronic TMD[1] and often worsen as TMD progresses.[10] These questionnaires will be completed on day 0.

(1) On days 0, 2, and 5, participants will be examined according to the Diagnostic Criteria for TMD (DC-TMD)[33] and will complete 2 numerical rating scales asking the following questions:
(2) On a scale of 1 to 10 where 1 is "poor sleep quality" and 10 is "excellent sleep quality," how would you rate your sleep last night?
(3) On a scale of 1 to 10 where 1 is "not at all stressed" and 10 is "very stressed," how would you rate your level of stress over the last 24 hours?

2.3.4. Pressure pain thresholds

Because NGF injection is known to sensitize mechanosensitive afferents,[23,44] and because lower PPTs at cranial sites are associated with increased risk of developing TMD[14] and fluctuate with the clinical disease course,[39] we will assess PPTs at 5 sites—overlying the masseter muscle, temporalis muscle, the temporomandibular joint, the trapezius muscle, and the lateral epicondyle. Three measures will be made at each site, with 1-minute rest between measurements at the same site, in pseudorandomized order using a commercially available algometer.

2.3.5. Peak alpha frequency

Scalp EEG will be collected using Brain Vision actiCAP with at least 32 channels, following the extended international 10 to 20 system,[28] a BrainAmp DC amplifier, and Brain Vision Recorder version 1.22.0101 software (all Brain Products GmbH, Munich, Germany). Auxiliary recordings will include skin conductance, respiration, and electrocardiogram (ECG). Participants will be asked to make facial muscle contractions such as clenching their teeth, blinking, and saccades, while EEG is recorded. This will take about 2 minutes and will be used to aid in automated artefact removal. Participants will then be told to relax their muscles and resting-state eyes-closed EEG will be recorded for 5 minutes and used for PAF calculation.

2.3.6. Corticomotor excitability

Rapid TMS will be used to map the primary motor cortical representation of the right masseter muscle and right extensor carpi radialis brevis (ECRB) muscle. Mapping of the right ECRB muscle is included to determine whether any changes in corticomotor excitability are restricted to the affected muscle. Single-pulse, biphasic stimuli will be delivered to the left hemisphere using a Magstim Super Rapid^2 Plus and a 70-mm figure-of-eight coil. Bipolar surface electrodes will be used to record electromyographic (EMG) activity.[8] EMG signals will be amplified (x2000), filtered (20–1000 Hz), and digitally sampled at 5 kHz. The scalp site that evokes the largest EMG response (motor-evoked potential, MEP) at a given TMS intensity will be determined for each muscle in each individual (termed the "hotspot") and the active (aMT—masseter muscle) or resting (rMT—ECRB muscle) motor threshold calculated. A 6 x 6-cm grid will be generated in the neuronavigation software for each muscle, centred to each participant's hotspot. 110 stimuli will be delivered at 2-sec intervals to pseudorandom locations over the grid at 120% of aMT for the masseter muscle and 120% of rMT for the ECRB muscle.

2.3.7. Intramuscular injection of nerve growth factor

After cleaning the skin with alcohol, a sterile solution of recombinant human NGF (dose of 5 μg [0.2 mL]) will be given as a bolus injection into the right masseter on days 0 and 2 using a 1-mL syringe with a disposable needle (27 G). Following the procedure of Costa et al.,[4] the needle will be inserted perpendicular to the masseter body until bony contact. The needle will then be retracted ~2 mm, aspiration performed, and NGF injected. Any individual who does not develop sensitivity to the NGF model, assessed by diary pain ratings and PPTs of the injected muscle, will be considered a nonresponder and excluded from analyses. Because NGF acts by sensitising mechanosensitive afferents, no change in PPTs after injection would argue in favour of model failure.

2.4. Outcome measures

2.4.1. Primary outcome

The primary outcome is pain sensitivity: participants are dichotomized as high- or low-pain sensitive based on the peak pain

severity from diary recordings.[12,35] That is, based on pain severity in the training set (n = 100), participants will be classified as the top 40% high- or bottom 40% low-pain sensitive. This classification can be further weighted (eg, very high, very low) as described in Aim 1.3.

2.4.2. Secondary outcomes

The secondary outcomes are pain severity (peak average daily pain severity based on diaries on a 0–10 scale) and pain duration, defined as the time between pain onset and complete resolution of pain (0 on a 0–10 scale for 2 consecutive days).

2.4.3. Biomarker candidates

Biomarker candidates are PAF at Day 0 and CME at Day 5. Because this is a discovery project, we will also examine PAF and CME at every day it is tested (see Aim 1.3 below).

2.5. Data processing

2.5.1. Peak alpha frequency and other electroencephalography metrics

All data processing will be performed using custom MATLAB scripts implementing EEGLAB[7] and FieldTrip toolboxes.[27] Data will be referenced to the average across all recording channels and segmented into 5-second epochs. These epochs are manually inspected and all epochs containing marked muscular artifacts are rejected. Channels with poor recordings will be rejected. Principal component analysis is then applied to identify and remove components relating to eye blinks, saccades, and ECG artifacts. Power spectral density will be derived in 0.20-Hz bins and the 2 to 40 Hz range will be extracted. Power spectral density will be extracted in sensor space around sensorimotor cortices (C3, Cz, C4, and neighboring electrodes), as well as sensorimotor ICA components demonstrating clear alpha peaks.[26,48,49] A Hanning taper will be applied to the data before calculating the spectra to reduce any edge artifacts similar to the approach taken in studies conducted by Mazaheri et al.[24–26] Peak alpha frequency is calculated using the center of gravity method, as we have done previously.[12]

2.5.2. Corticomotor excitability

All data processing is performed using a custom MATLAB script. Triangular linear interpolation is used to create a full surface map within a transformed 2D plane containing the stimulation coordinates and their corresponding peak-to-peak MEP amplitudes.[5,47] The resultant map is divided into 2500 partitions (50 × 50), with each partition assigned an approximated value based on the nearest acquired MEP data. Map area is determined as the ratio of the number of approximated partitions where the MEP exceeds 10% of the maximum MEP across all partitions. This cutoff reduces data variability. Map volume is then calculated as the sum of all MEPs (subtracted by the 10% level). This approach is described in full detail (including relevant equations) here.[47]

2.6. Statistical analyses

2.6.1. Aim 1.1: predicting pain sensitivity and optimizing the model

We will validate the PAF/CME biomarker signature and test the predictive accuracy using a nested control-test scheme. The sample of 150 subjects will first be randomly divided into an outer-training set (n = 100) and an outer-testing set (n = 50). The ratios of high- vs low-pain sensitive individuals will be matched between the 2 cohorts where "high pain sensitive" subjects are defined as the 40% of all subjects with the highest pain sensitivity, and "low pain sensitive" subjects are the 40% of all subjects with the lowest pain sensitivity. Next, the outer-training cohort will be split into 5-folds (20 subjects for each fold) for cross validation. Each fold of 20 subjects will be tested as an inner testing cohort based on the remaining 4 folds as the inner training cohorts. The research team at UMB will be blinded to the outcomes of pain sensitivity, severity, and duration in the outer-testing cohort. We expect the 5-fold cross validation will provide sound performance assessment with balanced variance-bias trade-off (see details in Ref. 15). We will consider multiple classifiers including logistic regression, support vector machine, gradient boosting, random forest, and neural networks. These predictive models along with the tuning parameters will be compared based on the performance of the 5-fold cross validation. The biomarkers may predict outcomes in a nonlinear fashion, and thus most machine learning models (eg, support vector machine and gradient boosting + random forest) will detect nonlinear functions. The predictive model with the highest performance (ie, the final model) based on the ability to classify the 40% most pain sensitive and the 40% least pain sensitive participants will be referred to as the "winning classifier." The parameters of the winning classifier will be fixed and used to predict the outcomes of the outer-testing set. After finalizing the predicted outcomes, the outcomes will be unblinded to the UMB team. We will compare the predicted outcomes with the true outcomes and assess the accuracy, sensitivity, specificity, as well as positive and negative predictive values. The predictive accuracy based on binary outcome prediction is used because it is more robust than mean squared error of a predictive model for continuous variables and is more commonly used in the field. Our target is to achieve an area under the curve of the receiver operating characteristic greater than or equal to 75% when applying the fixed classifier to the testing data set.

2.6.2. Aim 1.2: reportable ranges

The sensitivity, specificity, and accuracy of the PAF/CME biomarker will be based on the blinded prediction of the outer-training 50 samples. Reference intervals will be reported for the whole sample, including intervals for fast vs slow PAF and high vs low CME. These will be reported as tables, standardized by age, sex, and other factors. We will further report on the stability of these measures over time (Days 0, 2, and 5).

2.6.3. Aim 1.3: optimization

We will explore how the inclusion of other combinations of factors in the model affects performance characteristics. The auxiliary factors considered in the model will include questionnaire and diary data, PPTs, and other EEG data (theta, alpha, beta, low gamma power) using a model/variable selection procedure to further boost the performance of the model. The nested training-testing scheme will be used to determine the optimal pain sensitivity prediction model using the biomarkers.

2.6.3.1. Weighted accuracy

Because the low- and high-pain sensitive categories are determined based on a continuous pain scale, subjects with pain intensities near the median should be weighted less. Therefore, in

addition to the simple accuracy, the weighted accuracy will be calculated. The weight will be determined by the distance of pain levels to the high–low cut-off.

2.7. Automation and simplification of methods

In order for the biomarker signature to have application to large populations and settings, users must be able to rapidly collect and analyze data with minimal training. We will develop methods that automatically produce biomarker readouts with minimal human input, thus reducing bias associated with data input. Our goal will be to develop a method for automated signature calculation that achieves an intraclass coefficient of at least 80% compared to output from non-automated data processing and no significant difference betweenautomated and nonautomated based on bootstrap inference.

3. Discussion

Biomarkers with clinically meaningful predictive power are yet to be uncovered in musculoskeletal pain disorders. A predictive biomarker would have many important applications including the ability to detect those who are likely to transition to chronic pain before or soon after the onset of pain, facilitate early intervention of high-risk individuals, allow stratification of individuals in clinical trials, and promote the discovery and development of new therapeutics and preventatives. The PREDICT study will undertake analytical validation of a novel PAF and CME biomarker signature using a human model of the transition to sustained myofascial temporomandibular pain. The study will determine the sensitivity, specificity, and accuracy of this biomarker signature at predicting pain sensitivity and establish the reportable range of test results for biomarker detection. If successful, the study would deliver a candidate biomarker signature ready for advanced clinical validation in future studies.

The study design has a number of strengths. First, the use of the NGF pain model provides a highly standardized and clinically relevant model in which to undertake analytical validation of the PAF and CME biomarker signature. Second, the multisite nature of the study ensures blinded analyses of all data. Third, detection methods for the PAF and CME biomarker signature are entirely feasible for use in Phase II and III clinical trials and could be easily refined for broad implementation in healthcare settings. Finally, the methods and statistical analysis plan are prespecified to ensure reporting transparency.

In summary, preliminary evidence suggests combined resting-state peak alpha frequency and corticomotor excitability may have utility as a biomarker signature to predict pain sensitivity. The PREDICT study will provide valuable information on the performance characteristics of this biomarker signature, that if successful, would support validation in clinical populations.

Disclosures

D.A. Seminowicz and A.J. Furman have a patent pending for "A Simple and Portable Biomarker for Pain Sensitivity". The authors have no other conflicts of interest to declare.

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