

Evaluation of Plasma Calcitonin Gene-Related Peptide as a Biomarker for Painful Temporomandibular Disorder and Migraine

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Objective: To assess associations of plasma calcitonin gene-related peptide (CGRP) with chronic temporomandibular disorder (TMD) myalgia/arthritis or frequent/chronic migraine, alone and in combination, and to evaluate relations between the CGRP concentration and clinical, psychological, and somatosensory characteristics of participants.

Methods: The cross-sectional study selected four groups of adult volunteers: healthy controls (HCs), TMD without migraine, migraine without TMD, and TMD with migraine. Each group comprised 20 participants, providing 94% power to detect statistically significant associations with CGRP concentration for either TMD or migraine. TMD and headache were classified according to the Diagnostic Criteria for TMD and the International Classification for Headache Disorders, 3rd edition, respectively. Plasma CGRP was quantified with a validated high-sensitivity electrochemiluminescent Meso Scale Discovery assay. Questionnaires and clinical examinations were used to evaluate characteristics of TMD, headache, psychological distress, and pressure pain sensitivity. Univariate regression models quantified associations of the CGRP concentration with TMD, migraine, and their interaction. Univariate associations of the CGRP concentration with clinical, psychological, and pressure pain characteristics were also assessed.

Results: Among 80 participants enrolled, neither TMD nor migraine was associated with plasma CGRP concentration ($P = 0.761$ and $P = 0.972$, respectively). The CGRP concentration (mean \pm SD) was similar in all 4 groups: HCs 2.0 ± 0.7 pg/mL, TMD 2.1 ± 0.8 pg/mL, migraine 2.1 ± 0.9 pg/mL, and TMD with migraine 2.2 ± 0.7 pg/mL. CGRP concentration was positively associated with age ($P = 0.034$) and marginally with body mass index ($P = 0.080$) but was unrelated to other participant characteristics.

Conclusion: In this well-powered study, interictal plasma concentration of CGRP was a poor biomarker for TMD and migraine.

Keywords: orofacial pain, musculoskeletal pain, neuropeptide, temporomandibular joint disorders, headache

Introduction

Painful temporomandibular disorder (TMD) often co-exists with migraine.^{1,2} For example, TMD prevalence was 5 times higher in people with severe headache and migraine than in people without the headache (15.6% and 2.6%, respectively).³ Likewise, migraine is highly prevalent in TMD patients: in our recent study, migraine occurred in 52% of patients with chronic myogenous TMD.⁴ Currently, both disorders are defined solely by clinical criteria^{5,6} which, in the absence of specific biomarkers, limit the capacity for screening, diagnosis, determining prognosis, and predicting response to therapy.⁷

One of the most promising biomarker candidates for migraine is calcitonin gene-related peptide (CGRP). CGRP consists of 37 amino acids and has 2 isoforms, α and β , that have similar biological activity but differ by 3 amino acids. Peripheral somatosensory nerves and central nervous system (CNS) neurons mostly express α CGRP (denoted as CGRP

in this manuscript), while enteric and motor neurons almost exclusively express β CGRP.⁸ In the trigeminal system, CGRP released from peripheral nerve endings can lead to elevated synthesis of nitric oxide and sensitization of peripheral trigeminal nerves; the neuronal activation then drives central sensitization.⁹ A vast body of evidence supports a crucial role of CGRP in migraine pathophysiology.^{9–11} The initial clinical study found an elevated plasma level of CGRP in jugular venous blood during migraine attacks.¹² Administration of sumatriptan aborted the CGRP increase and reduced symptoms of migraine.¹³ Elevated CGRP in peripheral venous blood between attacks was subsequently reported for both episodic¹⁴ and chronic migraine.¹⁵ However, other studies could not reproduce findings of increased CGRP concentrations either in the external jugular vein or peripheral blood of migraineurs.^{16,17} The CGRP role in migraine was further supported by research where intravenous injection of CGRP led to migraine-like headache in individuals with history of migraine.^{18,19} Finally, CGRP receptor antagonists and humanized antibodies to CGRP or its receptor were efficacious in the treatment of migraine.²⁰

In contrast to compelling evidence of a causative role of CGRP in migraine, data on CGRP contribution to pathophysiology of painful TMD are scarce. CGRP-containing nerve endings are present in masseter muscle and in synovial membrane, articular disc, periosteum, and capsule of the temporomandibular joint (TMJ).^{21–23} Elevated CGRP concentrations were reported in the TMJ synovium in the presence of TMJ internal derangements²⁴ and in the TMJ synovial fluid in TMJ osteoarthritis.^{25,26} However, the CGRP level in blood of people with painful TMD has not been investigated.

In this study with a factorial design, we aimed to assess whether plasma concentration of CGRP in peripheral venous blood was associated with a factor of present or absent chronic myogenous/arthrogenous TMD, a factor of present or absent frequent/chronic migraine, or the interaction of these factors. The second aim of the study was to evaluate associations between the CGRP concentration and participants' phenotyping characteristics.

Methods

Study Design and Setting

The cross-sectional stratified sampling design selected 80 participants from 4 groups based on the presence or absence of chronic painful TMD and/or frequent/chronic migraine: (a) a healthy control group (HCs) included participants with neither painful TMD nor any migraine; (b) a TMD group included participants with chronic painful TMD, who did not have frequent/chronic migraine meeting the study migraine case definition described below; (c) a migraine (MIG) group included participants with frequent/chronic migraine who did not have chronic painful TMD (infrequent TMD, not meeting the study TMD case definition described below, was allowed); and (d) a TMD+MIG group included participants with both chronic painful TMD and frequent/chronic migraine. The study definitions for TMD and migraine cases were designed to enroll participants with clinically important painful TMD and migraine that likely reflect the population of patients seeking care in clinical settings. Each group consisted of 20 participants. Frequency matching was used during enrollment to create groups with approximately equal proportions of females ($\pm 10\%$ among the groups) and adults younger than 30 years ($\pm 10\%$ among the groups).

The participants were enrolled between June and December 2018 from the nearby community and the Orofacial Pain Clinic at the Adams School of Dentistry, the University of North Carolina at Chapel Hill. The study was approved by the university's Institutional Review Board and complied with the Declaration of Helsinki. The participants attended one study visit lasting approximately 2 hours. Prior to the visit, the participants were prescreened for eligibility either in person or via telephone. Potentially eligible participants received a package of questionnaires to complete before the visit. During the visit, participants provided informed consent and were further evaluated for eligibility. All eligible participants provided a blood specimen for quantification of plasma CGRP.

Participants

Participants were females and males 18 to 64 years of age who agreed to discontinue use of any pain medication (including TMD- and migraine-specific medications) for 3 days prior to the study visit. General exclusion criteria were self-reported and comprised severe hepatic, respiratory, immunologic, cardiovascular or psychiatric diseases, fibromyalgia, trigeminal autonomic cephalalgias, painful cranial neuropathies, odontogenic pain, head trauma or surgery within 6

weeks prior to the study visit, history of treatment for drug or alcohol abuse within 1 year prior to the study visit, use of botulinum toxin or anti-CGRP monoclonal antibody injections within 4 months prior to the study visit, use of opioids or barbiturate medications, use of daily preventive medications for chronic pain conditions, and pregnancy.

The diagnoses of TMD myalgia and arthralgia were based on the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD).⁵ To achieve enrollment of participants with chronic and moderate-to-severe facial pain, additional inclusion criteria were established for the TMD cases: (a) the presence of facial pain for at least 3 months (this criterion constitutes chronic pain); (b) at least 10 days with facial pain in the 30 days prior to the study visit; and (c) facial pain intensity in the past week of ≥ 30 on a numerical rating scale of “0” to “100” (this criterion constitutes moderate-to-severe pain intensity). A specific exclusion criterion for TMD participants was use of injection therapy for facial pain within 2 weeks prior to the study visit.

The headache diagnosis was based on the International Classification for Headache Disorders, 3rd edition (ICHD-3).⁶ In addition, migraine cases needed to have had at least 4 migraine days per month in the last 3 months. Specific exclusion criteria for participants with migraine were migraine onset after 50 years of age and use of interventions or devices, such as nerve blocks or transcranial magnetic stimulation, within 2 months prior to the study visit.

To qualify as HCs, in addition to the overall exclusion criteria, participants must not have experienced painful TMD or migraine within 1 year prior to the study visit and must not have had tension-type headache (TTH) on more than 4 days per month in the last 3 months. The detailed list of inclusion and exclusion criteria for all groups is provided in the [Supplementary Materials](#).

Clinical and Biopsychosocial Characteristics

Collected information comprised demographics, medical history, clinical characteristics of TMD and headache, emotional functioning, experimental pain sensitivity, and concomitant medications. Measurements of height and weight were also collected and used to calculate a body mass index (BMI).

Facial pain characteristics obtained from the DC/TMD clinical examination⁵ included classification of myalgia and/or arthralgia, frequency of facial pain, measurements of pain-free, maximum unassisted, and maximum assisted mouth opening, and familiar pain responses evoked by examination of the masticatory muscles and TMJs. TMD-related disability and interference in functioning were assessed using the Graded Chronic Pain Scale (GCPS),²⁷ and jaw function was evaluated with the Jaw Functional Limitation Scale (JFLS).^{28,29} The GCPS grade is derived from several variables: (1) the characteristic pain intensity computed as the mean, multiplied by 10, of the 3 pain items; (2) the pain interference score, computed as the mean, multiplied by 10, of 3 pain interference items; and (3) pain disability days. Based on these 3 variables, participants were classified into 6 chronic pain grades: 0 = no pain, I = low pain intensity and low pain-related disability, IIa = high pain intensity and low pain-related disability, IIb = high pain intensity and high activity interference, III = moderate pain-related disability, IV = severe pain-related disability. For analyses, the GCPS endpoint was dichotomized into a low-grade category including grades from 0 to IIa and a high-grade category including grades from IIb to IV. The JFLS is a validated questionnaire that measures limitations on 3 subscales: mastication (6 items), vertical jaw mobility (4 items), and verbal and emotional expression (8 items). Each item is rated on an 11-point scale from “no limitation” (0 points) to “severe limitation” (10 points). The subscales are computed as a mean response for all items in the subscale, while the total score is the average of all 18 items.

Type of headache was assessed via a structured headache interview² based on the ICHD-3.⁶ The structured headache interview recorded details of up to 3 different headaches. Information elicited for each headache included headache location, intensity, quality, duration, frequency, and aggravating factors. The final question asked about the average number of days per month with headache of any type during the past 3 months. For analyses, definite and probable diagnoses of primary headaches were combined into one category for each primary headache type (migraine or TTH). Headache-related disability and interference in functioning were assessed using the GCPS²⁷ and the Headache Impact Test-6 (HIT-6).³⁰ The 6-item HIT-6 was developed for use in both clinical practice and research. It was designed to measure the adverse impact of headache on social and role functioning, vitality, psychological wellbeing, and cognitive performance. The questionnaire also measures the severity of headache pain. Responders rate frequency of each headache-related burden using one of 5 responses: “never” (6 points), “rarely” (8 points), “sometimes” (10 points),

“very often” (11 points), and “always” (13 points). These responses are summed to produce the total HIT-6 score ranging from 36 to 78 points.

Psychological characteristics were assessed with the Hospital Anxiety and Depression Scale (HADS),³¹ the Perceived Stress Scale (PSS),³² and the Symptom Checklist 90-Revised (SCL-90R)³³ somatization subscale. The HADS was developed to screen for clinically significant anxiety and depressive symptoms in medically ill patients. Its depression and anxiety components consist of 7 items each. Responses are rated on a 4-point Likert scale and range from 0 to 3, including reversed items. The total score for each HADS component ranges from 0 to 21 points. The PSS contains 14 items and measures the degree to which situations in a responder’s life are appraised as stressful. The items in the PSS ask about feelings and thoughts during the last month. In each item, respondents rate how often they felt a certain way on a scale from “never” (0 points) to “very often” (4 points). The PSS scores are calculated by reversing responses to the 7 positively stated items and then summing across all scale items. The PSS total score ranges from 0 to 56 points. The SCL-90R instrument is used by professionals in mental health, medical, and clinical research settings. The SCL-90R somatization scale consists of 12 items and evaluates the degree of somatic symptoms experienced by responders. The responders are asked how much each symptom bothered them in the last 7 days. The responses are rated on a scale from “not at all” (0 points) to “extremely” (4 points). The total score is the average of all items.

Pressure pain thresholds (PPTs) were measured bilaterally over the temporalis, masseter, and trapezius muscles, the TMJs, and the lateral epicondyles with a pressure algometer (FDX-10, Wagner Instruments, CT), as described previously.³⁴ One pre-trial assessment was performed at each site, followed by additional assessments until 2 measures differing by less than 0.2 kg were obtained, or 5 assessments were administered. In either case, the average of the 2 closest values was recorded as the threshold estimate. Pressure stimuli were delivered at an approximate rate of 1 kg/s. The cutoff pressure for all body sites was 5 kg. The values from the right and left sides were averaged to obtain a single PPT per anatomical site.

Blood Collection and Plasma CGRP Measurement

For migraine participants, peripheral blood was collected interictally, ie, during periods between migraine attacks. In addition, all participants refrained from taking any pain medications for 3 days prior to the study visit. Blood specimens were collected from the antecubital vein into 8.5 mL BD P100 tubes (BD Biosciences, NJ, USA) coated with a K₂EDTA anticoagulant and a proprietary cocktail of protease inhibitors specifically formulated to stabilize plasma proteins. To separate plasma, the BD P100 tubes were centrifuged at 2500g for 20 minutes. Then, plasma was aliquoted into cryovials, snap-frozen in liquid nitrogen, and placed in a -80°C freezer for storage.

Plasma CGRP concentrations were quantified at Eli Lilly and Company laboratory (Indianapolis, IN, USA) using a validated high-sensitivity electrochemiluminescent Meso Scale Discovery assay described previously.³⁵ Calibrators were run in triplicate, and samples were run in duplicate. Three quality controls were prepared in sample buffer and run on each assay plate. CGRP concentrations of 20 pg/mL, 5 pg/mL, and 1 pg/mL were denoted High, Middle, and Low quality controls, respectively. The inter-assay coefficients of variation (%CV) for High, Middle, and Low quality controls across sample plates were 3.11%, 3.74%, and 5.45%, respectively. The average lower limit of detection for the plates was 0.148 pg/mL \pm 0.012 pg/mL. The laboratory staff was blinded to clinical information and group assignment.

Sample Size

For the sample size calculation, we used the results from a previous study by Cernuda-Morollón et al¹⁵ which demonstrated a mean effect estimate of 12.7 pg/mL greater CGRP concentration for participants with episodic migraine (46.4 pg/mL) compared to HCs (33.7 pg/mL) with a standard deviation of 16 pg/mL. For the current study, we used the “glimpower” procedure in SAS v9.4 to determine that a sample size of 80 participants (ie, 20 participants in each of the four groups) yielded 94% power to detect a statistically significant ($P < 0.05$) main effect of migraine compared to controls, independent of any TMD effect. Likewise, there was 94% power to detect an independent effect of TMD if it was of the same magnitude (ie, 12.7 pg/mL increase in CGRP compared to controls). For both effects, the same sample size provided power of 79% to detect statistically significant ($P < 0.05$) main effects as small as 10 pg/mL.

To estimate power for plausible interactions, we assumed the same mean 12.7 pg/mL increase (compared to controls) for each pain condition in the absence of the other pain condition and an increase (compared to controls) of 40.6 pg/mL for subjects with both pain conditions. The latter is equivalent to the mean 75 pg/mL CGRP concentration reported for chronic migraine cases by Cernuda-Morollón et al¹⁵ and is equivalent to synergy of 1.6-times the expected additive effects of migraine and TMD. Based on these assumptions, there was 56% power to detect the multiplicative interaction with $P < 0.05$. Power to detect the interaction increases to 80%, when synergy is 2.0-times the expected effect under additivity.

Statistical Methods

Distributions of CGRP concentrations in each of the four study groups were inspected with the aid of violin plots. Statistical outliers were defined as any concentrations that exceeded the third quartile plus 1.5 times the interquartile range (ie, 3.934 pg/mL) and the six values identified were truncated to 3.934. Descriptive statistics for other covariates were calculated for each study group and reported as percentage distribution of categorical variables or mean values and standard deviations of continuous variables.

To evaluate the first aim, CGRP concentration was used as the dependent variable in a least squares linear model that tested for main effects of two binary indicator variables for each case classification (ie, TMD present or absent and migraine present or absent) and their interaction. Covariates in the model were sex (a binary indicator, male or female), race (a binary indicator, White or other), age (a continuous variable), and a BMI (a continuous variable). Besides CGRP, other 22 participant characteristics, that did not have their values equal to 0 in one of the study groups, were then used as the dependent variables in similar univariate regression models (linear regression for the continuous characteristics and binary logistic regression for the binary characteristics). While a Bonferroni correction of $P \leq 0.002$ (ie, 0.05/23) provides sufficient protection against type I error for traditional judgments about statistical significance, P -values were reported without the Bonferroni correction to provide a more general guide to potentially important univariate associations.

For the second aim, univariate linear regression models in which the dependent variable was CGRP concentration transformed to a z-score estimated associations with age (a continuous variable, transformed to a z-score), sex (a binary indicator variable for females/males), race (a binary indicator variable for Whites/other races), and body mass index (a continuous variable, transformed to a z-score). Other participant characteristics that were continuous variables were transformed to z-scores and used as the independent variables in linear regression models with CGRP concentration, also transformed to a z-score, as the dependent variable. Each model also adjusted for sex, race, age, and a BMI.

Results

Participants

Of 154 participants prescreened, 80 met inclusion/exclusion criteria for one of the study groups and were enrolled in the study. The main reasons for non-enrollment were as follows: not meeting the study criterion for migraine frequency of at least 4 days per month ($n = 21$), using preventive migraine medications ($n = 15$), not being interested in the study ($n = 9$), and not meeting the study inclusion criteria for TMD ($n = 7$). The rest of the prescreened participants were excluded due to comorbid conditions such as cluster headache ($n = 1$), trigeminal neuralgia ($n = 1$), diabetes ($n = 1$), fibromyalgia ($n = 4$), hyperthyroidism ($n = 2$), and multiple sclerosis ($n = 1$), use of preventive pain medications ($n = 2$), use of pain medications within 3 days prior to the study visit ($n = 3$), use of opioids ($n = 2$), surgery within 6 weeks prior to the study visit ($n = 2$), alcohol abuse ($n = 1$), and difficulty to understand study instructions ($n = 1$). Most of the enrolled participants were young females (Table 1). The four study groups matched for age and sex (Table 1).

Although the absence of painful TMD and any migraine was a selection criterion for the HC group, episodic TTH was allowed, and 7 (35%) HCs had episodic TTH with mild headache intensity (Table 1).

In the TMD group, all participants had TMD myalgia and 19 (95%) of them also had TMJ arthralgia. In the last month, they experienced TMD pain almost daily with average intensity of 53.2 on a 0–100 scale and exhibited a restricted pain-free jaw opening (Table 1).

Table I Demographic and Clinical Characteristics in Four Participant Groups, Stratified by TMD and Migraine Status^a

Characteristic	Controls n = 20	TMD n = 20	Migraine n = 20	TMD + Migraine n = 20	TMD Effect P value	Migraine Effect P value	Interaction P value
DEMOGRAPHIC							
Age, years	34.1 (12.4)	32.5 (12.1)	33.9 (11.5)	35.9 (11.7)	0.948	0.556	0.495
Sex, female, n (%)	17 (85%)	16 (80%)	16 (80%)	18 (90%)	0.712	0.712	0.355
Race, white, n (%)	9 (45%)	14 (70%)	13 (65%)	14 (70%)	0.178	0.387	0.387
GENERAL HEALTH							
Body mass index (kg/m ²)	26.6 (6.2)	25.8 (5.0)	27.6 (7.8)	30.7 (8.9)	0.465	0.066	0.214
TMD							
Time since onset, y	0	13.6 (10.9)	8.8 (3.0)	16.3 (9.2)	n/a	n/a	n/a
Frequency in the last 30 days, d	0	26.0 (6.6)	2.2 (4.9)	22.7 (8.2)	n/a	n/a	n/a
Myalgia, n (%)	0 (0%)	20 (100%)	3 (15%)	20 (100%)	n/a	n/a	n/a
Arthralgia, n (%)	0 (0%)	19 (95%)	1 (5%)	19 (95%)	n/a	n/a	n/a
Myalgia and arthralgia, n (%)	0 (0%)	19 (95%)	1 (5%)	19 (95%)	n/a	n/a	n/a
GCPS: TMD pain intensity, 0–100 scale	0	53.2 (15.9)	7.0 (9.1)	50.5 (17.3)	n/a	n/a	n/a
GCPS: TMD pain interference, 0–100 scale	0	14.2 (13.2)	1.8 (5.0)	34.3 (28.1)	n/a	n/a	n/a
GCPS: TMD grade IIb–IV, n (%)	0 (0%)	1 (5%)	0 (0%)	8 (40%)	n/a	n/a	n/a
Pain-free jaw opening, mm	48.1 (7.4)	31.1 (6.8)	43.6 (7.5)	26.9 (12.5)	<0.001	0.032	0.787
Maximum unassisted jaw opening, mm	51.2 (6.0)	44.8 (8.3)	47.9 (7.7)	42.5 (13.5)	0.002	0.089	0.729
Maximum assisted jaw opening, mm	52.3 (6.0)	49.1 (6.9)	50.4 (6.6)	46.0 (11.4)	0.030	0.140	0.871
JFLS total score, 0–10 scale	0.0 (0.1)	2.3 (1.2)	0.6 (1.4)	2.6 (2.0)	<0.001	0.084	0.829
HEADACHE							
Time since migraine onset, y	0	15.3 (11.6)	13.6 (9.2)	19.4 (11.4)	n/a	n/a	n/a
Monthly frequency in the last 3 months, d	0.8 (1.2)	9.1 (9.5)	11.1 (5.4)	17.7 (8.2)	<0.001	<0.001	0.566
Episodic migraine, n (%)	0 (0%)	7 (35%)	15 (75%)	10 (50%)	n/a	n/a	n/a
Chronic migraine, n (%)	0 (0%)	0 (0%)	5 (25%)	10 (50%)	n/a	n/a	n/a
Migraine without aura, n (%)	0 (0%)	4 (20%)	12 (60%)	7 (35%)	n/a	n/a	n/a
Migraine with aura, n (%)	0 (0%)	4 (20%)	3 (15%)	5 (25%)	n/a	n/a	n/a
Episodic TTH, n (%)	7 (35%)	10 (50%)	6 (30%)	6 (30%)	0.513	0.256	0.513
Chronic TTH, n (%)	0 (0%)	5 (25%)	0 (0%)	2 (10%)	n/a	n/a	n/a
Medication overuse headache, n (%)	0 (0%)	0 (0%)	0 (0%)	2 (10%)	n/a	n/a	n/a
GCPS: Headache intensity, 0–100 scale	10.0 (15.4)	35.5 (22.1)	54.8 (13.3)	53.2 (9.5)	0.002	<0.001	<0.001
GCPS: Headache interference, 0–100 scale	3.0 (13.4)	18.2 (18.6)	54.8 (23.3)	45.3 (25.8)	0.468	<0.001	0.022
GCPS: Headache grade IIb–IV, n (%)	0 (0%)	4 (20%)	14 (70%)	13 (65%)	n/a	n/a	n/a
HIT-6 score, 36–78 scale	39.5 (6.6)	52.2 (8.9)	63.5 (6.6)	63.6 (7.7)	<0.001	<0.001	<0.001

Notes: ^aData are mean (SD) unless otherwise indicated. P values ≤ 0.002 are highlighted in a bold font. The statistical analysis was deemed not applicable for a variable when all its values in one of the groups were equal 0.

Abbreviations: GCPS, Graded Chronic Pain Scale; HIT-6, Headache Impact Test-6; JFLS, Jaw Functional Limitation Scale; n/a, not applicable; SD, standard deviation; TMD, temporomandibular disorder.

In the MIG group, 15 (75%) participants met ICHD-3 criteria for episodic migraine (with and without aura) and 5 (25%) met criteria for chronic migraine. They had average headache frequency of 11.1 days per month and average headache intensity of 54.8 on the 0–100 scale (Table 1).

In the TMD+MIG group, values of TMD characteristics resembled values seen in the TMD group. Migraine was chronic in 50% of the participants with average headache frequency of 17.7 days per month and headache intensity like in the MIG group (Table 1).

Among TMD and migraine groups, analyses of data for analgesic medications used during a month prior to the study visit showed that the most frequently reported medications were non-steroidal anti-inflammatory drugs and acetaminophen. In addition, the participants with migraine used triptans and muscle relaxants (Table 2).

Participants' Characteristics Associated with TMD and Migraine

As expected, TMD was statistically significantly associated with characteristics of facial pain, such as jaw openings and limitations in jaw function (P values < 0.002), and some characteristics of headache, such as headache monthly frequency, intensity, and impact ($P < 0.002$) (Table 1). Predictably, migraine was also associated with headache characteristics ($P < 0.001$), and TMD and migraine interacted in their associations with headache intensity and impact ($P < 0.001$) (Table 1).

Migraine was strongly associated with psychological factors but not with somatosensory characteristics. Compared with participants without migraine, migraineurs reported greater anxiety ($P = 0.001$), depression ($P < 0.001$), perceived stress ($P = 0.002$), and somatic symptoms ($P < 0.001$) (Table 2). In contrast, the TMD association with psychological distress was less pronounced, as the statistically significant association was noted with somatic symptoms only ($P < 0.001$) (Table 2). However, TMD was strongly associated with increased sensitivity to pressure pain at all sites assessed ($P < 0.001$).

Plasma CGRP Concentration

Violin plots of individual CGRP concentrations revealed similar distributions among the four study groups (Figure 1). Summary statistics for CGRP concentration (mean \pm SD) were similar in all 4 groups: 2.0 ± 0.7 pg/mL in HCs, 2.1 ± 0.8 pg/mL in participants with TMD, 2.1 ± 0.9 pg/mL in participants with migraine, and 2.2 ± 0.7 pg/mL in participants with both TMD and migraine (Table 2). In regression analysis including all participants, neither TMD nor migraine as main effects were associated with the plasma CGRP concentration ($P = 0.761$ and $P = 0.972$, respectively), and no statistically significant TMD \times migraine interaction was noted ($P = 0.696$) (Table 2).

Association Between Plasma CGRP Concentration and Participants' Characteristics

In analyses including all participants, participant age was the only characteristic statistically significantly associated with plasma CGRP concentration ($P = 0.034$), while the association with BMI showed a trend towards significance ($P = 0.080$) (Table 3). We found no statistically significant associations between the CGRP concentration and participant characteristics related to TMD, migraine, psychological distress, and experimental pain sensitivity (Table 4).

As PPTs were significantly lower in TMD cases, we analyzed associations of CGRP concentrations with PPTs within each study group and found no statistically significant results (Table 5). Also, we did not observe statistically significant PPT \times study group interactions in linear regression models for all study participants, where the CGRP concentration was a dependent variable (Table 5).

Discussion

Summary of the Main Findings

To our knowledge, this is the first study measuring plasma CGRP concentrations in peripheral venous blood of participants with chronic painful TMD. Given frequent co-occurrence of TMD and migraine, we sampled enough participants from four study groups to have ample statistical power to distinguish between potential influences of either condition or their co-occurrence, on circulating CGRP. Yet, neither chronic painful TMD nor migraine was associated

Table 2 Psychological and Experimental Pain Sensitivity Characteristics, Plasma CGRP Concentration, and Medication Use in Four Participant Groups, Stratified by TMD and Migraine Status^a

Characteristic	Controls n = 20	TMD n = 20	Migraine n = 20	TMD + Migraine n = 20	TMD Effect P value	Migraine Effect P value	Interaction P value
PSYCHOLOGICAL							
HADS anxiety, 0–21 scale	4.0 (2.9)	6.9 (4.5)	7.5 (3.4)	9.2 (5.0)	0.019	0.001	0.844
HADS depression, 0–21 scale	1.0 (1.1)	2.0 (2.1)	3.7 (2.2)	4.6 (4.4)	0.167	<0.001	0.927
Perceived stress scale, 0–56 scale	15.8 (7.7)	20.6 (7.5)	22.8 (7.5)	24.2 (9.6)	0.097	0.002	0.611
SCL-90R somatization, 0–4 scale	0.1 (0.2)	0.5 (0.5)	0.6 (0.4)	1.0 (0.5)	<0.001	<0.001	0.923
PRESSURE PAIN THRESHOLDS							
Temporalis PPT, 0–500 kPa	149.7 (102.7)	62.7 (31.5)	98.4 (53.9)	56.1 (36.0)	<0.001	0.044	0.170
Masseter PPT, 0–500 kPa	131.9 (58.4)	66.2 (25.0)	107.4 (45.2)	59.3 (40.8)	<0.001	0.059	0.437
TMJ mean PPT, 0–500 kPa	144.2 (60.0)	63.1 (29.5)	112.9 (54.7)	61.3 (43.4)	<0.001	0.076	0.234
Trapezius PPT, 0–500 kPa	286.3 (146.3)	122.3 (62.8)	213.3 (119.2)	116.4 (83.1)	<0.001	0.089	0.143
Lateral epicondyle PPT, 0–500 kPa	296.3 (160.4)	177.7 (82.8)	255.0 (135.7)	154.8 (97.2)	<0.001	0.124	0.798
MOLECULAR BIOMARKER							
CGRP concentration (pg/mL)	2.0 (0.7)	2.1 (0.8)	2.1 (0.9)	2.2 (0.7)	0.761	0.972	0.696
MEDICATION USE BY INDICATION							
TMD: NSAIDs/APAP, n (%)	0 (0%)	12 (60%)	0 (0%)	5 (25%)	n/a	n/a	n/a
TMD: Muscle relaxants, n (%)	0 (0%)	0 (0%)	0 (0%)	2 (10%)	n/a	n/a	n/a
Migraine: NSAIDs/APAP, n (%)	0 (0%)	4 (20%)	12 (60%)	17 (85%)	n/a	n/a	n/a
Migraine: Triptans, n (%)	0 (0%)	0 (0%)	7 (35%)	3 (35%)	n/a	n/a	n/a
Migraine: Muscle relaxants, n (%)	0 (0%)	0 (0%)	1 (5%)	1 (5%)	n/a	n/a	n/a
TTH: NSAIDs/APAP, n (%)	2 (10%)	11 (55%)	2 (10%)	7 (35%)	0.001	0.508	0.508

Notes: ^aData are mean (SD) unless otherwise indicated. P values ≤ 0.002 are highlighted in a bold font. The statistical analysis was deemed not applicable for a variable when all its values in one of the groups were equal 0.

Abbreviations: APAP, N-acetyl-para-aminophenol (acetaminophen); CGRP, calcitonin gene-related peptide; HADS, Hospital Anxiety and Depression Scale; n/a, not applicable; NSAIDs: non-steroidal anti-inflammatory drugs; PPT, pressure pain threshold; SCL-90R, Symptom Checklist 90-Revised; SD, standard deviation; TMD, temporomandibular disorder; TTH, tension-type headache.

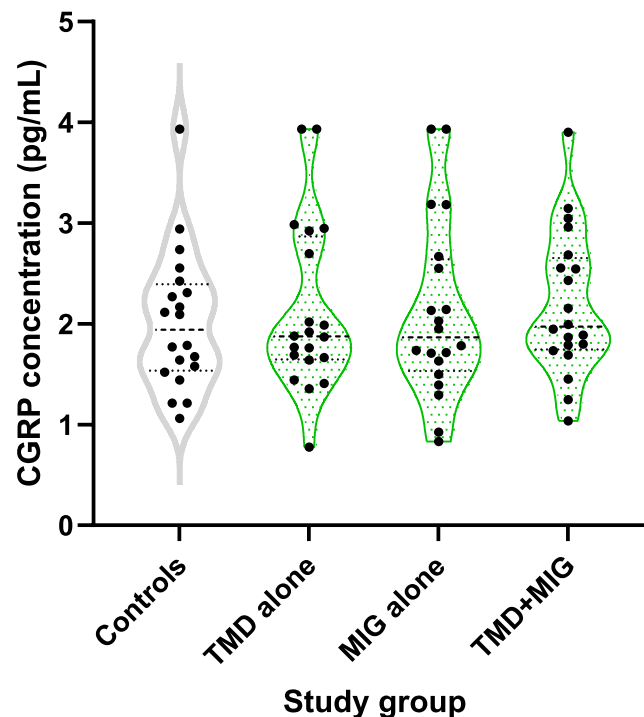


Figure 1 Individual plasma CGRP concentrations, stratified by a study group. Each dot represents an individual CGRP value. The middle line in each plot represents the median; the top and bottom lines represent the 25th and 75th percentiles.

Abbreviations: CGRP, calcitonin gene-related peptide; MIG, migraine; TMD, temporomandibular disorder.

with plasma CGRP concentrations. Moreover, the CGRP concentration was not associated with any of participant clinical, psychological, or pressure pain sensitivity characteristics. A positive association was found only with age, and a trend towards significance was observed for association with obesity.

Comparison of Psychological and Somatosensory Profiles of TMD and Migraine Participants

A vast body of evidence demonstrated associations of painful TMD and migraine with psychological factors, such as depression, anxiety, stress, and somatic awareness.^{36–42} Moreover, assessment of the patient psychological status is recommended by the Axis II protocol in the DC/TMD.⁵ However, not many studies compared psychological profiles of TMD and migraine patients. One recent research, utilizing the same factorial design as our study, found elevated anxiety and somatization in TMD patients and greater anxiety, depression, and somatization in migraineurs.⁴³ Our results are in full agreement with these findings, as we also observed an across-group gradient (HC < TMD < MIG < TMD+MIG) of

Table 3 Unadjusted Associations of Plasma CGRP Concentration with Participant Demographic Characteristics and a Body Mass Index^a

Characteristic	Estimate	P value
Age, years	0.24	0.034
Sex, female	0.22	0.476
Race, white	0.20	0.394
Body mass index, kg/m ²	0.20	0.080

Notes: ^aEstimates are regression coefficients from univariate linear regression models in which the dependent variable was CGRP concentration transformed to a z-score. Separate models estimated associations with age (a continuous variable, transformed to a z-score), sex (a binary indicator variable for females), race (a binary indicator variable for Whites), and a body mass index (a continuous variable, transformed to a z-score).

Table 4 Adjusted Associations of Plasma CGRP Concentration with Participant Characteristics^a

Characteristic	Estimate	P value
TMD		
Frequency in the last 30 days, d	0.08	0.509
GCPS: TMD pain intensity, 0–100 scale	0.06	0.592
GCPS: TMD pain interference, 0–100 scale	−0.04	0.748
GCPS: TMD grade, 0–4 scale	0.06	0.630
Pain-free jaw opening, mm	−0.12	0.296
Maximum unassisted jaw opening, mm	−0.10	0.374
JFLS total score, 0–10 scale	0.17	0.142
HEADACHE		
Monthly frequency in the last 3 months, d	−0.08	0.499
GCPS: Headache intensity, 0–100 scale	0.00	0.973
GCPS: Headache interference, 0–100 scale	−0.07	0.556
GCPS: Headache grade, 0–4 scale	−0.09	0.448
HIT-6 score, 36–78 scale	0.01	0.945
PSYCHOLOGICAL		
HADS anxiety, 0–21 scale	0.10	0.367
HADS depression, 0–21 scale	0.17	0.127
Perceived stress scale, 0–56 scale	0.17	0.141
SCL-90R somatization, 0–4 scale	0.12	0.297
PRESSURE PAIN THRESHOLDS		
Temporalis PPT, 0–500 kPa	0.07	0.544
Masseter PPT, 0–500 kPa	0.09	0.438
TMJ mean PPT, 0–500 kPa	0.03	0.771
Trapezius PPT, 0–500 kPa	0.02	0.850
Lateral epicondyle PPT, 0–500 kPa	0.02	0.867

Notes: ^aEstimates are regression coefficients from multivariable linear regression models in which the dependent variable (CGRP concentration) and predictor variables were transformed to a z-score, yielding standardized regression coefficients. For each regression model, estimates were adjusted by age (a continuous variable), sex (a binary indicator variable for females), race (a binary indicator variable for Whites), and a body mass index (a continuous variable).

Abbreviations: CGRP, calcitonin gene-related peptide; GCPS, Graded Chronic Pain Scale; HADS, Hospital Anxiety and Depression Scale; HIT-6, Headache Impact Test-6; JFLS, Jaw Functional Limitation Scale; PPT, pressure pain threshold; SCL-90R, Symptom Checklist 90-Revised; TMD, temporomandibular disorder.

Table 5 Adjusted Associations of Plasma CGRP Concentration with PPTs, Stratified by a Study Group

Characteristic	Controls n = 20		TMD n = 20		Migraine n = 20		TMD + Migraine n = 20		PPT × Study Group Interaction P value ^b
	Estimate ^a	P value	Estimate ^a	P value	Estimate ^a	P value	Estimate ^a	P value	
Temporalis PPT, 0–500 kPa	0.04	0.792	0.59	0.371	0.35	0.274	−0.04	0.943	0.820
Masseter PPT, 0–500 kPa	0.04	0.837	0.22	0.731	0.55	0.057	−0.07	0.822	0.404
TMJ mean PPT, 0–500 kPa	−0.01	0.974	0.06	0.921	0.33	0.241	−0.07	0.854	0.742
Trapezius PPT, 0–500 kPa	0.04	0.694	0.36	0.208	−0.63	0.248	0.13	0.461	0.640
Lateral epicondyle PPT, 0–500 kPa	−0.09	0.626	0.29	0.672	0.09	0.733	0.35	0.350	0.829

Notes: ^aEstimates are regression coefficients from separate linear regression models, one for each study group, in which the dependent variable (CGRP concentration) and predictor variables (PPTs) were transformed to a z-score, yielding standardized regression coefficients. For each regression model, estimates were adjusted by age (a continuous variable), sex (a binary indicator variable for females), race (a binary indicator variable for Whites), and a body mass index (a continuous variable). ^bPPT × study group interaction P values are from linear regression models for all study participants, using CGRP concentration z-score as the dependent variable with predictor variables PPT, study group, and their interaction. The models also adjusted for age (a continuous variable), sex (a binary indicator variable for females), race (a binary indicator variable for Whites), and a body mass index (a continuous variable).

Abbreviations: CGRP, calcitonin gene-related peptide; PPT, pressure pain threshold.

increasing estimates for all four psychological factors assessed. Additionally, our analysis also showed a more pronounced association of anxiety, depression, stress, and somatization with migraine than with painful TMD.

Elevated somatosensory perception in painful TMD and migraine, compared with healthy controls, was reported by many studies, although with some discrepancies between the reports.^{34,44–48} In both conditions, the differences in pain sensitivity were found not only in orofacial region but also in remote body sites, suggesting a generalized upregulation of nociceptive processing. In TMD, the largest effects were observed for PPTs, and that is why these quantitative sensory tests were chosen for our study.³⁴ As stated in a recent meta-analysis, PPTs were also reliably lower in patients with migraine than in healthy controls.⁴⁸ To our knowledge, none of the studies compared PPTs in participants with TMD and/or migraine. One study evaluated sensitivity to cold, heat, and mechanical cutaneous stimuli among healthy, TMD, and migraine participants stratified in a factorial design.⁴⁹ While cold and heat hyperalgesia was observed in all 3 patient groups, mechanical cutaneous allodynia was demonstrated only in TMD patients, with and without migraine. For all experimental modalities, the most pronounced hyperalgesia/allodynia was found in patients with concomitant TMD and migraine. Our PPT results agree with the above study, as we also observed an across-group gradient in PPTs (HC > MIG > TMD > TMD+MIG) with the statistically significant effect of TMD.

CGRP as a Biomarker for Migraine

A biomarker is

a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.⁵⁰

In clinical practice, a reliable biomarker could be used to identify risk of developing a disease, to screen for a subclinical disease, to diagnose an overt disease, to categorize disease severity, or to predict response to therapy.⁵¹

CGRP has been actively investigated as a putative biomarker for migraine.⁷ It was evaluated in ictal (ie, during the attacks) and interictal (ie, between the attacks) phases of migraine, in peripheral and jugular venous blood, and in episodic and chronic migraine.⁵² However, the results of the studies are inconsistent. The first study found elevated CGRP plasma concentrations in the external jugular vein of migraineurs during the attacks.¹² Another study confirmed the finding in peripheral blood collected from migraine patients ictally but not in the blood collected interictally.⁵³ However, the third study measuring CGRP concentrations in the jugular and peripheral blood both ictally and interictally did not reproduce the previous positive findings.¹⁷ Regarding interictal CGRP concentrations in peripheral blood, several more studies reported higher CGRP concentrations in patients with episodic and chronic migraine compared with healthy volunteers,^{14,15,54,55} but another study found no difference between migraineurs and HCs, between migraine with and without aura, between episodic and chronic migraine, and between ictal and interictal phases.¹⁶ Our study results also did not show interictally elevated CGRP concentrations in participants with frequent or chronic migraine. Further, we did not find any associations between the CGRP concentration and headache monthly frequency, intensity, chronic pain grade, and impact on quality of life.

The discrepancies between studies can be explained by differences in sample sizes, designs, participant selection criteria, blood processing protocols, and assays used for the CGRP measuring. For example, the blood CGRP has a short half-life of less than 10 minutes,⁵⁶ and that is why the use of protease inhibitors is important to prevent the peptide degradation. However, some studies used the protease inhibitors and others did not. Additionally, commercially available CGRP assays are variable and not well validated. Most assays might detect not only an entire peptide but also its fragments and relative peptides, such as β CGRP and amylin.⁷ Standardization of blood processing protocols and thorough validation of assays are needed to achieve better comparisons between studies.

CGRP as a Biomarker for TMD

The average CGRP concentration in our study was not elevated in participants with chronic painful TMD, and it was not associated with TMD characteristics. Despite our negative results, we consider CGRP's contribution to painful TMD plausible. This hypothesis is supported by findings of CGRP-containing nerve endings in the masseter muscle and TMJ^{21–23} and elevated CGRP concentrations in the TMJ with disc displacement or degenerative joint disorders.^{24–26}

Additional evidence for the CGRP role in painful TMD comes from animal studies. Supporting CGRP contribution to myogenous TMD, increased CGRP concentrations were found in the rat masseter muscle following injection of complete Freund's adjuvant (CFA).⁵⁷ In another study, pretreatment with a CGRP receptor antagonist before the CFA injection in the mouse masseter muscle reduced animal orofacial pain behavior and neuronal activation in the spinal trigeminal nucleus.⁵⁸ Establishing CGRP involvement in arthrogenous TMD, an increased number of TMJ-innervating CGRP-immunoreactive neurons was detected in the rat trigeminal ganglion in a carrageenan-induced TMJ arthritis model.⁵⁹ The elevated CGRP level in the trigeminal ganglion and spinal trigeminal nucleus was also confirmed in another model of TMJ arthritis in rats.⁶⁰ Additionally, injecting CGRP in the rat TMJ stimulated neuronal and glial expression of proteins involved in the development of peripheral and central sensitization.⁶¹ Considering all the evidence, we recognize the importance of CGRP's contribution to pathophysiology of painful TMD but regard the plasma concentration of CGRP in peripheral blood to be a poor biomarker for the disorder. Potentially, local CGRP concentrations in the masticatory muscles, TMJ, trigeminal ganglion, spinal trigeminal nucleus, and other regions of CNS implicated in central sensitization would be better correlates of painful TMD than the plasma CGRP concentration, although CGRP concentrations in those regions were not studied here.

CGRP and Ageing

In animal research, circulating concentrations of CGRP have been found to both increase and decrease with ageing.⁶² One possible explanation why plasma concentration of CGRP elevates as age advances could be increased production of CGRP by thyroid.^{63,64} On the other hand, decreased circulating CGRP in aged females could be a consequence of decreased production of sex steroid hormones.⁶⁵ Further, aging enhances negative effect of hypertension on CGRP expression in neurons and its release from nerves.⁶⁶ In two earlier studies that assessed a relation between circulating CGRP and age in people with and without migraine, no association was noted.^{15,16} The discrepancy with positive association found in our study could be explained by differences in participant selection criteria, eg, uncontrolled hypertension was exclusionary in our study but not in two other studies.

CGRP and Obesity

Although we found only borderline positive association of the CGRP concentration with a BMI, our finding is in line with the existing evidence on the role of CGRP in obesity. Mice lacking CGRP were protected against obesity induced by high-fat diet.⁶⁷ Plasma CGRP concentration was elevated in obese Zucker rats prior to the onset of obesity⁶⁸ and in obese women.⁶⁹ Relevant to our study population, obesity constitutes a risk factor for migraine⁷⁰ and was associated with frequency, severity, and clinical features of migraine attacks.⁷¹ Research on the role of obesity in painful TMD is scarce. One study found that TMD pain was associated with total body fat percentage in a univariate analysis, but the association was lost when corrected for co-variables in a multivariable model.⁷² Collectively, current evidence points towards CGRP as an important link between obesity and migraine.⁷³

Strengths and Limitations

Important strengths of this study were the use of the validated DC/TMD⁵ for the classification of TMD myalgia and arthralgia and the use of the structured, ICHD-3-based interview for classification of headache.² The use of the validated Meso Scale Discovery assay and blinding of the laboratory staff to participants' clinical data also strengthened the validity of study findings. Another strength was that the sample size was calculated a priori, providing sufficient statistical power to detect group differences that were plausible based on a previous study. Hence, the observed lack of statistically significant differences in CGRP concentrations between the study groups cannot be attributed to a type II error. The limitation of the study is reliance on participants' retrospective self-report for assessment of TMD and headache characteristics. The generalizability of our data was supported by enrollment of all consecutive eligible volunteers of any sex, race, and ethnicity. However, the selection criteria were designed for enrollment of participants with advanced symptoms typical for clinical settings and not fully representative of the conditions themselves.

Conclusion

In this well-powered study, the interictal concentration of CGRP in peripheral blood was a poor biomarker of these disorders. Further, the CGRP concentration was not associated with any of the clinical characteristics of painful TMD or migraine, characteristics of psychological distress, or measures of pressure pain sensitivity. Interestingly, it was positively associated with participant age and, borderline, with obesity.

Abbreviations

TMD, temporomandibular disorder; CGRP, calcitonin gene-related peptide; CNS, central nervous system; TMJ, temporomandibular joint; HCs, healthy controls; MIG, migraine group; DC/TMD, the Diagnostic Criteria for TMD; ICHD-3, the International Classification of Headache Disorders, 3rd edition; TTH, tension-type headache; BMI, body mass index; GCPS, Graded Chronic Pain Scale; JFLS, Jaw Functional Limitation Scale; HIT-6, Headache Impact Test-6; HADS, Hospital Anxiety and Depression Scale; PSS, Perceived Stress Scale; SCL-90R, Symptom Checklist 90-Revised; PPT, pressure pain threshold; SD, standard deviation; UNC-CH, University of North Carolina at Chapel Hill.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

KWJ and LRV are employees of Eli Lilly and Company and may or may not hold stock in the company. XC is an employee of AbbVie. The authors report no other conflicts of interest in this work.

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