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Calcitonin gene-related peptide in newly diagnosed idiopathic intracranial hypertension: a prospective, cross-sectional, case-control study of cerebrospinal fluid and plasma

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Abstract

Background Calcitonin Gene-Related Peptide (CGRP) is involved in migraine pain signaling, and blockage hereof is effective in migraine treatment. Headache in idiopathic intracranial hypertension (IIH) is often migraine-like but the underlying mechanisms are not understood. We report levels of CGRP in plasma and cerebrospinal fluid (CSF) of patients with newly diagnosed IIH to elucidate CGRP involvement in the pathogenesis of headache in IIH.

Method We consecutively enrolled patients suspected of having IIH in a prospective cohort at two Danish tertiary headache centers. Patients are confirmed to have IIH or disproven of it (non-IIH). We included non-IIH with primary headache disorders as headache controls to IIH cases. We also recruited sex-, age- and BMI-matched healthy controls (HC). All participants had CSF and blood drawn and CGRP was analyzed using a validated radioimmunoassay. CSF plasma-ratios were calculated. Between-group levels were compared with ANOVA or Kruskal-Walli's test. In sub-analyses we restricted comparison of HC to non-IIH/IIH with chronic migraine; we also compared IIH with versus without headache. We correlated CGRP to lumbar opening pressure (OP), and BMI, and assessed the correlation between CGRP in plasma and CSF. Generalized or linear regression was applied to adjust for confounding by BMI, age, and active smoking.

Results Comparing 97 patients with IIH, 52 non-IIH, and 37 HC, we found no between-group differences in CGRP levels in plasma ($p=0.78$), CSF ($p=0.79$), or in CSF:plasma-ratio ($p=0.13$). Adjusting for BMI, age, and smoking yielded similar results. CGRP levels were neither associated with having a migraine phenotype or chronic headache, nor with having any headache versus no headache in IIH. CGRP in plasma correlated with CGRP in CSF ($p<0.0001$). CGRP did not correlate with OP or BMI.

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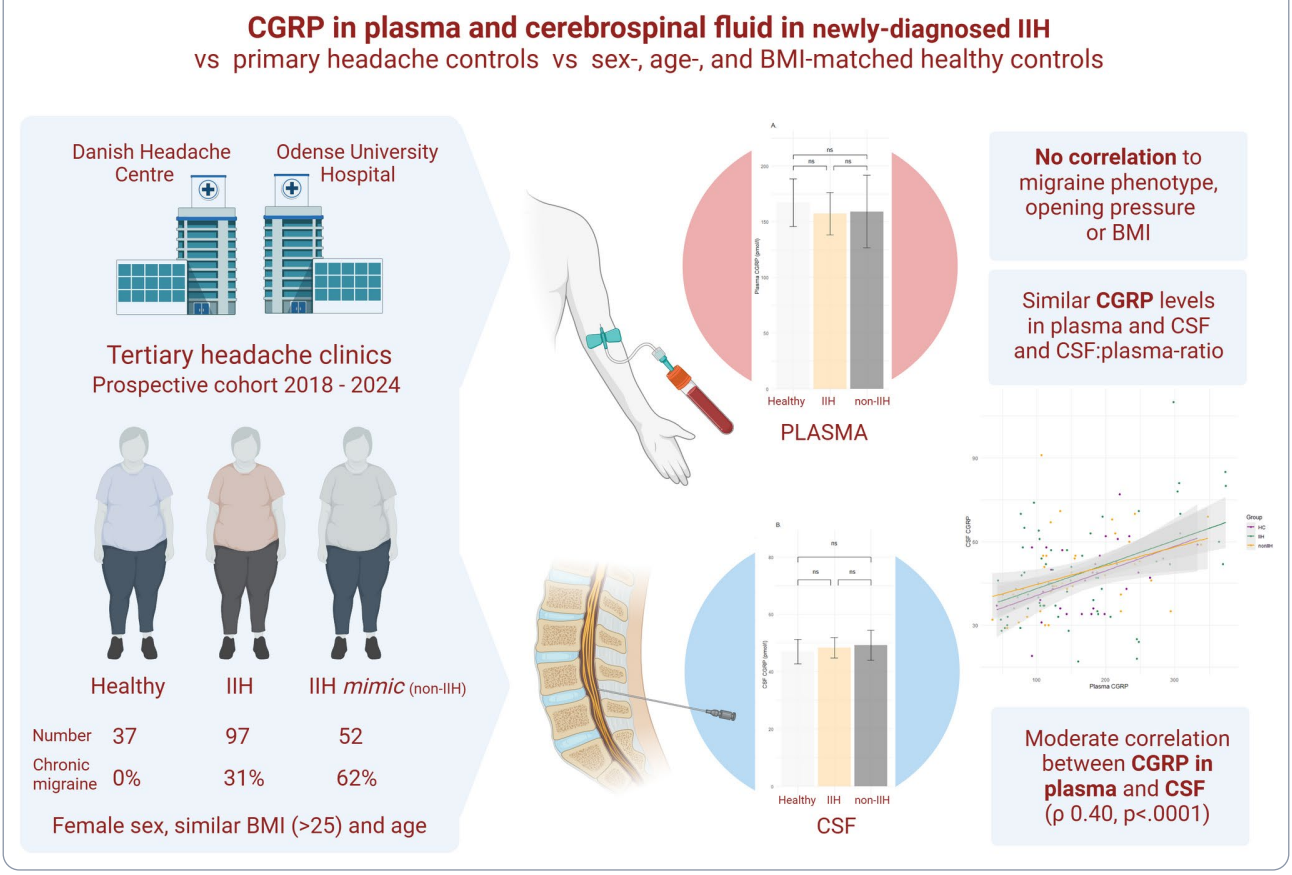
Conclusion CGRP levels in plasma and CSF and their ratios were comparable in IIH, non-IIH patients with headache, and sex-, age-, and BMI-matched HC. CGRP in plasma correlated with CGRP in CSF. Due to methodology, we probably measured basal resting CGRP. The role of CGRP in IIH-headache needs further clarification. A headache preventive effect in IIH of anti-CGRP targeted therapy remains a relevant unexplored area.

Keywords CGRP, IIH, Intracranial pressure, Secondary headache, Cerebrospinal fluid, Migraine

Graphical Abstract



Introduction

Headache burden is substantial in people with idiopathic intracranial hypertension (IIH), and drives impaired quality of life [1, 2]. First-line treatment of active IIH, acetazolamide, decreases intracranial pressure (ICP) and thereby papilledema, but it does not necessarily alleviate accompanying headaches [3] affecting the majority of patients [4, 5]. Furthermore, headaches frequently persist as post-IIH headache after ocular remission is achieved [5–7]. Mechanisms driving IIH headache are unknown and proper evidence on its management is nonexistent. Hence, evidence-based solutions for abortive and preventive headache treatment in IIH in both the active and remitted stages remain an urgent unmet need. Headache in IIH often has a migraine-like phenotype [4, 5]– not only in the 25–50% [4, 5, 7] with pre-existing migraine, but also in new-onset IIH headaches [7, 8], and in previously migraine-naïve patients with IIH [7]. This frequent resemblance of migraine has raised speculations of whether IIH and migraine share common pain pathways [9]. If so, IIH headache is expected to benefit from migraine-directed treatment. Indeed, expert consensus is to treat IIH headaches according to the phenotypic presentation [10, 11]. The neuropeptide Calcitonin Gene-Related Peptide (CGRP) is involved in migraine pain and monoclonal antibodies (mAbs) targeting the CGRP peptide or its receptor are efficacious preventive treatments of episodic and chronic migraine [12, 13]. CGRP mAbs were also reported efficacious as treatment of chronic post-IIH headaches refractive to other preventive therapies in two case series [14, 15] and a small open-label study [16]. Hitherto, no randomized controlled studies on CGRP mAbs in IIH exist.

CGRP is considered a marker of trigeminal activation [17, 18] which drives migraine headache. CGRP infusion provokes migraine headache in patients with migraine

[19–21]. Plasma levels of CGRP are reported elevated in patients with migraine [22, 23]– during spontaneous attacks [18, 24, 25] and during induced attacks in provocation models [26], however, conflicted by opposing findings [27–29]. Interictal levels in patients with episodic migraine (EM) are reported comparable to controls [24, 26, 29] or increased [25]. In chronic migraine, interictal levels were elevated [30] or comparable [29] to episodic migraine and headache-free controls. CGRP levels are also reported increased and associated with pain in non-headache pain pathologies and in several different tissues [31], hence, CGRP may not be specific for migraine. Two studies have reported peripheral concentrations of CGRP in IIH [32, 33]: In a Turkish study [32], serum CGRP was significantly higher in IIH compared to healthy controls (HC) and significantly lower than in patients with chronic migraine (CM). In an Austrian study [33], headache, particularly migraine-like, was consistently associated with higher plasma concentrations of CGRP in IIH [33]. No studies have reported CGRP levels in the cerebrospinal fluid (CSF) in IIH which is of particular interest given the disturbed homeostasis of CSF pressure in IIH and because of the proximity of CSF to sites of CGRP release in migraine: the trigeminal ganglion (TG) and meningeal trigeminal afferents.

If CGRP drives IIH headache this may inform future headache preventive strategies in IIH. We evaluated CGRP levels in plasma and CSF of patients with newly diagnosed IIH compared to two control groups: (i) *IIH mimics* with headache disorders, and (ii) sex-, age-, and BMI-matched HC. Our study complements the scarce evidence on CGRP involvement in IIH headaches and provides the first insights into CGRP in CSF in IIH.

Methods

Setting and study participants

At two tertiary headache centers in Denmark (Danish Headache Center, Rigshospitalet, and Department of Neurology, Odense University Hospital) we prospectively enrolled patients suspected of having IIH based on symptoms, clinical phenotype, objective findings, neuroimaging, and/or ophthalmic assessment. Specialists in neurology and neuro-ophthalmology diagnose IIH in consensus according to the 2013 diagnostic criteria [34] for IIH including definite IIH, probable IIH, definite or suggested IIH without papilledema (*IIH* hereafter). Patients diagnosed with IIH were naïve to ICP-lowering treatment at inclusion. Patients in whom IIH was initially suspected but eventually ruled out are called *'non-IIH'*.

Participants underwent standardized diagnostic investigation including a medical interview, clinical examination, magnetic resonance neuroimaging including cerebral venography, lumbar puncture in an outstretched lateral decubitus position with manometric opening pressure (OP) measurement, and withdrawal of CSF and routine blood analyses. Experienced neuro-ophthalmologists assessed patients with slit lamp examination, fundus photography, optic coherence tomography, and automated visual field examination. Papilledema severity was graded according to the modified Frisén scale [35].

For this study, patients enrolled in the period January 2018– May 2024 were included if plasma and/or CSF was obtained for research purposes. We excluded patients with pregnancy, secondary pseudotumor cerebri syndrome, IIH relapse, male sex, and treatment with CGRP mAb within five half-times since last injection. Non-IIH patients were excluded if they had no history of headache.

Age-, sex- and BMI-matched HC were recruited through social media in the period July 2020-May 2021. They had ≤ 1 weekly headache day, no symptoms associated with raised ICP, and optic disc edema was ruled out with fundus photography.

Headache and medication

Headache was phenotyped according to the ICHD-3 as migraine (with or without aura) or tension-type headache (TTH); in case of co-existing migraine and TTH, the patient was categorized as having migraine. Headache fulfilling criteria for both *IIH headache* and migraine were categorized as migraine, hence, in the IIH population we did not differ between 'true migraine' and 'migraine-mimicking' headaches. A category '*unclassifiable*' was used if the headache fulfilled neither migraine nor TTH criteria or if missing information disabled a headache diagnosis. We did not have information on headache existence and type at the very time of sampling.

Biological samples

Whole-blood and CSF was drawn during routine diagnostic investigation and was centrifuged as soon as possible within a maximum of one hour. Plasma was obtained after centrifugation at 2,000G x 10 min x 20 °C; CSF was centrifuged at 400G x 10 min x 20 °C. Both were frozen immediately at -80 °C until analysis.

Assay and validation

To validate the use of our assay for CSF we did a pilot test of 10 paired plasma- and CSF samples. CGRP was detectable and consistently lower in CSF (range 20–85 pmol/L) than in plasma (range 207–373 pmol/L) and levels correlated ($p < 0.001$, R $[2]_{adj}$ 0.57).

Staff blinded to diagnoses determined CGRP concentrations in plasma ($CGRP_{PLASMA}$) and CSF ($CGRP_{CSF}$) with a validated radioimmunoassay for human CGRP [36]. We used the in-house antibody AB4-2905-85 [36] and α-CGRP as calibrator. The tracer was prepared by

iodination of [Tyr0] α-CGRP (25–37) amide and purified by high-performance liquid chromatography. In brief, the samples and calibrators were incubated with antiserum at 4° for 90 h. The tracer was added followed by incubation for 48 h. Free and antibody-bound tracer were separated by Sac-Cel separation. Lower and upper limits of CGRP detection were 15 pmol/L and 400 pmol/L, respectively. Samples with values > 400 pmol/L were diluted if possible, and values were calculated. Coefficient of variation in percent (CV%) was assessed employing dedicated quality control samples at three different levels covering the measuring range. The inter-assay CV% was 29, 13, and 15, at 40, 200, and 378 pmol/L, respectively. The intra-assay CV% was 17, 3, and 3 at the same levels as for inter-CV%.

Statistical analysis

R 4.4.1 was used for statistics. Differences in categorical variables were assessed by X2 or Fisher's test. Data distribution of numeric variables was assessed by visual inspection of histograms and Quantile-Quantile plots. We compared normally distributed data using t-test or ANOVA; Welch's ANOVA in case of unequal variance as assessed with Bartlett's test. Non-normally distributed data was compared using Mann-Whitney U test or Kruskal-Walli's test; variance homogeneity was tested with Levene's test.

Proportions of CGRP values < 15 (lower limit of detection) were equally distributed among groups ($p = 0.28$) and handled as missing values ($n = 21$). Sensitivity analyses where these values were set at either 7.5 or 15 did not change the results. Exclusion of these values led to the entire exclusion of six patients who had values < 15 pmol/L in both plasma and CSF. One patient had $CGRP_{PLASMA}$ >400 pmol/L (upper limit of detection) and not enough material for dilution; this value was handled as missing. One non-IIH patient had $CGRP_{PLASMA}$ of 7701 pmol/L verified by several dilutions 1:10 and 1:20; this patient had received anti-CGRP receptor mAb (Erenumab) within five half times at time of sampling and was omitted from analyses. In general, missing values were excluded from analyses in which the variable appeared.

The primary end point was between-group difference in CGRP concentrations in plasma and CSF. Generalized linear regression models allowed subsequent adjustment for BMI, age, and smoking. We did some exploratory analyses of CGRP levels comparing (1) IIH with headache vs. non-IIH with chronic migraine vs. HC, (2) IIH with vs. without headache, and (3) IIH with chronic migraine-like headache versus HC. We correlated CGRP to LPOP and BMI, overall and within groups, using Spearman's or Pearson's correlation analysis as appropriate, and correlated levels in plasma and CSF using linear regression adjusting for BMI, age, smoking and participant group.

There was a mean 11.2% (95% CI 6.2–16.1) missing plasma and CSF sample results wherefore we did sensitivity analyses employing predictive mean matching multiple imputation ($m = 20$) assuming that results were missing at random (MAR).

Results

Of 368 patients enrolled in the cohort in the period 2018–2024 we included 97 patients with IIH, 52 non-IIH, and 37 HC for CGRP analyses. We excluded 210 patients for reasons given in Fig. 1; the most frequent reason was missing biological material ($n = 113$). Participant characteristics are given in Table 1. All were of female sex and had similar age and BMI; all had BMI > 25. More patients with IIH were actively smoking (40% vs. 21% in both non-IIH and HC, $p = 0.01$). Lumbar OP was higher in IIH than in non-IIH and HC ($p < 0.0001$). All non-IIH had headache, whereas 92% in IIH and 92% of HC had headache, $p = 0.04$. Headache frequency substantially differed: HC had median 1 (IQR 0.50–2.86, range 0–4) monthly headache days and none had chronic headache. Headache was chronic (≥ 15 days monthly) in 57% of IIH and 73% of non-IIH ($p = 0.07$). A migraine phenotype was observed in 77% non-IIH ($n = 40$) compared to 56% in IIH ($n = 54$) and 16% of HC ($n = 6$), $p < 0.0001$. Migraine prevention was prescribed in few cases: one IIH (candesartan) and three non-IIH (candesartan, $n = 2$, metoprolol, $n = 1$); one non-IIH was excluded due to use of anti-CGRP mAb (Erenumab).

CGRP levels in plasma and CSF

We observed no between-group differences in $CGRP_{PLASMA}$ or $CGRP_{CSF}$ (Fig. 2; Table 2). Mean (± SD; given in pmol/L) $CGRP_{PLASMA}$ in IIH was 158 (± 86), in non-IIH 154(± 77), and in HC 167 (± 66), $p = 0.744$. Mean (± SD) $CGRP_{CSF}$ was in IIH 49 (± 17), in non-IIH 49 (± 13), and in HC 47 (± 12), $p = 0.826$. Adjusting for BMI, age, and smoking did not change the results: Compared to HC as reference, $CGRP_{PLASMA}$ in IIH was 10% lower (0.90 [95% CI 0.73; 1.10], $p = 0.32$) and $CGRP_{CSF}$ was 3% higher (1.03 [95% CI 0.90;1.18], $p = 0.64$); in non-IIH, $CGRP_{PLASMA}$ was 8% lower (0.92 [95% CI 0.74; 1.15], $p = 0.48$) and $CGRP_{CSF}$ was 4% higher (1.04 [95% CI 0.90–1.21], $p = 0.58$), all estimates statistically insignificant Sensitivity analyses of the imputed data set gave similar between-group significance estimates of $CGRP_{PLASMA}$ and $CGRP_{CSF}$ (data not shown).

$CGRP_{PLASMA}$ was significantly higher than $CGRP_{CSF}$ ($p < 0.0001$, Table 2) but they correlated moderately (Spearman's ρ 0.40, $p < 0.0001$, Fig. 3). Adjusting for BMI, age, smoking, and participant group, the positive correlation remained significant with an increase of 0.08 pmol/L $CGRP_{CSF}$ by 1 pmol/L increase in $CGRP_{PLASMA,}$ which explained 17% of the variance of $CGRP_{CSF}$ (β 0.08,

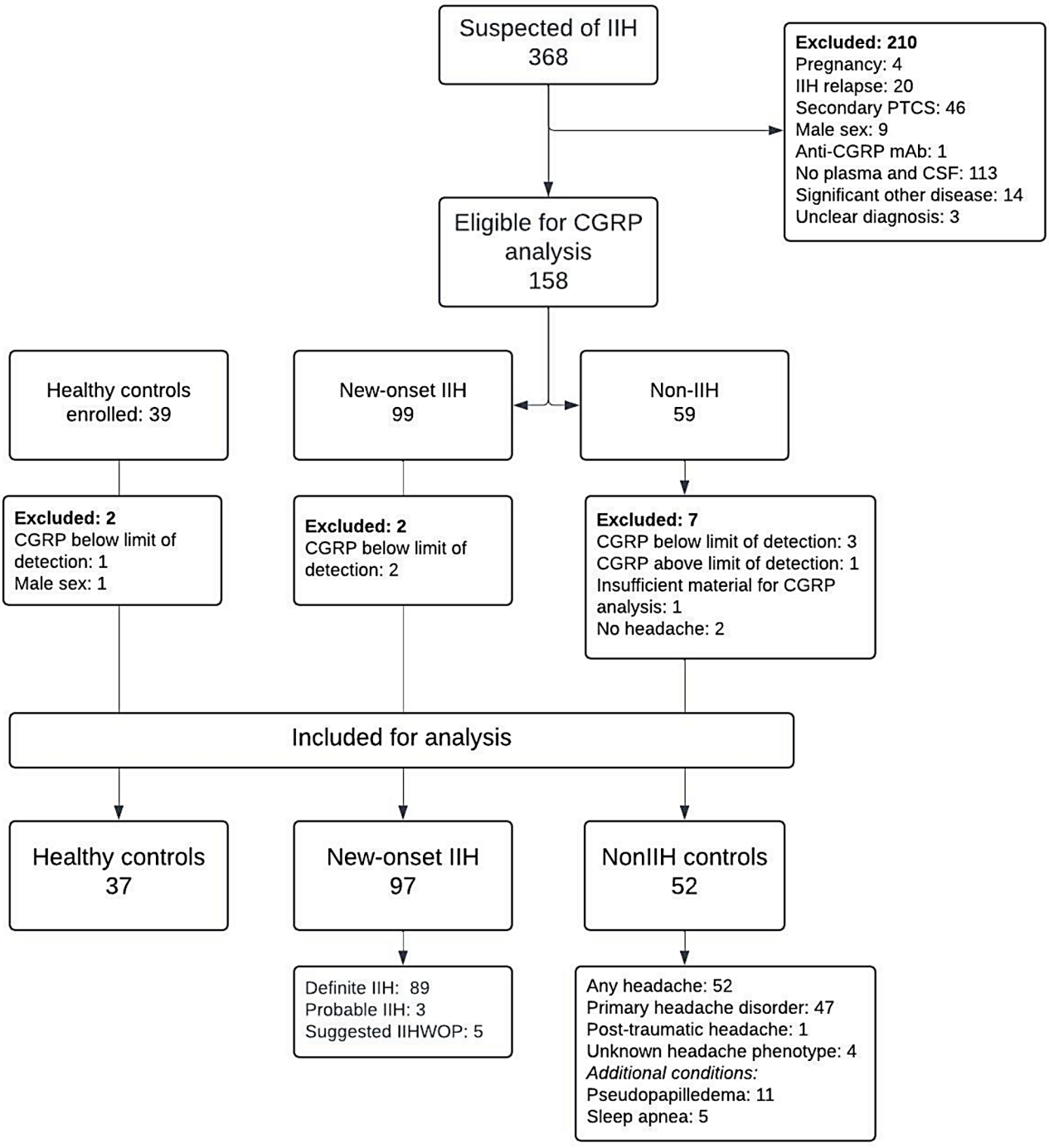


Fig. 1 Flowchart of participants excluded and included for CGRP analysis and their diagnoses. None

$p < 0.0001$, adjusted R [2] 0.17). We found no between-group differences in CSF: plasma-ratio (Table 2): Mean (± SD) CGRP CSF: plasma-ratio was in IIH 0.38 (± 0.20), in non-IIH 0.35 (± 0.17), and in HC 0.31 (± 0.15), $p = 0.139$.

CGRP, headache phenotype and -frequency

Three exploratory analyses restricting comparator groups for increased homogeneity regarding headache, migraine and chronicity showed no significantly different between-group CGRP levels in plasma or CSF. We compared (i) IIH with headache versus non-IIH with chronic migraine vs. HC (Fig. 4), (ii) IIH *without* versus *with* headache,

Table 1 Participant characteristics

	IIH *n* = 97	Non-IIH *n* = 52	Healthy controls *n* = 37	*p*-value
Age, median (IQR)	28 (23–36)	31 (26–36)	29 (24–33)	0.23
Female sex, *n* (%)	97 (100)	59 (100)	37 (100)	-
BMI, mean (± SD)	36.3 (6.1)	37.1 (7.3)	37.0 (6.3)	0.73
Active smoking, *n* (%)	38 (40)	12 (21)	7 (21)	0.01
Lumbar opening pressure (cm CSF), median (IQR), [range]	35 (29–44) [19–66]	22 (19–28) [13–51]	19 (16–23) [12–31]	< 0.0001
Papilledema grade[ϒ], *n* (%)				-
Grade 0	5 (5)	52	37	
Grade 1	20 (21)	0	0	
Grade 2	33 (34)	0	0	
Grade 3	25 (26)	0	0	
Grade 4	13 (14)	0	0	
Previous headache history, *n* (%)	52 (54)	36 (69)	13 (35)	< 0.01
Headache, *n* (%)	89 (92)	52 (100)	34 (92)	0.06
No headache, *n (%)*	8 (8)	0	3 (8)	
Migraine-like headache, *n* (%)	54 (56)	41 (79)	6 (16)	< 0.0001
Tension type-like headache, *n* (%)	23 (24)	6 (12)	28 (76)	
Unclassifiable/another headache, *n* (%)	12 (12)	5 (10)	0	
Primary headache disorder, *n* (%)	77 (79)	47 (90)	34 (92)	0.10
Chronic headache[¥], *n* (%)	55 (57)	40 (77)	0	0.02
Chronic migraine-like headache[¥], *n* (%)	30 (31)	32 (62)	0	< 0.001
Headache preventive therapy, *n*	1	3	0	-
Hormonal contraception, *n* (%)	38 (39%)	24 (46%)	16 (43%)	0.701

[ϒ] Papilledema reported with the modified Frisén grade (0–5). In 13 patients with IIH, papilledema was graded by an ophthalmologist rather than a neuro-ophthalmologist. [¥] Chronic (migraine) headache: ≥15 days monthly. p-value reported for the comparison of IIH versus non-IIH (HC omitted, since none with chronic headache)

Missing data: BMI: 4 (IIH, *n* = 2; non-IIH, *n* = 2; HC, *n* = 0); lumbar opening pressure: 8 (IIH, *n* = 0; non-IIH, *n* = 5; HC, *n* = 3); smoking: 7 (IIH, *n* = 2; non-IIH, *n* = 2; HC, *n* = 3); papilledema grade: 11 (IIH, *n* = 11); Papilledema: 1 (IIH, *n* = 1);

Fig. 5A-B, and (iii) IIH with chronic migraine versus HC, Fig. 5C-D. Results are given in Table 3.

CGRP, opening pressure, and BMI

Adjusting for participant group, BMI, and smoking, an OP increase of 1 cm CSF decreased $CGRP_{PLASMA}$ with 0.27 pmol/L ($p = 0.708$) and decreased $CGRP_{CSF}$ with 0.07 pmol/L ($p = 0.615$). There was no overall correlation between LPOP and $CGRP_{PLASMA}$ (Spearman's ρ -0.01, $p = 0.855$) or $CGRP_{CSF}$ (Spearman's ρ 0.057, $p = 0.51$). Group-wise analyses of $CGRP_{CSF}$ and LPOP in IIH (Spearman's ρ 0.128, $p = 0.293$), non-IIH (Spearman's ρ 0.004, $p = 0.98$), and in patients with migraine-like headache regardless of group (Spearman's ρ 0.095, $p = 0.425$) showed no difference either.

Comparing 10th percentiles of participants with the highest versus the lowest LPOP ($n = 18$ in each, high OP range 45.0–66.0 cm CSF; low OP range 11.5–17.0 cm CSF) showed median (IQR) $CGRP_{PLASMA}$ of 114 (83–193) versus 145 (118–195) pmol/L ($p = 0.268$) and mean(± SD) $CGRP_{CSF}$ 47 (± 15) versus 46 (± 13) pmol/L ($p = 0.929$). Overall, BMI did not correlate with $CGRP_{PLASMA}$ or with $CGRP_{CSF}$ (Pearson's $r = 0.06$, $p = 0.43$ for both).

Discussion

We observed comparable levels of CGRP in plasma and CSF in newly diagnosed IIH compared to *IIH mimics* with primary headache disorders (non-*IIH*) and sex-, age- and BMI-matched healthy controls. In sub-analyses, a chronic migraine-like headache phenotype was not reflected in significantly different CGRP levels. This contrasts the only other two studies reporting CGRP levels in IIH [32, 33]: In the Turkish study [32], serum CGRP in IIH of unknown duration was significantly elevated compared to age- and sex-matched HC with similar BMI, comparable to those with episodic migraine (significantly lower BMI), and significantly lower than in chronic migraine (similar BMI); age and sex was similar in all control groups. Quite opposite, the Austrian study [33] found that plasma CGRP at time of IIH diagnosis was *lower* in IIH than in HC, who were of significantly younger age and male sex, though – BMI was not reported. In that study, patients with episodic migraine also had lower CGRP than HC. In our observations, IIH and non-IIH also had numerically lower $CGRP_{PLASMA}$ than HC, but statistically insignificant (comparison 1, Table 3). In the Austrian study, headache of any phenotype but particularly migranious headache was consistently associated with higher levels of CGRP in IIH during six months follow-up (eight samples). All Turkish IIH patients had migraine phenotype headaches as per inclusion criteria. At ophthalmic remission, CGRP was higher in patients with persistent post-IIH headache compared to those with headache resolution. Information on pre-existing migraine and its duration in these sub-groups was not given, however, highly relevant for the interpretation of whether the observed CGRP differences are driven by a pre-existing primary 'true' migraine or were induced by IIH; headaches had lasted for a much shorter time in IIH compared to controls, though, intuitively indicating more new-onset headaches in IIH. Austrian patients with IIH with extreme CGRP values exceeding upper limit of detection all had a history of pre-existing migraine except for one ($n = 5/6$), yet CGRP remained significantly higher in IIH *with* versus *without* any headache after adjustment for pre-existing migraine. We also saw numerically higher values in IIH *with* versus *without* headache,

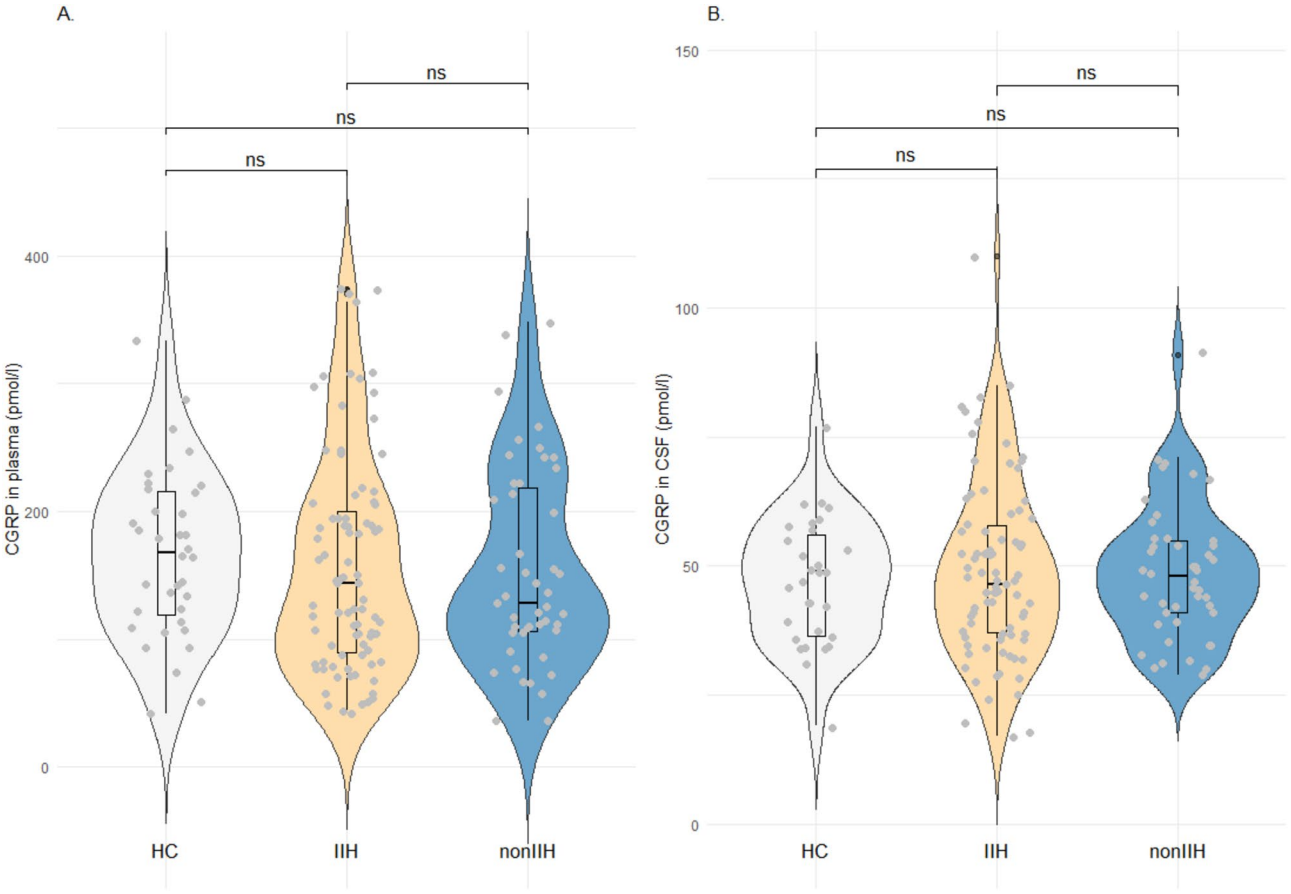


Fig. 2 Concentrations of CGRP in plasma and CSF. Violin plots with indications of individual observations (grey dots), median, interquartile range, and range showing the concentrations of CGRP (pmol/L) in plasma (**A**) and CSF(**B**) grouped by sex-, age-, and BMI-matched healthy controls (HC, *grey*), patients with IIH (*yellow*), and patients with headache but without IIH (non-IIH, *blue*). *ns* = non-significant comparison (ANOVA)

Table 2 CGRP concentration in plasma, cerebrospinal fluid, and their ratio

	IIH (*n* = 97)	**Non-IIH (*n* = 52)**	**Healthy controls (*n* = 37)**	***p*-value**
Plasma (pmol/L)	*n* = 87	*n* = 47	*n* = 36	0.744
Mean (± SD)	158 (± 86)	154 (± 77)	167 (± 66)	
Median (IQR)	144 (90–200)	128 (106–218)	168 (119–216)	
Range	42–374	36–348	42–333	
CSF (pmol/L)	*n* = 82	*n* = 45	*n* = 31	0.826
Mean (± SD)	49 (± 17)	49 (± 13)	47 (± 12)	
Median (IQR)	47 (37–58)	48 (41–55)	49 (37–56)	
Range	17–110	29–91	19–77	
CSF: plasma ratio	*n* = 72	*n* = 40	*n* = 30	0.139
Mean (± SD)	0.38 (± 0.20)	0.35 (± 0.17)	0.31 (± 0.15)	
Median (IQR)	0.35 (0.23–0.53)	0.33 (0.25–0.39)	0.28 (0.20–0.35)	
Range	0.07–1.05	0.12–0.89	0.17–0.88	

however, insignificant. As opposed to the Turkish study, IIH headaches in the Austrian and our study were phenotypically heterogeneous. In sub-analyses restricted to IIH patients with *migraine*-like headaches we found numerically but non-significantly higher CGRP levels in plasma compared to HC as did the Austrian group more convincingly. Methodological differences may contribute to explain the discrepancies between our and the previous observations as will be discussed further in the limitations section.

CGRP, cerebrospinal fluid and opening pressure

CGRP in CSF has never been reported in IIH but was elevated in patients with chronic migraine compared to controls in three studies [23]. We observed substantially lower CGRP levels in CSF compared to plasma with overall median CSF:plasma-ratio of 0.32 (range 0.07–1.05). We also found strong evidence of a moderate correlation of CGRP in the two compartments raising speculations on some degree of interference. CGRP concentrations in CSF versus jugular vein blood are reported both significantly higher [37] and similar [38] in rodents. To our

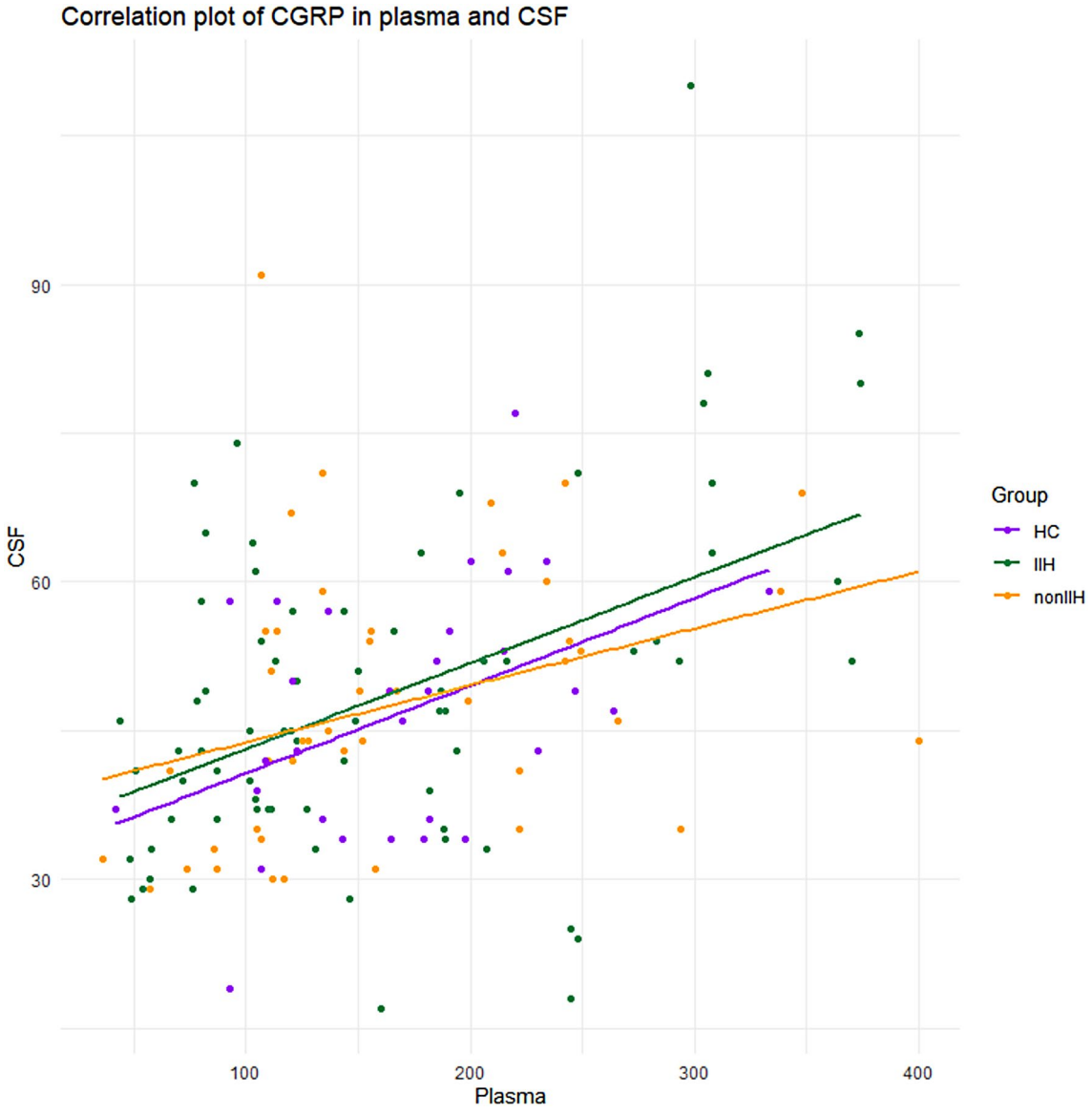


Fig. 3 Correlation between CGRP in plasma and CSF. Correlation between CGRP in plasma and CSF in IIH (green), non-IIH (orange), and healthy controls (HC, purple), $p < 0.0001$

knowledge, $CGRP_{CSF}$ in human studies have never been compared to systemic levels [39–41]. A central question is the origin and role of CGRP in the CSF and its relation to (i) systemic CGRP levels, (ii) nociceptive signaling in the peripheral nervous system, and (iii) ICP. A recent rodent model demonstrated how CSF constitutes a direct communicative pathway between the central nervous system and the TG [42]. Cortex-derived and CSF-borne solutes activated the TG through direct entry of CSF at the TG root. CSF flow augmented by cortical spreading depressions (CSD) even had a predilection for the TG as opposed to the parasagittal dural space. Also, altered CSF proteome, including a two-fold increase in CGRP, explained the substantial TG activation that followed the CSD. In this context, centrally derived CGRP activated peripheral trigeminal neurons. This complements evidence pointing to a peripheral origin of ganglion-activating CGRP: In one rodent model, primary meningeal trigeminal afferents were responsible for CGRP increase in the CSF and jugular vein blood following an intra-cisternal inflammatory stimulus [37]. In another rodent model, CGRP applied directly onto the exterior dura mater led to increased CGRP levels in both CSF and jugular vein blood [38], but convincing permeance of the dura mater in the opposite direction (from the subarachnoid side) was not confirmed ex vivo. However, central stimuli (CSD) have been shown to stimulate meningeal trigeminal axons previously [43]. Together, it

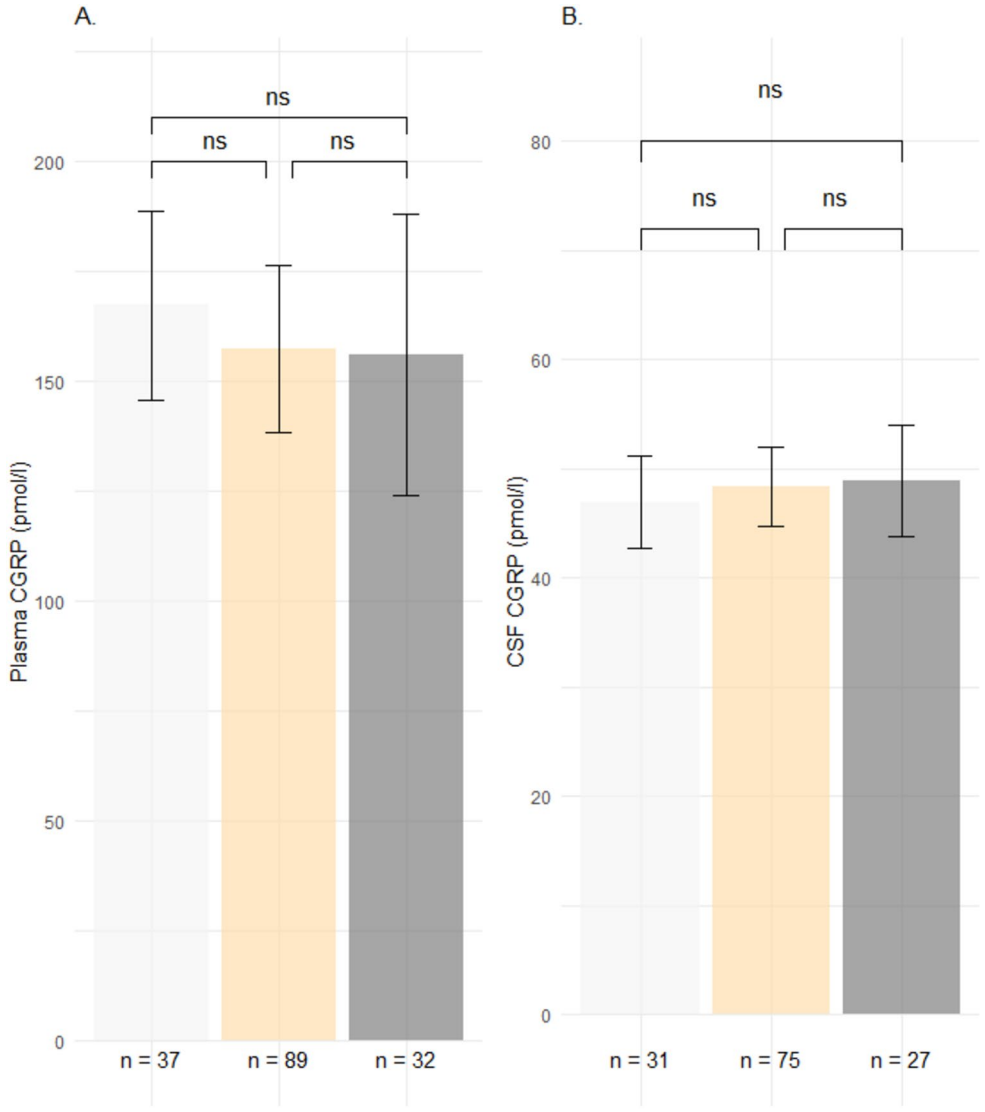


Fig. 4 Sub-analyses of CGRP in plasma and CSF depending on migraine headache phenotype and chronicity. Barplots showing mean (95% confidence intervals) CGRP in healthy controls (light grey) versus IIH with headache (yellow) versus non-IIH with chronic migraine (dark grey) in plasma (**A**) and CSF (**B**); *ns* = not significant

seems that release of CGRP can originate both centrally and peripherally. Whether afferent nociceptive signaling is fused unidirectionally and exclusively at meningeal primary trigeminal afferents is unclear but it seems that bidirectional exchange of neuropeptides in the central-peripheral interface is possible which is relevant to the understanding of CGRP existence and function in CSF relative to blood.

Another central question is whether and how the trigemino-vascular system is affected by ICP and vice versa. Intracranial hypertension has been hypothesized to cause migraine headache by mechanical and/or chemical stimulation of afferent meningeal innervation for decades [44, 45] which, in IIH, is plausible given the congested venous sinuses. Electric stimulation of the superior sagittal sinus led to increased systemic CGRP levels in cats [46]. Furthermore, Meckel's cave, which harbors the TG, is observed dilated, bilobed and indented in IIH compared to age- and sex-matched controls [47], likely due to pressure-induced mechanical distension of this intradural cistern. Any functional impact of such structural changes is uncertain. The opposite scenario of CGRP affecting ICP is also obscure; in rodents, CGRP injected into the cisterna magna reduced CSF efflux via meningeal lymphatic vasculature [48]. In another rodent study, choroid plexus stimulated with CGRP responded with increased cAMP [49]. Our data showed no correlation between OP and CGRP in line with the Austrian study [33], in which CGRP levels furthermore remained constant over 6 months in which time ICP decrease expectedly happened

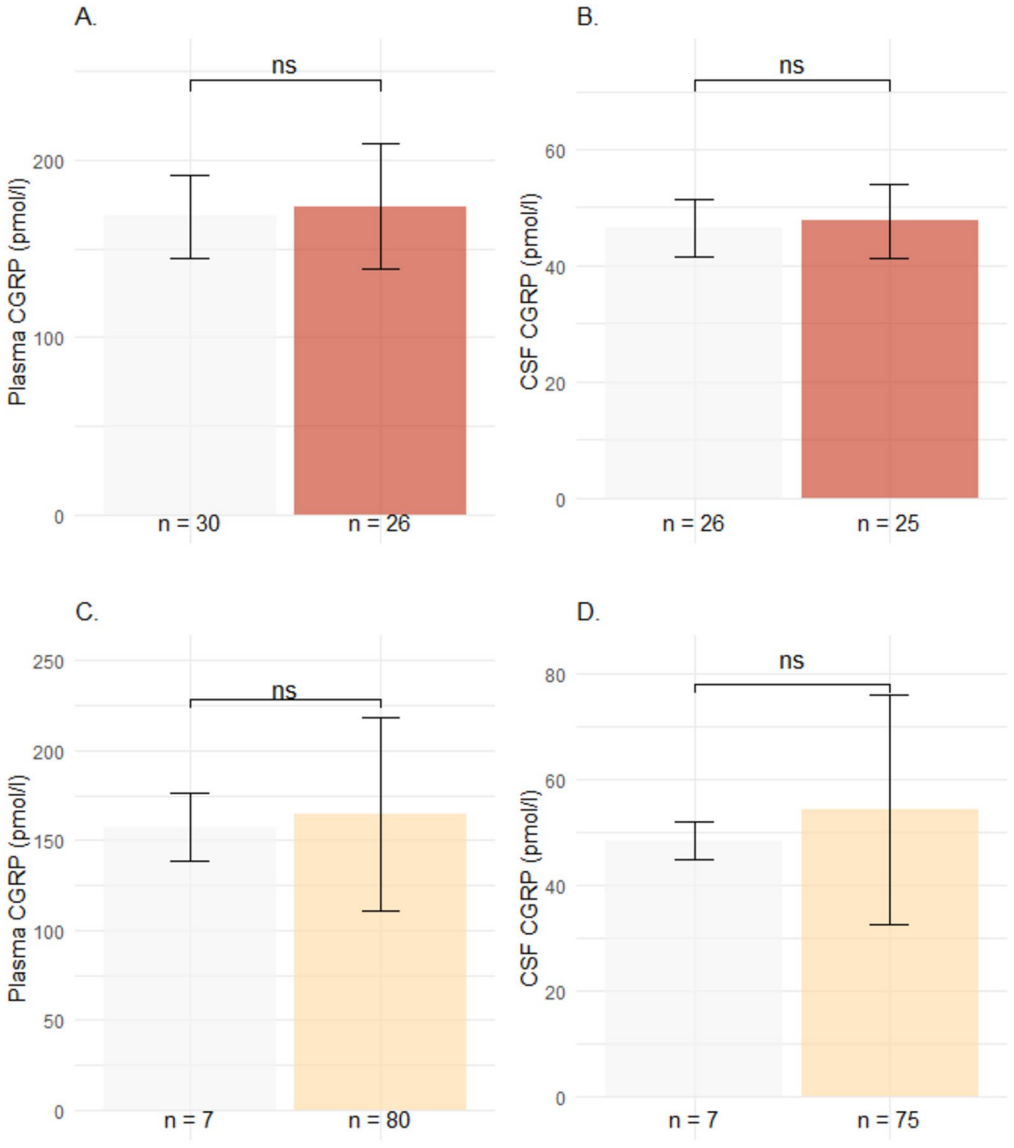


Fig. 5 Barplots showing mean (95% confidence interval) CGRP in patients with IIH with chronic migraine-like headache (red) versus healthy controls (grey) in plasma (**A**) and CSF (**B**), and IIH *with* (yellow) versus *without* (grey) headache in plasma (**C**) and CSF (**D**); *ns* = not significant

in most patients. In the first case series on anti-CGRP mAb treatment of IIH headache [14] sustained headache alleviation was observed despite relapse of papilledema supporting that CGRP is indeed involved in high pressure headaches. All patients in that series presented with migraine-like headaches at IIH diagnosis, and 57% (4/7) of patients had pre-existing migraine. This questions again whether headaches were aggravated or unveiled 'true migraine' rather than 'pure' high pressure headaches – if that distinction is sensible. Any link between ICP, noxious neuropeptides and trigeminal activation remains to be demonstrated.

Alternative drivers of IIH headache

It is possible that CGRP is not the main driver of headaches in (all) patient with IIH. As proposed by *Krajnc* et al.[33] it may be constricted to a subpopulation of patients prone to migraine(-like) pathophysiology. Also, although CGRP can cause migraine it is not an absolute prerequisite for migraine induction [50] – numerous and downstream signaling pathways are at play. Furthermore, the metabolic and hormonal disruption characterizing IIH may also drive headache. Several deviating metabolites in CSF and urine correlated with the burden and resolution of headache in IIH [51], although not confirmed in another metabolomics study of new-onset IIH [52].

Table 3 Sub-analyses of CGRP levels in plasma and CSF depending on headache existence, migraine phenotype, and chronicity

	Plasma (pmol/L)	*p*-value	CSF (pmol/L)	*p*-value
Comparison 1, *mean (± SD)*				
IIH with headache	157 ± 87, *n* = 80		48 ± 16, *n* = 75	
Non-IIH with chronic migraine	159 ± 88, *n* = 28		49 ± 14, *n* = 26	
Healthy controls	167 ± 66, *n* = 36	0.834	47 ± 12, *n* = 31	0.836
Comparison 2, *median (IQR)*				
IIH with headache	160 (118–189), *n* = 80		45 (41–63), *n* = 75	
IIH without headache	129 (87–205), *n* = 7	0.708	47 (37–58), *n* = 7	0.752
Comparison 3, *mean (± SD)*				
IIH with chronic migraine	174 ± 92, *n* = 26		48 ± 16, *n* = 25	
Healthy controls without migraine	168 ± 66, *n* = 30	0.803	46 ± 13, *n* = 26	0.755

Strengths and limitations

We present the hitherto largest IIH population investigated for CGRP and the first study to report CGRP in CSF in IIH. Patients were well-characterized being diagnosed at highly specialized tertiary centers and were enrolled at time of diagnosis equaling treatment-naivety. Matched healthy controls is an obvious strength as is their rare provision of OP and CSF. The size of the dataset could be of insufficient statistical power, especially in sub-analyses. However, our IIH sample size surpassed previous studies. A particular and unique strength was the comparable BMI. In humans, obesity is associated with increased CGRP [53], and in rodents, obesity increases nociceptive activation [54] and sensory processing of the trigeminal system [55].

Our study has some important limitations. Sampling was performed for diagnostic purposes and therefore not standardized regarding time of the day, fasting, menstrual cycle, or headache status. There is some evidence to suggest an effect on CGRP of fasting [56, 57], meals [53], circadian variability [56], and hormonal fluctuations during the menstrual cycle [58–60]. Also, CGRP levels decrease significantly within hours following migraine ictus [24, 26] reaching basal levels two hours after spontaneous resolution [24] or after termination by triptans [18, 61]. Hence, peripheral CGRP levels are sensitive to timing of sampling in relation to headache. The Austrian and Turkish studies compared IIH to controls in a headache-free interval without recent use of abortive therapy. Being investigated for suspected IIH, often because of ongoing headaches, it is likely that our non-HC participants were temporally close to headache attacks and use of abortive therapy. CGRP would expectedly be even higher in non-HC compared to HC, which it was not – not even in sub-analyses restricted to those with chronic migraine. Methodological issues could explain this. Meticulously planned preanalytical procedures are pivotal for CGRP measurements [62]. CGRP is readily degraded by extracellular peptidases: in plasma, the half-life in the initial fast phase of exponential decay is 7 minutes [63], whereas degradation in CSF is less clear [64]. The neuro-anatomical origin of basal resting state CGRP levels seem to differ from stimulus-induced CGRP peaks [37]. Our protocol may have disabled measurement of stimulus induced CGRP peaks and left us with the basal CGRP tone. Sample processing of up to 24 h by 4 C yielded CGRP levels comparable to immediately frozen samples in a meta-analysis [65], but we handled samples at ambient temperature. Decay of CGRP during long-term sample storage (up to seven years in the present cohort) could mitigate findings [65]. Finally, our assay was limited by cross-reactivity by α- and β-CGRP. Together, the Austrian study seems to have applied the most appropriate methodology biochemically given their swift sample processing in a cold chain and short duration of sample storage; however, our study was superior regarding sample size and BMI-, age-, and sex-matching healthy controls. Reassuringly, our results are comparable to a previous study using the same assay in which between-group differences in CGRP levels were detected in cluster headache [66]. Other studies using this assay report numerically lower CGRP values [67, 68]; they adhered to slightly different pre-analytical sample handling.

Clinical and future perspectives

Publication bias favors positive findings. Our negative findings do not necessarily imply non-response to anti-CGRP mAb therapy. CGRP levels are not consistently shown elevated in EM and CH, yet, these patients do have effect of anti-CGRP mAbs [12, 13]. A recent meta-analysis found that higher BMI, obesity, and psychiatric comorbidity were negative predictors of response to anti-CGRP mAb therapy [69]. This could raise concern for lower response-rates in IIH given their obese phenotype and high burden of psychiatric comorbidity [70] – factors that already limit the repertoire of headache preventive options in IIH. Nevertheless, great response rates were observed in the 55 patients receiving open-label treatment with Erenumab for chronic post-IIH headache [16]. A proper double-blinded randomized controlled trial of CGRP mAbs in IIH is needed to clarify this pertinent clinical question.

Conclusion

We found no difference in levels of CGRP in plasma or CSF in IIH compared to healthy BMI-, age-, and sex-matched controls and *IIH mimics* with primary headache disorders. Neither did CGRP levels differ depending on headache phenotype, chronicity, lumbar opening pressure, or BMI. Our observations may reflect basal CGRP tone rather than the stimulated state and emphasizes that CGRP is at most partly mediating headache but not driving it. Investigation of the effect of anti-CGRP mAbs on headache in IIH is still relevant to explore.

Abbreviations

ANOVA	Analysis of Variance
BMI Body	Mass Index
CGRP	calcitonin gene-related peptide
CI	confidence interval
CM	chronic migraine
CSD	cortical spreading depression
CSF	cerebrospinal fluid
CV	coefficient of variation
EM	episodic migraine
HC	healthy controls
ICP	intracranial pressure
IIH	idiopathic intracranial hypertension
IIHWOP	idiopathic intracranial hypertension without papilledema
mAb	Monoclonal antibody
OP	Opening pressure (lumbar puncture)
TG	trigeminal ganglion
TTH	tension type headache

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Author contributions

NSH collected and cleansed data, was involved in the conception and study design, did statistical analyses, and drafted and revised the manuscript; JJK collected data and revised the manuscript, LKB and NRJ facilitated CGRP-CSF pilot testing, and CGRP analyses, and revised the manuscript; DB collected data, was involved in conception and study design and revised the manuscript; RHJ was involved in the conception and study design, data analyses and revised the manuscript. All authors approved the submitted version and have agreed to be personally accountable for its content.

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Data availability

Anonymized data is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the local ethical committee (Region of Southern Denmark, S-20170058). We adhered to the tenets of the Helsinki declaration and Danish Law. All patients gave their informed written consent. HC were compensated financially (270 Euro and reimbursement of transportation) for the inconvenience of participation.

Competing interests

The authors declare that they have no competing interests

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