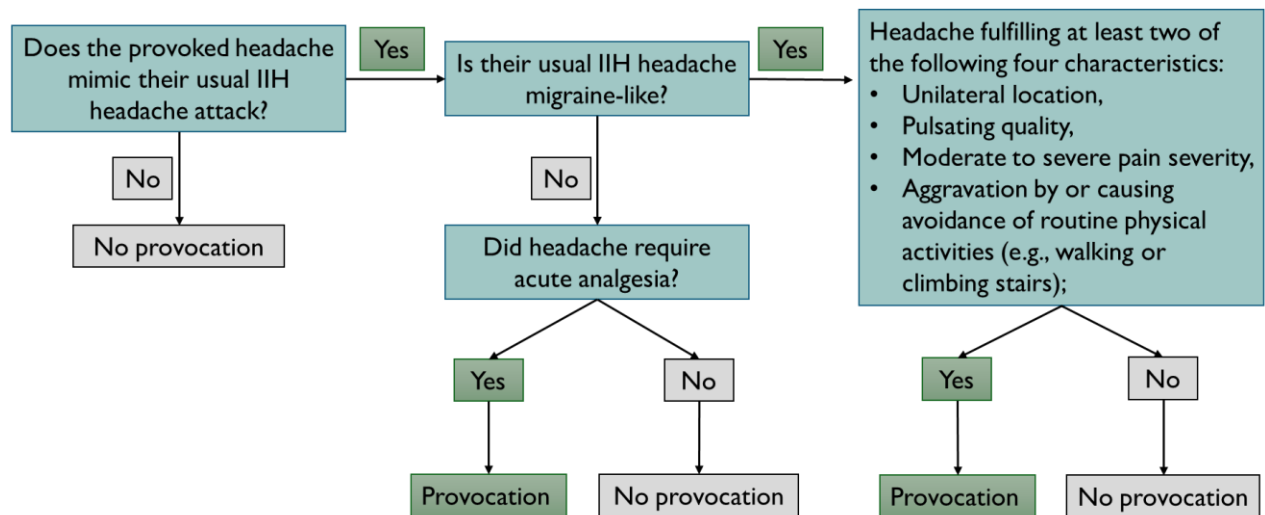


Supplemental material



Supplementary Fig. 1 Flowchart showing the criteria that were used to define a calcitonin gene-related peptide (CGRP)-induced idiopathic intracranial hypertension (IIH) headache attack.

Supplementary Table 1 Levels of cytokines at baseline in participants.

Cytokine	Visit 1	Visit 2	P- value	Range of Quantification
	Mean (SD) pg/mL, n			
CCL11	67.4 (21.9), 16	54.4 (15.8) , 16	0.063	0.2 - 15625.0
CCL13	83.4 (40) , 16	77.2 (25.2) , 16	0.564	0.1 - 1953.1
CCL19	188.6 (129.8), 15	190.9 (134.8), 15	0.954	1.0 - 976.6
CCL2	284.7 (95.4) , 16	271.6 (57.5) , 16	0.584	0.2 - 3906.3
CCL3	6.4 (2.2) , 16	6.3 (2) , 16	0.746	0.0 - 1953.1
CCL4	60.4 (18.3) , 16	59.6 (15.2) , 16	0.851	0.1 - 3906.3
CCL7	0.6 (0.3) , 16	0.5 (0.3) , 16	0.138	0.1 - 1953.1
CCL8	30.5 (12.7) , 16	31.5 (12.9) , 16	0.825	0.0 - 1953.1
CSF1	128.8 (12.7) , 16	131.5 (20) , 16	0.565	0.2 - 3906.2
CSF3	167.4 (64.1) , 16	233.6 (268.5) , 16	0.368	16.2 - 62500.0
CXCL10	115.3 (76.8) , 16	126.9 (97.9) , 16	0.585	0.1 - 4344.6
CXCL11	52.3 (40.5) , 16	39.6 (11.2) , 16	0.243	0.1- 1953.1
CXCL12	179.3 (60) , 16	171.2 (62.6) , 16	0.464	30.5 - 31250.0
CXCL8	24.2 (69) , 16	18.4 (51.2) , 16	0.223	0.3 - 2908.3
CXCL9	61.3 (39) , 16	50.3 (19) , 16	0.172	0.2 - 2591.1
EGF	55.3 (91.8) , 16	49.4 (33.9) , 16	0.801	0.5 - 976.6
FLT3LG	94.1 (37.8) , 16	89.3 (34.7) , 16	0.291	1.0 - 3906.3
HGF	290.3 (108) , 16	293 (70.3) , 16	0.901	0.2 - 15624.8
IFN- γ	0.3 (0.5) , 16	0.8 (2.3) , 16	0.389	0.0 - 3906.3
IL-10	5.4 (3.1) , 16	7.2 (8.9) , 16	0.453	0.5 - 31251.5
IL-13	2.3 (2) , 16	2.1 (1.6) , 16	0.126	0.5 - 5589.9
IL-15	10.1 (2.2) , 16	11.7 (4.6) , 16	0.152	1.0 - 15624.5
IL-17C	23.6 (13.9) , 16	21.8 (16) , 16	0.608	0.7 - 7812.5
IL-18	331.8 (55.3) , 16	308.9 (78.6) , 16	0.205	0.5 - 15461.8
IL-27	7.8 (6) , 16	9.8 (11.8) , 16	0.399	0.5 - 31460.1
IL-4	0.2 (0.2), 9	0.2 (0.2), 10	0.664	0.1 - 3906.2
IL-6	7.9 (5.7) , 16	11.1 (15.7) , 16	0.494	0.1 - 3906.3
IL-7	1.5 (1.1) , 16	1.5 (0.8) , 16	0.898	0.2 - 1953.1
LTA	10.1 (3) , 16	9.7 (2.4) , 16	0.463	0.1 - 3906.3
MMP1	547.9 (563.5) , 16	347.3 (209.4), 16	0.13	1.0 - 7812.5
MMP12	172 (55.2) , 16	164.9 (71) , 16	0.454	7.6 - 15625.0
OLR1	91.4 (213) , 16	49.7 (26) , 16	0.417	1.0 - 3906.3
OSM	2.9 (2.7) , 16	3.5 (3) , 16	0.428	0.1 - 1953.1
TGF- α	5.5 (1.5) , 16	4.9 (0.8) , 16	0.094	1.1 - 1953.1
TNF- α	13.8 (2.7) , 16	13.8 (3.2) , 16	0.93	3.8 - 15625.0
TNFSF10	491.8 (95.6) , 16	476 (68.6) , 16	0.471	1.0 - 7812.5
TNFSF12	418.7 (89.5) , 16	394.9 (76.8) , 16	0.21	3.8 - 15625.0
VEGF-A	315.7 (130.7) , 16	290.5 (61.9) , 16	0.32	0.5 - 7812.5

Abbreviations: CCL11: C-C motif chemokine 11 or Eotaxin, CCL13: C-C motif chemokine 13, CCL19: C-C motif chemokine 19, CCL2: C-C motif chemokine 2, CCL3: C-C motif chemokine 3, CCL4: C-C motif chemokine 4, CCL7: C-C motif chemokine 7, CCL8: C-C motif chemokine 8, CSF1: Macrophage colony-stimulating factor 1, CSF3: Granulocyte colony-stimulating factor, CXCL10: C-X-C motif chemokine 10, CXCL11: C-X-C motif chemokine 11, CXCL12: Stromal cell-derived factor 1, CXCL8: Interleukin-8, CXCL9: C-X-C motif chemokine 9, EGF: Epidermal growth factor, FLT3LG: Fms-related tyrosine kinase 3 ligand, HGF: Hepatocyte growth factor, IFN- γ : Interferon gamma, IL-10: Interleukin-10, IL-13: Interleukin-13, IL-15: Interleukin-15, IL-17C: Interleukin-17C, IL-18: Interleukin-18, IL-27: Interleukin-27, IL-4: Interleukin-4, IL-6: Interleukin-6, IL-7: Interleukin-7, LTA: Lymphotoxin-alpha, MMP1: Matrix metalloproteinase-1 or Interstitial collagenase, MMP12: Matrix metalloproteinase-12 or macrophage metalloelastase, OLR1: Oxidized low-density lipoprotein receptor 1, OSM: Oncostatin M, TGF- α : Transforming growth factor alpha, TNF- α : Tumor necrosis factor alpha, TNFSF10: Tumor necrosis factor ligand superfamily member 10, TNFSF12: Tumor necrosis factor ligand superfamily member 12, VEGF-A: Vascular endothelial growth factor A.

Supplemental Table 2 Comparison of baseline cytokine levels on the CGRP visit between participants who developed CGRP-provoked headache (H+) and those who did not (H-).

Cytokine	CGRP visit Baseline H+	CGRP visit Baseline H-	P- value
	Mean (SD) pg/mL, n		
CCL11	63.7 (17.1), 7	60.1 (18.8), 9	0.697
CCL13	76.0 (23.9), 7	74.4 (26.5) , 9	0.902
CCL19	138.8 (32.1), 6	224.0 (166.4) , 9	0.17
CCL2	262.8 (34.5), 7	261.6 (58.8) , 9	0.962
CCL3	6.4 (1.8), 7	5.8 (1.8) , 9	0.518
CCL4	57.1 (17.3), 7	56.6 (8.6) , 9	0.948
CCL7	0.6 (0.3), 7	0.5 (0.1) , 9	0.246
CCL8	26.5 (11.6), 7	26.8 (6.0) , 9	0.952
CSF1	134.8 (14.9), 7	123.6 (11.2) , 9	0.125
CSF3	171.9 (88.2), 7	150.3 (61.0) , 9	0.592
CXCL10	122.0 (84.3), 7	95.1 (64.1) , 9	0.498
CXCL11	36.1 (11.2), 7	36.5 (10.1) , 9	0.935
CXCL12	179.4 (43.7), 7	175.5 (70.2) , 9	0.894
CXCL8	35.4 (77.1), 7	5.5 (1.4) , 9	0.345
CXCL9	50.4 (8.3), 7	47.8 (25.7) , 9	0.782
EGF	40.9 (19.8), 7	21.8 (14.9) , 9	0.058
FLT3LG	92.7 (38.1), 7	90.7 (39.8) , 9	0.917
HGF	303.2 (93.4), 7	277.0 (61.2) , 9	0.535
IFN- γ	0.2 (0.1), 7	0.4 (0.7) , 9	0.261
IL-10	4.2 (1.0), 7	5.2 (2.4) , 9	0.306
IL-13	2.1 (2.2), 7	2.1 (1.2) , 9	0.967
IL-15	11.1 (2.8), 7	9.8 (2.4) , 9	0.357
IL-17C	27.9 (17.7), 7	16.5 (5.6) , 9	0.143
IL-18	291.9 (43.3), 7	334.5 (61.6) , 9	0.127
IL-27	9.2 (7.3), 7	7.4 (5.3) , 9	0.585
IL-4	0.1 (0.0), 5	0.3 (0.3) , 5	0.178
IL-6	6.9 (5.3), 7	7.3 (3.0) , 9	0.861
IL-7	1.2 (0.3), 7	1.1 (0.6) , 9	0.669
LTA	9.0 (2.1), 7	11.1 (2.7) , 9	0.116
MMP1	311.1 (241.8), 7	323.4 (212.4) , 9	0.917
MMP12	156.1 (47.5), 7	175.1 (73.8) , 9	0.543
OLR1	57.6 (30.5), 7	44.8 (23.5) , 9	0.38
OSM	4.2 (3.6), 7	2.9 (2.5) , 9	0.414
TGF- α	5.0 (1.1), 7	4.9 (0.8) , 9	0.815
TNF- α	14.0 (2.3), 7	13.2 (3.0) , 9	0.554
TNFSF10	496.2 (66.1), 7	472.3 (54.0) , 9	0.454
TNFSF12	382.4 (83.4), 7	414.4 (103.2) , 9	0.504
VEGF-A	296.8 (78.7), 7	264.9 (49.7) , 9	0.371

Abbreviations: CCL11: C-C motif chemokine 11 or Eotaxin, CCL13: C-C motif chemokine 13, CCL19: C-C motif chemokine 19, CCL2: C-C motif chemokine 2, CCL3: C-C motif chemokine 3, CCL4: C-C motif chemokine 4, CCL7: C-C motif chemokine 7, CCL8: C-C motif chemokine 8, CSF1: Macrophage colony-stimulating factor 1, CSF3: Granulocyte colony-stimulating factor, CXCL10: C-X-C motif chemokine 10, CXCL11: C-X-C motif chemokine 11, CXCL12: Stromal cell-derived factor 1, CXCL8: Interleukin-8, CXCL9: C-X-C motif chemokine 9, EGF: Epidermal growth factor, FLT3LG: Fms-related tyrosine kinase 3 ligand, HGF: Hepatocyte growth factor, IFN- γ : Interferon gamma, IL-10: Interleukin-10, IL-13: Interleukin-13, IL-15: Interleukin-15, IL-17C: Interleukin-17C, IL-18: Interleukin-18, IL-27: Interleukin-27, IL-4: Interleukin-4, IL-6: Interleukin-6, IL-7: Interleukin-7, LTA: Lymphotoxin-alpha, MMP1: Matrix metalloproteinase-1 or Interstitial collagenase, MMP12: Matrix metalloproteinase-12 or macrophage metalloelastase, OLR1: Oxidized low-density lipoprotein receptor 1, OSM: Oncostatin M, TGF- α : Transforming growth factor alpha, TNF- α : Tumor necrosis factor alpha, TNFSF10: Tumor necrosis factor ligand superfamily member 10, TNFSF12: Tumor necrosis factor ligand superfamily member 12, VEGF-A: Vascular endothelial growth factor A.

Supplementary Table 3 Cytokines showing significant changes in the primary comparison (calcitonin gene-related peptide (CGRP) visit: baseline vs ictal) with corresponding Benjamini–Hochberg false discovery rate (FDR)-adjusted p-values.

Cytokine	Raw p-value	FDR-adjusted p-value
IL-27	0.001	0.038
HGF	0.007	0.133
OSM	0.011	0.133
IL-6	0.014	0.133
FLT3LG	0.022	0.167
CCL4	0.038	0.207
TGF- α	0.042	0.207
IL-17C	0.044	0.207
CCL8	0.049	0.207

FDR-adjusted p-values were calculated using the Benjamini–Hochberg method across all cytokines passing quality control (n = 38) for the primary comparison of within-participant changes between baseline and ictal sampling during the calcitonin gene-related peptide (CGRP) visit in participants who developed a headache. Only cytokines showing significant changes in the primary analysis are shown. Abbreviations: CGRP: Calcitonin gene-related peptide, FDR: False discovery rate, IL-27: Interleukin-27, HGF: Hepatocyte growth factor, OSM: Oncostatin M, IL-6: Interleukin-6, FLT3LG: Fms-related tyrosine kinase 3 ligand, CCL4: C-C motif chemokine 4, TGF- α : Transforming growth factor alpha, IL-17C: Interleukin-17C, CCL8: C-C motif chemokine 8.