Received: 29 November 2022 | Accepted: 8 December 2022

DOI: 10.1111/ene.15678

european journal of neurology
the official journal of the european academy of neurology

ORIGINAL ARTICLE

Response to erenumab assessed by Headache Impact Test-6 is modulated by genetic factors and arterial hypertension: An explorative cohort study

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Funding information
Area Formazione accademica, Ricerca e Innovazione (AFRI), Ente Ospedaliero Cantonale

Abstract

Background and purpose: Response predictors to erenumab (ERE) in migraine patients would benefit their clinical management. We investigate associations between patients' clinical characteristics and polymorphisms at calcitonin receptor-like receptor (*CALCRL*) and receptor activity-modifying protein 1 (*RAMP1*) genes and response to ERE treatment measured as clinically meaningful improvement on the Headache Impact Test-6 (HIT-6) score.

Methods: This post hoc analysis of a prospective, multicenter, investigator-initiated study involves 110 migraine patients starting ERE 70 mg/month. Demographics, medical history, and migraine-related burden measured by HIT-6 score were collected during 3 months before and after ERE start. Selected polymorphic variants of *CALCRL* and *RAMP1* genes were determined using real-time polymerase chain reaction. Logistic regression models identified independent predictors for response to ERE, defined as HIT-6 score improvement ≥ 8 points (HIT-6 responders [HIT-6 RESP] vs. HIT-6 nonresponders).

Results: At Month 3, 58 (52.7%) patients were HIT-6 RESP. Comorbid hypertension predicted a lower probability of being HIT-6 RESP (odds ratio [OR] = 0.160, 95% confidence interval [CI] = 0.047–0.548, $p = 0.003$). Compared to major alleles, minor alleles *CALCRL* rs6710852G and *RAMP* rs6431564G conferred an increased probability of being HIT-6 RESP (for each G allele: OR = 2.82, 95% CI = 1.03–7.73, $p = 0.043$; OR = 2.10, 95% CI = 1.05–4.22, $p = 0.037$). *RAMP1* rs13386048A and *RAMP1* rs12465864G decreased this probability (for each rs13386048A, OR = 0.53, 95% CI = 0.28–0.98, $p = 0.042$; for each rs12465864G, OR = 0.32, 95% CI = 0.13–0.75, $p = 0.009$). A genetic risk score based on the presence and number of identified risk alleles was independently associated with HIT-6 RESP (OR = 0.49, 95% CI = 0.33–0.72, $p = 0.0003$), surviving Bonferroni correction.

Conclusions: Response to ERE was associated with comorbid hypertension and specific allelic variants in *CALCRL* and *RAMP1* genes. Results require confirmation in future studies.

KEYWORDS
anti-CGRP antibodies, erenumab, hypertension, migraine, patient-reported outcomes, treatment response

INTRODUCTION

Migraine is a highly prevalent neurological disorder with genetic predisposition, representing the second leading cause of years lived with disability worldwide [1]. The monoclonal antibody erenumab (ERE) is a migraine-preventive therapy targeting the calcitonin gene-related peptide (CGRP) receptor implicated in migraine pathogenesis [2]. ERE reduces migraine attacks at least comparably to previous migraine-preventive drugs, with a generally more favorable tolerability profile [3, 4]. Nonetheless, some patients do not adequately respond to ERE treatment, according to clinical trials and real-world data [5–9]. Therefore, clinic and/or genetic factors predicting individual response to ERE would significantly contribute to optimize migraine management and related costs.

Migraine is a multifaceted disease manifesting as pain attacks with variable frequency, intensity, and duration and variably accompanied by aura and dysautonomic, cognitive, and hypersensitivity symptoms, all contributing to migraine burden [10]. Along this line, it has been recently highlighted how the recommended primary endpoints for clinical trials in migraine prevention (for instance, reduction in monthly migraine days [MMDs] or responder rate) may not exhaustively inform about the effectiveness of preventive drugs in real-life clinical settings [11]. This perspective, patient-reported outcomes (PRO), may provide comprehensive insight into patient perceptions of migraine impact and the effects of preventive therapies without any interposed interpretations [12, 13]. The short-form Headache Impact Test-6 (HIT-6) is a validated and extensively used PRO measure to assess the negative impact of headaches on normal daily activity [14].

In the present study, we aim to investigate in a cohort of 110 episodic and chronic migraine patients clinical and genetic factors associated with response to ERE, based on the detection of clinically meaningful improvements on the HIT-6 score after 3 months of treatment.

METHODS

This is a post hoc analysis of a previous investigator-initiated study whose methods are reported in detail elsewhere [15]. It was a multicenter, observational, prospective, exploratory study including consecutive episodic or chronic migraine patients [16] aged between 18 and 70 years, with at least eight documented days with migraine per month for at least 3 months and failure, intolerability, or contraindication to at least two migraine-preventive therapies approved in Switzerland, who were started with ERE according to the clinical judgment of their treating neurologists and independently from study participation between December 2019 and September 2020. Main exclusion criteria were botulin toxin injections within 4 months before inclusion, having started/changed the dose of one migraine-preventive medication within 2 months before inclusion, being affected with primary or secondary headaches other than migraine, and having contraindications to ERE, including uncontrolled arterial hypertension.

Patients were evaluated at first ERE 70 mg injection and 3 months thereafter; meanwhile, they continued to fill in a headache diary. Sociodemographic characteristics and migraine history as well as a blood sample for genetic analysis were collected at baseline. During the 3 months before and after ERE start, the number of MMDs, the monthly number of days with triptan/nonsteroidal analgesic use, average pain intensity and attack duration, and presence of medication overuse as well as adverse events were also collected.

To investigate migraine-related disability and its impact on daily life, at baseline and 3-month evaluations patients filled in the short-form HIT-6 [14]. The HIT-6 is a widely used PRO measure that quantifies the impact of headaches on daily activities. It comprises six items, assessing how often in the past month headache-related pain was severe, headaches impacted daily activities, and headaches caused the desire to lie down, fatigue, irritability, or difficulties in concentration. Each of these items allows five categorial answers ("never," "rarely," "sometimes," "very often," or "always"), and each of these answers is linked to a numerical score (6, 8, 10, 11, and 13, respectively), resulting in a final summed score ranging from 36 to 78.

Responders to ERE were defined as those patients who improved by at least 8 points on the HIT-6 score after 3 months of ERE treatment [17]. Both 6 and 8 points have been considered as thresholds for meaningful change for the HIT-6 in patients with chronic migraine or tension-type headache, and we opted for the more conservative definition between the two. In addition, our choice to use a threshold of ≥8 points for HIT-6 score reduction is in line with the results obtained using the median split method for turning a continuous variable into a categorical one, the median value of HIT-6 score changes in our cohort of migraine patients being equal to 8.5 [18]. Accordingly, migraine patients were divided into two groups: patients with HIT- 6 score changes <8 (HIT-6 NRESP) and those with HIT-6 score changes ≥ 8 (HIT-6 RESP).

We hypothesized that clinical and genetic profiles of HIT-6 RESP (responders) differ from those of HIT-6 NRESP (nonresponders). Accordingly, objectives of this post hoc analysis were to investigate associations between patients' clinic characteristics as well as selected polymorphisms at *CALCRL* and *RAMP1* genes (see Genotyping section) and HIT-6 RESP/NRESP status.

Genotyping

The criteria for single nucleotide polymorphism (SNP) selection and genotyping methods have been published elsewhere [15]. Briefly, 15 SNPs at *CALCRL* and *RAMP1* genes were selected from Variation Viewer (https://www.ncbi.nlm.nih.gov/variation/view) based on minor allele frequency of >10%. Genotyping of *CALCRL* and *RAMP1* polymorphisms was performed by real-time polymerase chain reaction using Applied Biosystems TaqMan Pre-Designed SNP Genotyping assays (*CALCRL* rs696574 assay ID: C___8726655_10; *CALCRL* rs6710852 assay ID: C_189160430_10;

CALCRL rs3213738 assay ID: C__27470324_10; *RAMP1* rs302680 assay ID: C___1071215_20; *RAMP1* rs13386048 assay ID: C__31241845_10; *RAMP1* rs12995100 assay ID: C__31241852_10; *RAMP1* rs12465864 assay ID: C__11739774_10; *RAMP1* rs7590387 assay ID: C__26481962_10; *RAMP1* rs75822777 assay ID: C_101309358_10; *RAMP1* rs302676 assay ID: C___1071223_30; *RAMP1* rs11673847 assay ID: C_176017176_10; *RAMP1* rs6431564 assay ID: C___2149740_10; *RAMP1* rs4663269 assay ID: C___2149726_10; *RAMP1* rs7603344 assay ID: C__11739137_10; *RAMP1* rs7578855 assay ID: C__31241858_10). Genotyping was performed blinded to all clinical data.

Statistical analysis

Categorical variables are reported as absolute (*n*) and relative frequency (%), whereas continuous variables are presented as mean with SD. To compare differences of clinical variables between the two patient groups (HIT-6 NRESP vs. HIT-6 RESP), the Student *t*-test was applied for continuous variables with equal variances and the Welch *F*-test for those with unequal variances. The chi-squared test was used for assessing differences in the distribution of categorical variables. Clinical variables with a *p*-value <0.1 from univariate logistic analyses were included in multivariate logistic regression models to identify factors independently associated with HIT-6 RESP status. The association between SNPs and HIT-6 RESP was assessed by logistic regression analysis assuming an additive genetic model of inheritance (i.e., each variant allele has an equal contribution to the outcome). To this end, genotypes from each SNP were coded as 0, 1, or 2 according to the number of variant alleles, and each SNP was modeled as a continuous variable. Then, a genetic risk score (GRS) was constructed as an unweighted score, calculated on the basis of total number of risk alleles of being HIT-6 NRESP at SNPs found to be significant in the logistic regression analysis adjusted by confounding clinical variables (cutoff of $p < 0.1$ from respective univariate analyses). All statistical analyses were performed using MedCalc version 13.3.3 (MedCalc Software). Given the exploratory nature of this study, we reported nominal statistical associations ($p < 0.05$). Adjusted *p*-values based on the Bonferroni correction were also considered to avoid chance findings due to multiple testing of 16 comparisons (15 SNPs and one GRS), and the significance was lowered to $p < 0.0031$ (i.e., 0.05/16).

Standard protocol approvals, registrations, and patient consents

The study conformed with the World Medical Association Declaration of Helsinki and was approved by the local ethics committees of the participating centers (Cantonal Ethics Committee Bern, Comitato Etico Canton Ticino [lead], BASEC 2019-01393). Written informed consent to use clinical data was obtained from all participants. This study is registered in Registry of all Projects in Switzerland and the study registry of Ente Cantonale Ospedaliero, Ticino, Switzerland (ID19-54).

RESULTS

One hundred thirteen patients were screened, and 110 patients (91 [82.7%] females, 55 [50%] with chronic migraine) were included and treated with ERE 70 mg monthly. Tables 1 and 2 report the characteristics of study participants stratified according to ERE responder status.

At Month 3, 58 (52.7%) patients had an improvement of ≥8 points and were classified as HIT-6 RESP, whereas 52 (47.3%) had an improvement of <8 points and were classified as HIT-6 NRESP. Compared to HIT-6 NRESP, at Month 3 HIT-6 RESP had greater therapeutic benefits in terms of absolute mean number of MMDs (5.2 [4.1] vs. 13.7 [9.3], $p < 0.0001$), reduction in mean number of

TABLE 1 Univariate association analysis of continuous variables with HIT-6 score reduction of ≥ 8

Clinical variable	HIT-6 score reduction <8, *n* = 52	HIT-6 score reduction ≥8, *n* = 58	*p*
Age, years, mean (SD)	47.6 (14.7)	46.4 (13.0)	0.672
Age at migraine onset, years, mean (SD), *n* = 107	18.7 (10.4)	16.9 (8.5)	0.338
BMI, kg/m^2, mean (SD), *n* = 109	24.3 (5.0)	23.1 (3.5)	0.146
Failed preventive medications, *n* (%)	4.6 (3.2)	3.8 (2.2)	0.163
First-degree relatives with migraine, *n* (%)	1.1 (1.0)	1.3 (1.4)	0.415
Attack duration, baseline, h, mean (SD), *n* = 109	20.7 (26.7)	22.8 (25.9)	0.679
MIDAS, baseline score, mean (SD)	64.0 (55.4)	68.9 (58.3)	0.649
Pain intensity, baseline score, mean (SD)	7.7 (1.5)	8.1 (1.5)	0.191
Monthly days with triptan use, baseline, mean (SD)	6.5 (8.3)	7.4 (7.2)	0.532
Monthly days with use of nontriptan analgesics, baseline, mean (SD)	10.7 (11.2)	6.2 (7.2)	**0.015**

Note: Probability value in bold is statistically significant.

Abbreviations: BMI, body mass index; HIT-6, Headache Impact Test-6; MIDAS, Migraine Disability Assessment Scale.

TABLE 2 Univariate association analysis of categorical variables with HIT-6 score reduction of ≥ 8

Clinical variable	HIT-6 score reduction < 8, *n* (%)	HIT-6 score reduction ≥ 8, *n* (%)	*p*
Gender			
Female	41 (78.8)	50 (86.2)	0.443
Male	11 (21.2)	8 (13.8)	
Menopause in women, *n* = 91			
Absent	24 (58.5)	35 (70.0)	0.358
Present	17 (41.5)	15 (30.0)	
Pregnancy, *n* = 91			
No	15 (36.6)	23 (46.0)	0.489
Yes	26 (63.4)	27 (54.0)	
Working status			
Employed	29 (55.8)	41 (70.7)	0.102
Unemployed	16 (30.8)	15 (25.9)	
Retired	7 (13.3)	2 (3.4)	
Smoking status			
Never	27 (51.9)	34 (58.6)	0.605
Past	11 (21.2)	13 (22.4)	
Current	14 (26.9)	11 (19.0)	
Alcohol intake, *n* = 107			
No	28 (54.9)	29 (51.8)	0.898
Yes	23 (45.1)	27 (48.2)	
Physical activity, *n* = 109			
Absent	33 (63.5)	27 (47.4)	0.135
Present	19 (36.5)	30 (52.6)	
Civil status			
Single	15 (28.8)	15 (25.9)	0.872
Married	27 (51.9)	33 (56.9)	
Other	10 (19.2)	10 (17.2)	
Insomnia			
Absent	24 (46.2)	25 (43.1)	0.673
Present + medication	14 (26.9)	13 (22.4)	
Present − medication	14 (26.9)	20 (34.5)	
Snoring			
Absent	37 (71.2)	38 (65.5)	0.668
Present	15 (28.8)	20 (34.5)	
Anxiety			
Absent	21 (40.4)	28 (48.3)	0.523
Present	31 (59.6)	30 (51.7)	
Depression			
Absent	18 (34.6)	29 (50.0)	0.151
Present	34 (65.4)	29 (50.0)	
Chronic pain			
Absent	39 (75.0)	44 (75.9)	0.907
Present	13 (25.0)	14 (24.1)	
Hypertension			
Absent	37 (71.2)	53 (93.0)	**0.006**
Present	15 (28.8)	4 (7.0)	

TABLE 2 (Continued)

Clinical variable	HIT-6 score reduction < 8, *n* (%)	HIT-6 score reduction ≥ 8, *n* (%)	*p*
Other comorbidities			
Absent	32 (61.5)	36 (62.1)	0.889
Present	20 (38.5)	22 (37.9)	
Head trauma, *n* = 109			
Absent	46 (90.2)	47 (81.0)	0.281
Present	5 (9.8)	11 (19.0)	
Migraine form			
Episodic	19 (36.5)	25 (43.1)	0.612
Chronic	33 (63.5)	33 (56.9)	
Current therapy			
No	11 (21.2)	19 (32.8)	0.250
Yes	41 (78.8)	39 (67.2)	
Aura, *n* = 109			
Absent	33 (63.5)	39 (68.4)	0.731
Present	19 (36.5)	18 (31.6)	
Use of triptans			
No	21 (40.4)	13 (22.4)	0.067
Yes	31 (59.6)	45 (77.6)	
Medication overuse			
No	26 (50.0)	33 (56.9)	0.594
Yes	26 (50.0)	25 (43.1)	

Note: Probability value in bold is statistically significant.
Abbreviation: HIT-6, Headache Impact Test-6.

MMDs versus baseline (10.3 [7.4] vs. 6.0 [9.7], $p < 0.001$), monthly days with triptan and nontriptan analgesic use (2.7 [3.5] vs. 5.0 [6.8], $p = 0.033$ and 3.4 [4.4] vs. 7.4 [8.9], $p = 0.005$, respectively), and proportion of subjects with chronic migraine and with medication overuse (1.7 vs. 40.4%, $p < 0.0001$ and 3.4 vs. 32.7%, $p = 0.0001$, respectively).

Factors associated with response to ERE therapy

At univariate analysis, baseline monthly days with use of nontriptan analgesics (HIT-6 RESP: 6.2 ±7.2 vs. HIT-6 NRESP: 10.7 ±9.5 $p = 0.015$) and comorbid arterial hypertension (HIT-6 RESP: 4 [7%] vs. HIT-6 NRESP: 15 [28.8%] $p = 0.006$) were associated with the HIT-6 responder status (Tables 1 and 2). When including these in a multivariate logistic regression model, only comorbid arterial hypertension maintained association (HIT-6 RESP: odds ratio [OR] =0.160, 95% confidence interval [CI] =0.047–0.548, $p = 0.003$; Table 3).

At univariate logistic regression analysis, minor alleles of three SNPs at *CALCRL*, rs696574T, rs6710852G, and rs3213738C, were found to confer an increased probability of being HIT-6 RESP, whereas minor alleles of two SNPs at *RAMP1*, rs13386048A and rs12465864G, decreased this likelihood (for respective crude ORs and 95% CIs, see Table 4).

TABLE 3 Multivariate logistic regression analysis of clinical factors in predicting Headache Impact Test-6 score reduction of ≥ 8

Clinical variable	OR (95% CI)	*p*
Monthly days with use of nontriptan analgesics before ERE start	0.949 (0.898–1.002)	0.059
Hypertension		
Absent	1 (ref)	
Present	0.160 (0.047–0.548)	**0.003**
Use of triptans		
No	1 (ref)	
Yes	1.445 (0.504–4.138)	0.493

Note: Multivariate logistic regression analysis of clinical variables with a significance level of $p < 0.1$ at the respective univariate analysis. Probability value in bold is statistically significant.
Abbreviations: CI, confidence interval; ERE, erenumab; OR, odds ratio; ref, reference.

After adjustments for clinical confounders, *CALCRL* rs6710852G allele was confirmed to confer an increased probability of being HIT-6 RESP compared to rs6710852T (for each G allele, OR = 2.82, 95% CI = 1.03–7.73, $p = 0.043$; Table 4). Conversely, *RAMP1* rs13386048A and *RAMP1* rs12465864G alleles decreased the probability of HIT-6 RESP status compared to *RAMP1* rs13386048G and

TABLE 4 Association analysis of SNPs with HIT-6 score reduction of ≥ 8

SNP	HIT-6 score reduction ≥ 8, *n* (%)	HIT-6 score reduction < 8, *n* (%)	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)[a]	*p*
CALCRL rs696574						
CC	36 (62.8)	42 (80.8)	2.24 (1.04–4.81)	**0.039**	1.85 (0.82–4.16)	0.139
TC	19 (32.8)	9 (17.3)				
TT	3 (5.2)	1 (1.9)				
CALCRL rs6710852						
TT	40 (69.0)	46 (88.5)	3.37 (1.28–8.92)	**0.014**	2.82 (1.03–7.73)	**0.043**
TG	16 (27.6)	6 (11.5)				
GG	2 (3.4)	0 (0)				
CALCRL rs3213738						
TT	43 (74.1)	46 (88.5)	2.67 (1.01–7.05)	**0.047**	2.37 (0.86–6.54)	0.095
TC	13 (22.4)	6 (11.5)				
CC	2 (3.4)	0 (0)				
RAMP1 rs302680						
AA	43 (74.1)	40 (76.9)	1.19 (0.56–2.51)	0.654	1.19 (0.52–2.73)	0.679
GA	13 (24.2)	11 (21.2)				
GG	2 (3.4)	1 (1.9)				
RAMP1 rs13386048						
GG	29 (50.0)	16 (30.8)	0.55 (0.32–0.97)	**0.037**	0.53 (0.28–0.98)	**0.042**
GA	23 (39.7)	26 (50.0)				
AA	6 (10.3)	10 (19.2)				
RAMP1 rs12995100						
TT	10 (17.2)	13 (25.0)	1.56 (0.86–2.82)	0.143	1.51 (0.80–2.87)	0.205
TC	33 (56.9)	31 (59.6)				
CC	15 (25.9)	8 (15.4)				
RAMP1 rs12465864						
AA	44 (75.9)	28 (53.8)	0.42 (0.20–0.88)	**0.021**	0.32 (0.13–0.75)	**0.009**
AG	13 (22.4)	22 (42.3)				
GG	1 (1.7)	2 (3.8)				
RAMP1 rs7590387						
CC	19 (32.8)	16 (30.8)	0.72 (0.42–1.21)	0.216	0.68 (0.38–1.22)	0.198
GC	31 (53.4)	21 (40.4)				
GG	8 (13.8)	15 (28.8)				
RAMP1 rs75822777						
GG	28 (48.3)	25 (48.1)	1.16 (0.65–2.07)	0.622	0.74 (0.38–1.43)	0.371
GA	23 (39.7)	24 (46.2)				
AA	7 (12.1)	3 (5.8)				
RAMP1 rs302676						
TT	40 (69.0)	27 (51.9)	0.53 (0.26–1.10)	0.090	0.54 (0.25–1.19)	0.129
TC	17 (29.3)	24 (46.2)				
CC	1 (1.7)	1 (1.9)				
RAMP1 rs11673847						
GG	37 (63.8)	36 (69.2)	1.12 (0.56–2.27)	0.75	1.31 (0.59–2.89)	0.504
GA	20 (34.5)	14 (26.9)				
AA	1 (1.7)	2 (3.8)				

TABLE 4 (Continued)

SNP	HIT-6 score reduction ≥ 8, *n* (%)	HIT-6 score reduction < 8, *n* (%)	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)[a]	*p*
RAMP1 rs6431564						
AA	14 (26.9)	13 (22.4)	1.33 (0.74–2.40)	0.304	2.10 (1.05–4.22)	**0.037**
AG	31 (59.6)	33 (56.9)				
GG	7 (13.5)	12 (20.7)				
RAMP1 rs4663269						
TT	12 (23.1)	13 (22.4)	1.15 (0.64–2.09)	0.625	1.19 (0.63–2.24)	0.586
TC	32 (61.5)	33 (56.9)				
CC	8 (15.4)	12 (20.7)				
RAMP1 rs7603344						
AA	26 (50.0)	29 (50.0)	0.89 (0.47–1.66)	0.710	0.84 (0.42–1.65)	0.608
AG	22 (42.3)	27 (46.6)				
GG	4 (7.7)	2 (3.4)				
RAMP1 rs7578855						
TT	20 (38.5)	24 (41.4)	0.73 (0.43–1.25)	0.252	0.71 (0.40–1.27)	0.249
CT	20 (38.5)	28 (48.3)				
CC	12 (23.1)	6 (10.3)				

Note: Association analysis of SNPs was performed by using the additive genetic model. Probability values in bold are statistically significant.
Abbreviations: CI, confidence interval; HIT-6: Headache Impact Test-6; OR, odds ratio; SNP, single nucleotide polymorphism.
[a]Logistic regression analysis adjusted by monthly days with use of nontriptan analgesics before erenumab start, hypertension, and use of triptans.

RAMP1 rs12465864A, respectively (for each rs13386048A allele, OR = 0.53, 95% CI = 0.28–0.98, $p = 0.042$; for each rs12465864G allele, OR = 0.32, 95% CI = 0.13–0.75, $p = 0.009$; Table 4). Additionally, the *RAMP* rs6431564G allele, which was found nonsignificant in unadjusted analysis, after correction for clinical confounders was found to significantly increase the probability of being HIT-6 RESP compared to *RAMP* rs6431564A (for each G allele, OR = 2.10, 95% CI = 1.05–4.22, $p = 0.037$; Table 4).

GRS analysis

To evaluate the cumulative effects of SNPs on the association with the HIT-6 responder status, we built a GRS based on total number of alleles conferring an increased risk of being HIT-6 NRESP (risk alleles) at the four SNPs found to be significant in the adjusted logistic regression analysis (i.e., *CALCRL* rs6710852T, *RAMP1* rs13386048A, *RAMP1* rs12465864G, and *RAMP1* rs6431564A). The proportion of HIT-6 RESP for each score group showed a decreasing trend (p for chi-squared trend = 0.0008) from lower to higher sum risk scores: 100% (score = 1), 70.0% (score = 2), 63.3% (score = 3), 51.7% (score = 4), 42.1% (score = 5), 29.4% (score = 6), 0% (score = 7; Figure 1). None of the migraine patients was found to carry 0 or 8 risk alleles for HIT-6 NRESP. In the multivariate analysis adjusted for clinical confounders (Table 5), GRS was found to be an independent predictor of HIT-6 RESP (OR = 0.49, 95% CI = 0.33–0.72, $p = 0.0003$). The association of GRS was significant even after correction for multiple testing (threshold p-value for Bonferroni correction = 0.0031). In addition, arterial hypertension remained independently associated with HIT-6 RESP (presence vs. absence, OR = 0.09, 95% CI = 0.02–0.38, $p = 0.001$).

DISCUSSION

Migraine is a complex disorder strongly routed on a genetic predisposition, with an estimated heritability of 40%–60% and 123 genomic loci modulating migraine risk identified so far [19]. It is therefore conceivable that interindividual variability in treatment response commonly seen among migraine patients in clinical practice and pharmacological studies also relies on genetic heterogeneity [15].

In recent years, great interest has been focused on CGRP pathway polymorphisms as risk factors for migraine susceptibility; however, little is known about the clinical relevance of these polymorphisms and their effect on the response to antimigraine treatment [20]. In line with this hypothesis, our main finding is that selected SNPs at *CALCRL* and *RAMP1* genes modulate response to ERE 70 mg. Particularly, *CALCRL* rs6710852G and *RAMP* rs6431564G minor alleles respectively conferred three and two times higher probability of being HIT-6 RESP compared to the corresponding major alleles. Conversely, minor alleles rs13386048A and rs12465864G in the *RAMP1* gene each decreased this likelihood by approximately 50%. No data are currently available on the association of these four intronic SNPs with migraine susceptibility, or on whether these may exert a role in regulating gene expression, for instance, by influencing

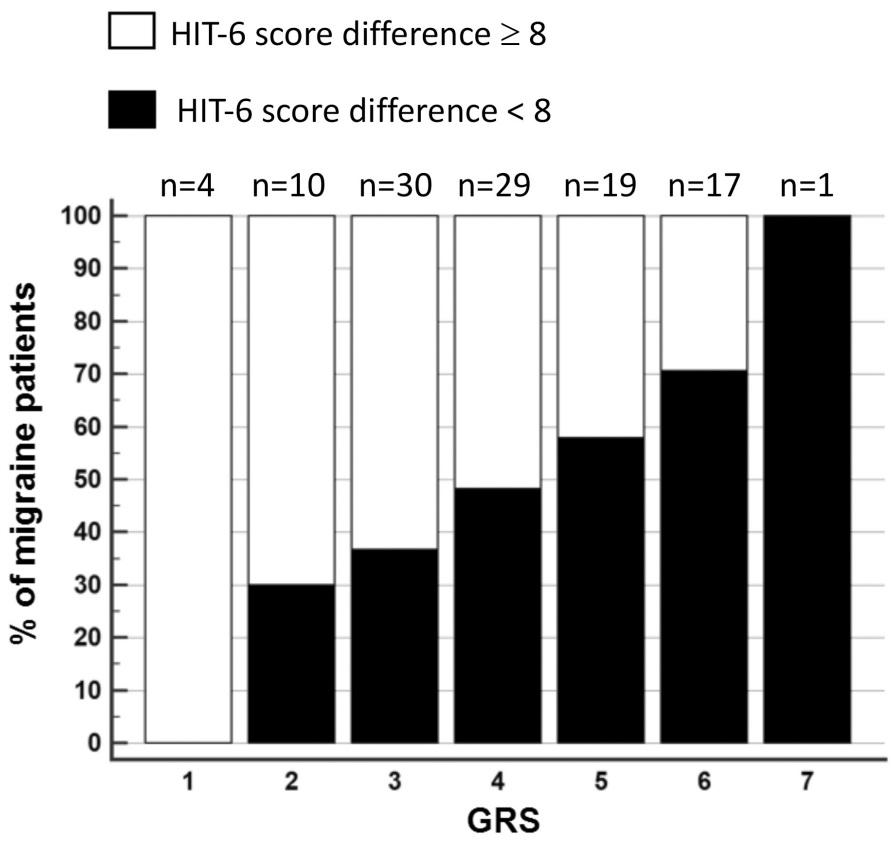

FIGURE 1 Genetic risk score (GRS) stratified according to Headache Impact Test-6 (HIT-6) score difference of ≥8 and <8

TABLE 5 Multivariate logistic regression analysis of clinical factors and GRS in predicting HIT-6 score reduction of ≥ 8

Variable	OR (95% CI)	*p*
Monthly days with use of non-triptan analgesics before ERE start	0.95 (0.90–1.01)	0.101
Hypertension		
Absent	1 (ref)	
Present	0.09 (0.02–0.38)	**0.001**
Use of triptans		
No	1 (ref)	
Yes	2.45 (0.74–8.06)	0.140
GRS, per unit increase[a]	0.49 (0.33–0.72)	**0.0003**

Note: Probability values in bold are statistically significant.

Abbreviations: CI, confidence interval; ERE, erenumab; GRS, genetic risk score; HIT-6, Headache Impact Test-6; OR, odds ratio; ref, reference.

[a]Based on total number of risk alleles (i.e. *CALCRL* rs6710852T, *RAMP1* rs13386048A, *RAMP1* rs12465864G and *RAMP1* rs6431564A).

splicing or regulatory processes. On the other hand, these SNPs may be in linkage with functional polymorphisms (as yet unidentified), that are the causative genetic determinants for response to ERE. Previous findings highlighted that merging multiple single genetic variants with minor effects into a GRS can increase power and reduce bias of genetic association studies [21, 22]. Therefore, we constructed a GRS based on SNPs at *CALCRL* and *RAMP1* loci to estimate the cumulative contribution of risk alleles, that is, those associated with nonresponder status, which was found to be independently associated with HIT-6 RESP status. Specifically, the cumulative score of risk alleles at the four SNPs (i.e., *CALCRL* rs6710852, *RAMP1* rs13386048, *RAMP1* rs12465864, and *RAMP1* rs6431564) was found to inversely associate with HIT-6 RESP. In other words, the higher the number of risk alleles for being HIT-6 NRESP, the lower the probability of a clinical meaningful improvement in HIT-6 total score. It is noteworthy that the GRS remained significant after multiple comparison correction, a result in line with the notion of a higher statistical power of the GRS approach than the single-SNPs analysis method [21–23].

The second main finding of our study is that migraine patients with arterial hypertension had a >90% reduction in the probability of responding to ERE, compared to migraine subjects not affected by arterial hypertension. This association has not been highlighted by other studies so far and needs confirmation by other studies, particularly due to the small sample size of the present one [5, 24].

Because CGRP is a potent physiological vasodilator widely represented in the human body, potential cardiovascular side effects including arterial hypertension represented a major concern with anti-CGRP treatment strategies. In the postmarketing setting, ERE was found to be associated with an increased risk of new onset or worsening arterial hypertension [25], which has now been included among warnings on the ERE label by the US Food and Drug Administration (FDA) [26]. Additionally, an increase in both systolic and diastolic blood pressure was recently reported in a large population of patients treated with ERE or fremanezumab over 1 year, and the effect was evident from the first follow-up measure 3 months after treatment start [27].

Unfortunately, blood pressure values were not systematically collected during the present study, which was conducted before the FDA label change [26]. At the moment, we can only speculate that ERE might have interfered with arterial blood pressure regulation by a direct effect on vessels or through an interaction with antihypertensive treatments. Consequent worsening of preexisting hypertension might have in turn worsened migraine and/or favored a concomitant component of headache attributable to arterial hypertension. Also, we cannot exclude ERE-unrelated contributing factors, such as insufficient treatment of arterial hypertension and/or antihypertensive drugs' side effects favoring headache. Interestingly, other relevant comorbid conditions such as chronic pain, anxiety, and depression failed to show association with poor ERE response in our analysis, further suggesting a specific mechanism related to arterial hypertension.

Reduction in the frequency of MMDs represents an important measure in the efficacy of migraine prophylaxis. However, as frequency is only one among diverse migraine facets, MMDs might suboptimally capture migraine impact on an individual. Accordingly, various clinical and regulatory guidelines increasingly encourage the use of PRO tools to monitor migraine treatment [28]. The HIT-6 is a widely used PRO measure and has been appointed by the American Headache Society as one of the three most relevant tools for assessing migraine prophylaxis benefits. Registration trials showed a benefit of ERE on various migraine-specific PRO measures including HIT-6 [6, 7, 9, 29]. Interestingly, a recent paper found that in these trials, PRO measures indicated better migraine-related quality of life in individuals treated with ERE compared to those receiving placebo and having the same number of MMDs [30]. This strongly supports the existence of treatment benefits beyond MMD reduction that translate into improvements in health-related quality of life. Our findings support this line of evidence. Actually, the present study shows that specific allelic variants at *CALCRL* and *RAMP* genes and comorbid arterial hypertension are associated with treatment response defined as a meaningful improvement on the HIT-6 score. This was a post hoc analysis of another study in which instead we could not identify any clinical or genetic factors associated with response to ERE in terms of 50% reduction in MMDs. We believe this result was driven by the use of an outcome measure exclusively focused on migraine frequency, which likely neglected other important migraine features, thus masking clinically relevant treatment effect modifiers. Importantly, we used a rather conservative definition of HIT-6 responders (i.e., improvement by at least 8 points), and HIT-6 RESP also showed converging benefits on various outcome measures including MMDs, use of acute treatments, and the proportion of subjects with chronic migraine and medication overuse.

Our study is not without limitations. Our post hoc analyses require replication in new, larger studies with different populations of migraine patients and control groups for potential confounding. However, the association of the GRS and arterial hypertension with the responder status survived adjustment for confounders and correction for multiple comparisons in a rather small population, compatible with a clinically relevant modulation effect. We also acknowledge that our GRS modeling did not weigh the effect size of the different risk alleles, as it was based on their presence or absence. In addition, the lack of an independent cohort of ERE-treated patients precluded the possibility of validating the developed GRS as a predictor of HIT-6 score response. On the other hand, our pharmacogenetic study was not designed to assess the role of the investigated SNPs as risk factors for migraine susceptibility; therefore, we cannot exclude that genotype or allele frequencies of some of the SNPs investigated may differ between migraineurs and control subjects. This important issue deserves further investigation in large case–control genetic studies. Also, arterial hypertension diagnosis was based on patients' medical history, and blood pressure was not monitored during our study, thus preventing us from better understanding the mechanisms by which arterial hypertension was found to be a risk factor for poorer response to ERE. ERE was used at the dose of 70 mg monthly for 3 months, thus possibly underestimating responders to ERE 140 mg monthly, and importantly, responders after longer treatment periods, also according to recently updated European Headache Association guidelines [31].

In conclusion, our study found that response to ERE treatment as measured by a meaningful improvement in migraine-related functional disability on the HIT-6 is modulated by specific allelic variants at *CALCRL* and *RAMP* genes and by comorbid arterial hypertension. Although a more comprehensive analysis of CGRP pathway polymorphisms should be conducted in future studies to develop and validate a clinically useful genetically based model for prediction of ERE response, our results highlight that the GRS approach may be an effective tool to investigate the impact of the genetic background on migraine treatment response. If appropriately confirmed, our results will likely have major clinical and research implications.

ACKNOWLEDGMENTS

We would like to thank Liliane Petrini for helping with the manuscript submission.

CONFLICT OF INTEREST

The authors have no competing interests. C.Z. is the recipient of a grant for senior researchers provided by Area Formazione Accademica, Ricerca e Innovazione, Ente Ospedaliero Cantonale. C.Z.'s and C.G.'s employer, Ente Ospedaliero Cantonale, has received compensation for their speaking activities, consulting fees, or research grants from Almirall, Biogen Idec, Bristol Myers Squibb, Lundbeck, Merck, Novartis, Sanofi, Teva Pharma, and Roche. C.J.S.'s employer, Inselspital, Bern University Hospital, has received compensation for his speaking activities, consulting fees, or research grants from his participation in consulting activities, on advisory boards, and as a speaker for Novartis, Eli Lilly, Teva Pharmaceuticals, Allergan, Almirall, Amgen, Lundbeck, MindMed, and Grünenthal. He is a part-time employee at Zynnon.

DATA AVAILABILITY STATEMENT

Individual deidentified participant data will be shared on reasonable request by professionals in this field. Data used for the statistical analysis may be received from the statistician on request.

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How to cite this article: Zecca C, Terrazzino S, Para D, et al. Response to erenumab assessed by Headache Impact Test-6 is modulated by genetic factors and arterial hypertension: An explorative cohort study. *Eur J Neurol*. 2023;30:1099-1108. doi:10.1111/ene.15678