Zecca Chiara (Orcid ID: 0000-0002-9990-3431)
Viana Michele (Orcid ID: 0000-0003-4356-8109)
Gobbi Claudio (Orcid ID: 0000-0002-7554-0664)

Response to erenumab assessed by HIT-6 is modulated by genetic factors and arterial hypertension - an explorative cohort study

Chiara Zecca MD[1,2]; Salvatore Terrazzino MD[3], Davide Para MD[1]; Giovanna Campagna MD[1], Michele Viana MD[1,4]; Christoph J. Schankin MD[5]; Claudio Gobbi MD[1,2]

[1] Department of Neurology, Neurocenter of Southern Switzerland, Ospedale Regionale di Lugano, Ente Ospedaliero Cantonale (EOC), Lugano, Switzerland;

[2] Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano, Switzerland;

[3] Department of Pharmaceutical Sciences and Interdepartmental Research Center of Pharmacogenetics and Pharmacogenomics (CRIFF), University of Piemonte Orientale "A. Avogadro", Novara, Italy;

[4] Headache Group, Department of Basic and Clinical Neurosciences, King's College London, London, UK;

[5] Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern Switzerland

Corresponding Author

Prof. Chiara Zecca

chiara.zecca@eoc.ch

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AUTHOR CONTRIBUTIONS

Chiara Zecca

Salvatore Terrazzino

Davide Para

Giovanna Campagna

Michele Viana

Christoph J. Schankin

Claudio Gobbi

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CONFLICT OF INTEREST STATEMENT

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ABSTRACT

Background: Response predictors to erenumab (ERE) in migraine patients would benefit their clinical management. We investigate associations between patients' clinic characteristics and polymorphisms at CALCRL and RAMP1 genes and response to ERE treatment measured as clinically meaningful improvement of the headache impact test 6 (HIT-6) score.

Methods: Post-hoc analysis of a prospective, multicenter, investigator-initiated study involving 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 calcitonin receptor-like receptor and receptor activity-modifying protein-1 genes were determined using Real-time PCR. Logistic regression models identified independent predictors for response to ERE, defined as HIT-6 score improvement ≥8 points (HIT-6 responders [HIT-6RESP], vs. HIT-6 non-responders [HIT-6NRESP]).

Results: At month 3, 58 (52.7%) patients were HIT-6RESP. Comorbid hypertension predicted a lower probability of being HIT-6RESP [OR (95%CI] 0.160 (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-6RESP [for each G allele: OR (95%CI): 2.82(1.03-7.73), p=0.043; OR 95%CI): 2.10(1.05-4.22), p=0.037]. RAMP1 rs13386048A and RAMP1 rs12465864G decreased this probability [for each rs13386048A, OR (95%CI): 0.53(0.28-0.98), p=0.042; for each rs12465864G, OR(95%CI): 0.32(0.13-0.75), p=0.009). A genetic risk score based on the presence and number of identified risk alleles resulted independently associated with HIT-6RESP (OR, 0.49; 95%CI, 0.33-0.72; p= 0.0003) surviving Bonferroni's correction.

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

Keywords: erenumab; hypertension; migraine; patients reported outcomes, treatment response

INTRODUCTION

Migraine is a highly prevalent neurological disorder with genetic predisposition representing the second 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 favourable 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, 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 (MMD) or responder rate) may not exhaustively inform about the effectiveness of preventive drugs in real-life clinical setting [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 (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 at investigating in a cohort of 110 episodic and chronic migraine patients clinic and genetic factors associated with response to ERE, based on the detection of clinically meaningful improvements in 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, with at least 8 documented days with migraine per month for at least 3 months and failure, intolerability or contraindication to at least 2 migraine preventive therapies approved in Switzerland, that were started with ERE according to the clinical judgement 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, or having contraindications to ERE, including uncontrolled arterial hypertension.

Patients were evaluated at first ERE 70 mg injection and 3 months thereafter, meanwhile continuing to fill in a headache diary. Socio-demographic 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/non-steroidal analgesics 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 Headache Impact test (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 last month headache-related pain was severe, headaches impacted daily activities or 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 that improved by at least 8 points in 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, being the median value of HIT-6 score changes in our cohort of migraine patients 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 clinic and genetic profiles of HIT-6 RESP (responders) differ from those of HIT-6 NRESP (non-responders). 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 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 (MAF) of more than 10%. Genotyping of CALCRL and RAMP1 polymorphisms was performed by real-time PCR 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 frequencies (%), while continuous variables are presented as means with standard deviation (SD). In order to compare differences of clinical variables between the two patient groups (HIT-6NRESP vs HIT-6RESP), the Student's t-test was applied for continuous variables with equal variances and the Welch's 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 withHIT-6RESP status. The association between SNPs and HIT-6RESP 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 modelled 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-6NRESP at SNPs found to be significant in the logistic regression analysis adjusted by confounding clinical variables (cut-off of $p< 0.1$ from respective univariate analyses). All statistical analyses were performed using MedCalc Version 13.3.3 (MedCalc Software, Mariakerke, Belgium). 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 1 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 (RAPS) and the study registry of Ente Cantonale Ospedaliero, Ticino, Switzerland (ID19-54).

Data Availability

Individual de-identified 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.

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-6RESP, whereas 52 (47.3%) had an improvement of <8 points and were classified as HIT-6NRESP. Compared to HIT-6NRESP, at month 3 HIT-6RESP 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 MMDs vs baseline [10.3 (7.4) vs 6.0 (9.7), p<0.001], monthly days with triptan and non-triptan analgesics 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], 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 non-triptan analgesics (HIT-6RESP: 6.2±7.2 vs HIT-6 NRESP: 10.7±9.5 p=0.015) and comorbid arterial hypertension (HIT-6RESP: 4 [7%] vs HIT-6NRESP: 15 [28.8%] p=0.006) were associated with the HIT-6 responder status (Table 1 and Table 2). When including these in a multivariate logistic regression model, only comorbid arterial hypertension maintained association (HIT-6RESP: OR [95%CI] 0.160 [0.047-0.548], p=0.003) (Table 3).

At univariate logistic regression analysis, minor alleles of three SNPs at CALCRL, including rs696574T, rs6710852G and rs3213738C, were found to confer an increased probability of being HIT-6RESP, while minor alleles of two SNPs at RAMP1, including rs13386048A and rs12465864G, decreased this likelihood (respective crude OR [95%CI], Table 4).

After adjustments for clinical confounders, CALCRL rs6710852G allele was confirmed to confer an increased probability of being HIT-6RESP compared to rs6710852T [for each G allele, OR (95%CI): 2.82 (1.03-7.73), p=0.043, Table 4]. Conversely, RAMP1 rs13386048A and RAMP1 rs12465864G alleles decreased the probability of HIT-6RESP status compared to RAMP1 rs13386048G and RAMP1 rs12465864A, respectively [for each rs13386048A allele, OR (95%CI): 0.53 (0.28-0.98), p=0.042; for each rs12465864G allele, OR (95%CI): 0.32 (0.13-0.75), p=0.009, Table 4). Additionally, the RAMP rs6431564G allele, which resulted non-significant at unadjusted analysis, after correction for clinical confounders was found to significantly increase the probability of being HIT-6RESP compared to RAMP rs6431564A [for each G allele, OR (95%CI): 2.10 (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-6NRESP (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-6RESP for each score group showed a decreasing trend (p for Chi-square 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's correction= 0.0031). In addition,

arterial hypertension remained independently associated with HIT-6RESP [presence vs absence, OR (95%CI): 0.09 (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 anti-migraine 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 each 3 and 2 times higher probability of being HIT-6RESP compared to the corresponding major alleles. Conversely, minor alleles rs13386048A and rs12465864G in the RAMP1 gene decreased by approximately 50% each this likelihood. No data are currently available on the association of these four intronic SNPs with migraine susceptibility, or if these may exert a role in regulating gene expression, for instance, by influencing 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 genetic risk score (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, i.e. those associated with a non-responder 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-6RESP. In other words, the higher the number of risk alleles of being HIT6 NRESP, the lower the probability of a clinical meaningful improvement in HIT-6 total score. Noteworthy, the GRS remained significant after multiple comparisons 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 more than 90% reduction in the probability to respond 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].

Since 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 post-marketing 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 in the ERE label by FDA [26]. Additionally, an increase in both systolic and diastolic blood pressure was recently reported in a large population of patients treated with erenumab or fremanezumab over one 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 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 anti-hypertensive treatments. Consequent worsening of pre-existing hypertension might have in turn worsened migraine and/or favoured 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 anti-hypertensive 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 just 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 3 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 MMDs reduction which 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 in the HIT6 score. This was a post-hoc analysis of another study in which instead we could not identify any clinic or genetic factors associated with response to ERE in terms of 50% reduction in MMDs. We believe this result was in fact 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-6RESP also showed converging benefits on various outcome measures including MMD, 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 modelling did not weight 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 patient's medical history and blood pressure was not monitored during our study thus preventing us from better understanding the mechanisms by which arterial hypertension resulted to be a risk factor for poorer response to ERE. ERE was used at the dose of 70 mg monthly for three 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 by HIT6 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 genetic-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.

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Figure caption:

Fig. 1 Genetic risk score stratified according to HIT-6 score difference ≥8 and <8

GRS: genetic risk score.

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

Clinical variable	**HIT-6 score reduction <8 Mean (SD) N= 52**	**HIT-6 score reduction ≥8 Mean (SD) n= 58**	**p-value**
Age, years (SD)	47.6 (14.7)	46.4 (13.0)	0.672
Age at migraine onset, (n=107), years (SD)	18.7 (10.4)	16.9 (8.5)	0.338
BMI (n=109), kg/m^2 (SD)	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 (n=109), hours (SD)	20.7 (26.7)	22.8 (25.9)	0.679
MIDAS, baseline, score (SD)	64.0 (55.4)	68.9 (58.3)	0.649
Pain intensity, baseline, score (SD)	7.7 (1.5)	8.1 (1.5)	0.191
Monthly days with triptan use, baseline, days (SD)	6.5 (8.3)	7.4 (7.2)	0.532
Monthly days with use of non-triptan analgesics, baseline, days (SD)	10.7 (11.2)	6.2 (7.2)	**0.015**

p-value in bold is statistically significant. HIT-6: Headache Impact Test-6 (HIT-6). SD, standard deviation.

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-value
Gender, n (%)			0.443
Female	41 (78.8)	50 (86.2)	
Male	11 (21.2)	8 (13.8)	
Menopause in women (n=91), n (%)			0.358
Absent	24 (58.5)	35 (70.0)	
Present	17 (41.5)	15 (30.0)	
Pregnancy (n=91), n (%)			0.489
No	15 (36.6)	23 (46.0)	
Yes	26 (63.4)	27 (54.0)	
Working status, n (%)			0.102
Employed	29 (55.8)	41 (70.7)	
Unemployed	16 (30.8)	15 (25.9)	
Retired	7 (13.3)	2 (3.4)	
Smoking status, n (%)			0.605
Never	27 (51.9)	34 (58.6)	
Past	11 (21.2)	13 (22.4)	
Current	14 (26.9)	11 (19.0)	
Alcohol intake (n=107), n (%)			0.898
No	28 (54.9)	29 (51.8)	
Yes	23 (45.1)	27 (48.2)	
Physical activity (n=109), n (%)			0.135
Absent	33 (63.5)	27 (47.4)	
Present	19 (36.5)	30 (52.6)	
Civil status, n (%)			0.872
Single	15 (28.8)	15 (25.9)	
Married	27 (51.9)	33 (56.9)	
Other	10 (19.2)	10 (17.2)	

Clinical variable	HIT-6 score reduction <8 n (%)	HIT-6 score reduction ≥8 n (%)	p-value
Insomnia, n (%)			0.673
Absent	24 (46.2)	25 (43.1)	
Present + medication	14 (26.9)	13 (22.4)	
Present - medication	14 (26.9)	20 (34.5)	
Snoring, n (%)			0.668
Absent	37 (71.2)	38 (65.5)	
Present	15 (28.8)	20 (34.5)	
Anxiety, n (%)			0.523
Absent	21 (40.4)	28 (48.3)	
Present	31 (59.6)	30 (51.7)	
Depression, n (%)			0.151
Absent	18 (34.6)	29 (50.0)	
Present	34 (65.4)	29 (50.0)	
Chronic pain, n (%)			0.907
Absent	39 (75.0)	44 (75.9)	
Present	13 (25.0)	14 (24.1)	
Hypertension, n (%)			**0.006**
Absent	37 (71.2)	53 (93.0)	
Present	15 (28.8)	4 (7.0)	
Other comorbidities, n (%)			0.889
Absent	32 (61.5)	36 (62.1)	
Present	20 (38.5)	22 (37.9)	
Head trauma (n=109), n (%)			0.281
Absent	46 (90.2)	47 (81.0)	
Present	5 (9.8)	11 (19.0)	
Migraine form, n (%)			0.612

Clinical variable	HIT-6 score reduction <8 n (%)	HIT-6 score reduction ≥8 n (%)	p-value
Episodic	19 (36.5)	25 (43.1)	
Chronic	33 (63.5)	33 (56.9)	
Current therapy, n (%)			0.250
No	11 (21.2)	19 (32.8)	
Yes	41 (78.8)	39 (67.2)	
Aura (n=109) , n (%)			0.731
Absent	33 (63.5)	39 (68.4)	
Present	19 (36.5)	18 (31.6)	
Use of triptans, n (%)			0.067
No	21 (40.4)	13 (22.4)	
Yes	31 (59.6)	45 (77.6)	
Medication overuse, n (%)			0.594
No	26 (50.0)	33 (56.9)	
Yes	26 (50.0)	25 (43.1)	

p-values in bold are statistically significant. HIT-6: Headache Impact Test-6 (HIT-6). SD, standard deviation.

Table 3 Multivariate *logistic regression analysis* of clinical factors in predicting HIT-6 score reduction of ≥8

Clinical variable	OR (95% CI)	p-value
Monthly days with use of non-triptan 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

Multivariate logistic regression analysis of clinical variables with a significance level of $p<0.1$ at the respective univariate analysis. p-value in bold is statistically significant. ERE, erenumab. HIT-6: Headache Impact Test-6 (HIT-6). CI, confidence interval. OR, odds ratio. ref, reference.

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-value	Adjusted OR* (95% CI)	p-value
CALCRL rs696574						
CC	36 (62.8)	42 (80.8)				
TC	19 (32.8)	9 (17.3)	2.24 (1.04-4.81)	**0.039**	1.85 (0.82-4.16)	0.139
TT	3 (5.2)	1 (1.9)				
CALCRL rs6710852						
TT	40 (69.0)	46 (88.5)				
TG	16 (27.6)	6 (11.5)	3.37 (1.28-8.92)	**0.014**	2.82 (1.03-7.73)	**0.043**
GG	2 (3.4)	0 (0)				
CALCRL rs3213738						
TT	43 (74.1)	46 (88.5)				
TC	13 (22.4)	6 (11.5)	2.67 (1.01-7.05)	**0.047**	2.37 (0.86-6.54)	0.095
CC	2 (3.4)	0 (0)				
RAMP1 rs302680						
AA	43 (74.1)	40 (76.9)				
GA	13 (24.2)	11 (21.2)	1.19 (0.56-2.51)	0.654	1.19 (0.52-2.73)	0.679
GG	2 (3.4)	1 (1.9)				
RAMP1 rs13386048						
GG	29 (50.0)	16 (30.8)				
GA	23 (39.7)	26 (50.0)	0.55 (0.32-0.97)	**0.037**	0.53 (0.28-0.98)	**0.042**
AA	6 (10.3)	10 (19.2)				
RAMP1 rs12995100						
TT	10 (17.2)	13 (25.0)				
TC	33 (56.9)	31 (59.6)	1.56 (0.86-2.82)	0.143	1.51 (0.80-2.87)	0.205
CC	15 (25.9)	8 (15.4)				

RAMP1 rs12465864						
AA	44 (75.9)	28 (53.8)				
AG	13 (22.4)	22 (42.3)	0.42 (0.20-0.88)	**0.021**	0.32 (0.13-0.75)	**0.009**
GG	1 (1.7)	2 (3.8)				
RAMP1 rs7590387						
CC	19 (32.8)	16 (30.8)				
GC	31 (53.4)	21 (40.4)	0.72 (0.42-1.21)	0.216	0.68 (0.38-1.22)	0.198
GG	8 (13.8)	15 (28.8)				
RAMP1 rs75822777						
GG	28 (48.3)	25 (48.1)				
GA	23 (39.7)	24 (46.2)	1.16 (0.65-2.07)	0.622	0.74 (0.38-1.43)	0.371
AA	7 (12.1)	3 (5.8)				
RAMP1 rs302676						
TT	40 (69.0)	27 (51.9)				
TC	17 (29.3)	24 (46.2)	0.53 (0.26-1.10)	0.090	0.54 (0.25-1.19)	0.129
CC	1 (1.7)	1 (1.9)				
RAMP1 rs11673847						
GG	37 (63.8)	36 (69.2)				
GA	20 (34.5)	14 (26.9)	1.12 (0.56-2.27)	0.75	1.31 (0.59-2.89)	0.504
AA	1 (1.7)	2 (3.8)				
RAMP1 rs6431564						
AA	14 (26.9)	13 (22.4)				
AG	31 (59.6)	33 (56.9)	1.33 (0.74-2.40)	0.304	2.10 (1.05-4.22)	**0.037**
GG	7 (13.5)	12 (20.7)				
RAMP1 rs4663269						
TT	12 (23.1)	13 (22.4)				
TC	32 (61.5)	33 (56.9)	1.15 (0.64-2.09)	0.625	1.19 (0.63-2.24)	0.586
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

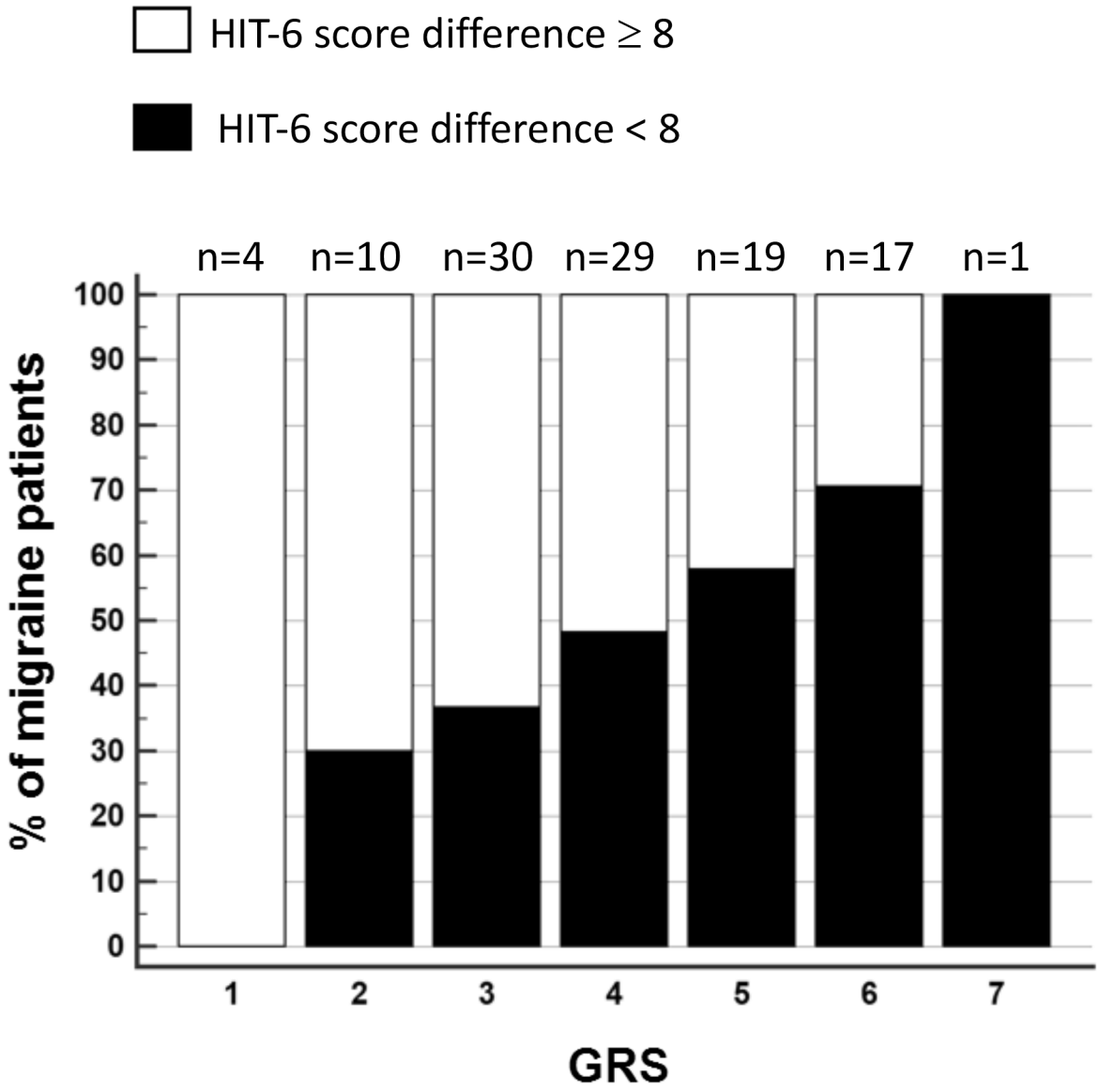
AG	22 (42.3)	27 (46.6)				
GG	4 (7.7)	2 (3.4)				
RAMP1 rs7578855						
TT	20 (38.5)20	24 (41.4)				
CT	(38.5)	28 (48.3)	0.73 (0.43-1.25)	0.252	0.71 (0.40-1.27)	0.249
CC	12 (23.1)	6 (10.3)				

*Logistic regression analysis adjusted by monthly days with use of non-triptan analgesics before erenumab start, hypertension and use of triptans. Association analysis of SNPs was performed by using the additive genetic model. p-values in bold are statistically significant. HIT-6: Headache Impact Test-6 (HIT-6). CI, confidence interval. OR, odds ratio.

Table 5 Multivariate *logistic regression analysis* of clinical factors and genetic risk score (GRS) in predicting HIT-6 score reduction of ≥8

Variable	**OR (95% CI)**	**p-value**
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	0.49 (0.33-0.72)	**0.0003**

*Based on total number of risk alleles (i.e. CALCRL rs6710852T, RAMP1 rs13386048A, RAMP1 rs12465864G and RAMP1 rs6431564A). p-values in bold are statistically significant. ERE, erenumab; HIT-6: Headache Impact Test-6 (HIT-6). CI, confidence interval. OR, odds ratio. ref, reference.



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