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Plasma SuPAR and therapeutic response to erenumab in migraine: a REFORM study

William K. Karlsson[1,2], Rune H. Christensen[1,2,3], Haidar M. Al-Khazali[1,2,3], Thomas Kallemose[4], Baker N. Jawad[4], Ove Andersen[2,4,5], Messoud Ashina[1,2] and Håkan Ashina[1,3*]

Abstract

Background Soluble urokinase-plasminogen activator receptor (suPAR) is a biomarker of systemic inflammation and elevated in plasma of individuals with migraine with aura. As inflammatory cytokines can upregulate calcitonin gene-related peptide (CGRP), suPAR levels might be linked to response to CGRP-targeting therapies. Therefore, we investigated whether plasma suPAR levels are associated with response to the CGRP-receptor antagonist erenumab.

Methods In this single-center, prospective study, adults with ≥ 4 monthly migraine days received 140 mg erenumab subcutaneously every 4 weeks for 24 weeks. Blood samples were collected at baseline, Week 24 (end of treatment), and Week 48 (24 weeks post-treatment). Responders were defined as achieving a ≥ 50% reduction in monthly migraine days from baseline to weeks 13–24. Associations between baseline suPAR and treatment response were analyzed using logistic and linear regression. Longitudinal changes in suPAR were assessed using linear mixed models.

Results The study included 623 participants with migraine (mean age 44.1 ± 12.3 years; 90.4% female) and 154 healthy controls. Among participants, 183 (29.4%) had migraine with aura, and 406 (65.2%) had chronic migraine. Baseline plasma suPAR levels were not associated with response to erenumab in the total migraine population (odds ratio [OR] 0.83, 95% confidence interval [CI] 0.64 to 1.07; $p = 0.14$) or in the aura subgroup (OR 0.73, 95% CI 0.48 to 1.10; $p = 0.14$). Plasma suPAR levels were significantly higher in non-responders compared to responders at Week 48 (7.5% higher, 95% CI 3.3 to 11.5%; $p = 0.005$). Non-responders with aura had higher suPAR concentrations than controls at baseline (difference 10.1%; 95% CI 3.0 to 17.8%; $p = 0.023$) and Week 24 (8.7%; 95% CI 1.6 to 16.2%; $p = 0.047$). These differences persisted at Week 48 (12.4%; 95% CI 4.6 to 20.7%; $p = 0.013$). No longitudinal changes in suPAR concentrations were observed.

Conclusions We did not find an association between baseline plasma suPAR levels and response to erenumab. Plasma suPAR concentrations remained stable, even among participants with aura. These findings suggest that systemic low-grade inflammation, as measured by suPAR, does not influence treatment efficacy.

Trial registration Pre-registered on ClinicalTrials.gov (NCT04603976 and NCT04674020).

Keywords Biomarker, Blood, CGRP, Efficacy, Inflammation, Predictors

*Correspondence:
Håkan Ashina
haakan.ashina@regionh.dk
[1]Department of Neurology, Danish Headache Center, Copenhagen University Hospital– Rigshospitalet, Copenhagen, Denmark
[2]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]Translational Research Center, Copenhagen University Hospital– Rigshospitalet, Copenhagen, Denmark
[4]Department of Clinical Research, Copenhagen University Hospital Amager and Hvidovre, Hvidovre, Denmark
[5]Department of Emergency Medicine, Copenhagen University Hospital Amager and Hvidovre, Hvidovre, Denmark

Introduction

Migraine is a ubiquitous neurologic disorder with an incompletely elucidated, multifaceted pathogenesis [1]. Therapeutic advances have introduced monoclonal antibodies (mAbs) directed against calcitonin gene-related peptide (CGRP) signaling, which have demonstrated efficacy for migraine prevention in randomized controlled trials and real-world studies [2]. However, their effectiveness varies considerably among patients [3, 4], and a reliable biomarker for predicting therapeutic response has yet to be established [5].

Soluble urokinase plasminogen activator receptor (suPAR) is a circulating glycoprotein implicated in chronic inflammation and endothelial dysfunction [6, 7]. Elevated serum and plasma suPAR levels have been reported in diverse pathologies, ranging from neurologic disorders to cardiovascular disease [6, 8, 9]. Recently, our lab reported higher plasma suPAR levels in migraine with aura compared to healthy controls (HCs) [10]. Moreover, adjusted analyses showed suPAR levels were higher in participants with aura compared to those without aura [10], corroborating findings from an earlier study [11]. These findings are particularly interesting, as mounting evidence from human neuroimaging studies implicates neuroinflammation in migraine with aura [12–14].

Neurogenic inflammation, a central process in migraine pathophysiology, involves the release of CGRP from nerve endings of trigeminal sensory neurons [15]. CGRP is a potent vasodilator and key mediator of migraine that can experimentally trigger both migraine attacks and aura [15, 16]. Its expression in trigeminal ganglion neurons is upregulated by pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) [17, 18]. Levels of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, are positively correlated with suPAR levels [6]. Therefore, suPAR levels reflect systemic inflammatory activity that may be able to influence CGRP-mediated mechanisms in migraine. If systemic inflammatory activity, reflected by plasma suPAR levels, is associated with treatment outcomes, suPAR could serve as a biomarker for predicting response and provide insights into the underlying mechanisms of migraine. However, this potential association has not been previously investigated.

To this end, we aimed to examine whether plasma suPAR concentrations are associated with the therapeutic response to erenumab in adults with migraine. Erenumab, a first-in-class anti-CGRP receptor mAb, was used for this study as it was the first and most widely available CGRP-targeting treatment during the study period. We also compared plasma suPAR levels across erenumab responders, non-responders, and HCs and evaluated longitudinal changes during the 48-week study period. Given that erenumab is prescribed for both individuals with and without aura, we investigated the full patient cohort to evaluate the potential of plasma suPAR as a predictor of treatment response in a real-world setting. Additionally, we specifically analyzed the migraine with aura subgroup due to the previously reported higher suPAR levels in this subtype of migraine [10, 11].

Methods

Study design

The longitudinal, prospective, observational data presented herein is part of the larger Registry for Migraine (REFORM) study (pre-registered with ClinicalTrials.Gov identifiers NCT04603976 and NCT04674020) conducted at the outpatient clinic of a national referral hospital [19].

The study included four distinct periods: a screening period (weeks −6 through −5), a 4-week baseline period (weeks −4 to day 1), a 24-week treatment period with erenumab (day 1 to week 24), and a 24-week follow-up period after treatment discontinuation (weeks 25 to 48). During the treatment period, participants received subcutaneous erenumab at a dose of 140 mg every 4 weeks for a total of 24 weeks as part of a separate, open-label, single-arm, phase IV trial Biomarker and Genetic Predictors of Erenumab Treatment Response (INTERROGATE; ClinicalTrials.Gov identifier: NCT04265755). Participants who discontinued erenumab during the treatment period, or commenced any treatments targeting CGRP signaling during the follow-up period, were withdrawn from the study. Participants were allowed to initiate other preventive medications for migraine during the follow-up period.

A formal power calculation was not conducted, as this exploratory analysis followed the pre-defined sample size of the parental REFORM study [19]. To balance feasibility and statistical power, the pre-defined aim was to collect blood samples from at least 600 consecutive participants with migraine at baseline (collected at the screening visit) and Week 24 (± 2 weeks), with additional samples from at least 200 consecutive participants who reached Week 48 (± 4 weeks). Additionally, at least 150 HCs were to provide a single baseline sample [19].

Participants

Eligible participants were adults aged 18 years or older with a diagnosis of migraine without aura, migraine with aura, or chronic migraine in accordance with the International Classification of Headache Disorders, 3rd edition (ICHD-3) [20]. Furthermore, participants had to experience four or more monthly migraine days (MMDs) prior to enrollment [19].

Participants were excluded if they were unable to distinguish migraine from other headaches, had migraine onset after 50 years of age, a history of post-traumatic headache, hemiplegic migraine, cluster headache, or

secondary headache disorders, except for medication-overuse headache. Participants who had previously used erenumab at any time or any other anti-CGRP mAbs within three months before enrollment were ineligible. Concomitant medications, including onabotulinumtoxinA, were permitted if the dosage remained stable for at least two months before screening. Participants receiving systemic immunosuppressants and those who did not receive 1st erenumab dose were also excluded. Further exclusions applied specifically to analyses of erenumab response, excluding participants who did not receive all six injections, had fewer than four MMDs at baseline, or completed fewer than 21 headache diary entries per month during the baseline period or weeks 13–24. The full list of inclusion and exclusion criteria for participants with migraine is available in the Supplementary Table S1.

As a reference, we included a HC group without a personal or family history of headache disorders, except for infrequent episodic tension-type headache (see Supplementary Table S2).

Clinical data

At the screening visit, site investigators collected detailed information on participant demographics, clinical characteristics, and medical and treatment history using a semi-structured interview. During the 4-week baseline period, participants maintained a detailed paper diary to classify migraines and tension-type headaches according to the ICHD-3 criteria. The diary recorded headache occurrence, duration, location, severity, associated symptoms (e.g., nausea, vomiting, sensitivity to light and sound), exacerbation with physical activity, medication use, and aura presence. Participants received both oral and written instructions to differentiate between migraine and tension-type headaches. After this period, they continued using a simplified diary throughout the 24-week treatment phase and the subsequent 24-week follow-up, documenting headache and migraine occurrence, aura, and medication use. As blood samples were collected irrespective of whether participants were in the interictal or ictal phase, we also recorded data at the time of sample collection on the presence of headache, headache characteristics, accompanying symptoms, and use of acute medications within the preceding 72 h. This information was collected to allow adjustment for ictal status and recent non-steroidal anti-inflammatory drug (NSAID) intake in the analyses. Additional information on the semi-structured interview, headache diaries, and data collected in relation to blood sampling has been reported elsewhere [19].

Plasma SuPAR measurements

Blood samples were collected into 9 mL dipotassium ethylenediaminetetraacetate (K_2EDTA) tubes by antecubital phlebotomy. Following collection, tubes were centrifuged at 2200 × g for 10 min at 4 °C, and the plasma was aliquoted into cryotubes and stored at − 80 °C until analysis. To maintain blinding, coded labeling and random arrangement of cryotubes were applied before assay initiation. Samples from each participant, along with corresponding controls, were stored together and processed on the same assay plates to minimize inter-assay variability.

Plasma suPAR levels were quantified using the suPARnostic® enzyme-linked immunosorbent assay (ELISA) (ViroGates A/S, Birkerød, Denmark), following the manufacturer's instructions [21]. The suPARnostic® ELISA is a double monoclonal antibody sandwich assay where samples and peroxidase-conjugated anti-suPAR are mixed in the included white mixing plate before incubation in the anti-suPAR precoated optically clear microwells. The kit contains a 96-well plate, including dedicated wells for at least three standards, one blank, and one curve control. Absorbance was read at 450 nm with a reference filter at 650 nm using a microplate reader. Each assay run included five recombinant suPAR calibration standards, a blank, and a curve control. Standards, blanks, and controls were measured in duplicates in accordance with the manufacturer's instructions [21]. The lower limits of detection and quantification were both 0.4 ng/mL for this assay, and the measured intra- and inter-assay coefficients of variations were 2.2% and 2.3%, respectively. All analyses were performed between March and April 2023 at the Department of Clinical Research, Copenhagen University Hospital Hvidovre, Denmark. Two experienced research technicians, who remained blinded to clinical information, carried out the measurements.

Samples with markedly elevated suPAR levels (> 6.0 ng/mL) were excluded from the analyses ($n = 1$). This threshold was chosen to minimize confounding effects from severe systemic inflammation to an extent associated with increased mortality risk in acute care settings [22]. Moreover, samples obtained outside the pre-defined time windows (Week 24 ± 2 weeks and Week 48 ± 4 weeks) were also excluded.

Outcomes and variables

The primary aim was to investigate whether baseline plasma suPAR was associated with each of our co-primary outcomes:

i. ≥ 50% reduction in MMDs.
ii. The absolute reduction in MMDs.

Secondary outcomes were the relative differences (%) in plasma suPAR levels at baseline, Week 24, and Week 48, comparing:

i. Participants with migraine vs. HCs.
ii. Responders, non-responders, and HCs.
iii. Longitudinal changes in plasma suPAR across timepoints in responders and non-responders.

Exploratory analyses examined whether changes in plasma suPAR from baseline to Week 24 correlated with absolute reduction in MMDs, monthly headache days (MHDs), moderate-to-severe MHDs, and monthly aura days. All outcomes were analyzed in the overall population and the migraine with aura subgroup.

Treatment response was assessed by comparing headache diaries from baseline to the mean of Weeks 13–24. Responders were defined as participants with a ≥ 50% reduction in MMDs from baseline (based on the mean of Weeks 13–24), while non-responders had < 50% reduction.

A month was defined as a 28-day interval. A migraine day was recorded if participants self-reported migraine, aura with headache, or used acute migraine-specific medication (triptans, ergot alkaloids, lasmiditan, or gepants). We categorized participants as having migraine with aura or chronic migraine, following the ICHD-3 criteria [20]. Medication-overuse was defined as the use of triptans, combination analgesics, ergotamines, or opioids on at least 10 days per month, or acetaminophen or NSAIDs on at least 15 days per month, over more than three consecutive months. Previous failure of preventive treatment was defined as a lack of efficacy despite administration at the minimal effective dose and duration suggested by the European Headache Federation guidelines [23]. Moreover, somatic and psychiatric comorbidities were noted through participant self-report and review of medical records.

Guided by the literature [4, 6, 24–26], we adjusted the analyses for potential confounders, including age, sex, body mass index (BMI), smoking status, chronic migraine, ictal status at time of blood sampling (defined as migraine headache, non-migraine headache, or headache free), use of any NSAIDs (monotherapy or combination drug) within 72 h of blood sampling, medication-overuse, preventive medication use, multiple (≥ 3) preventive medication failures, and current comorbidities. These comorbidities comprised autoimmune disorders, daily low back pain, daily neck pain, hypertension, other cardiovascular conditions, anxiety, and depression.

Statistical analysis

Continuous variables were summarized using means ± standard deviations or medians with interquartile ranges, as appropriate. Histograms and quantile-quantile (QQ) plots assessed the normal distribution of continuous variables. Categorical variables were presented as counts with percentages. Baseline differences were compared using t-tests, Mann–Whitney *U* test, or Pearson's Chi-squared test. The time to collection of blood samples was compared between responders and responders using the Mann–Whitney *U* test.

Logistic regression was used to investigate associations between baseline plasma suPAR and achieving a ≥ 50% reduction in mean MMDs from baseline to weeks 13 through 24. The results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Linear regression was also applied with absolute reduction in MMDs as the outcome variable, with results expressed as mean absolute reduction in MMD per ng/ml increase in suPAR (mean MMD reduction) with 95% CIs. We assessed the model unadjusted, adjusted for each potential confounder, and adjusted for all potential confounders. For the linear regression model, baseline MMDs were included with a natural spline with one knot in each model.

A linear mixed-effects regression model was used to compare plasma suPAR levels between treatment responders, non-responders, and HCs and assess changes in suPAR concentrations over time. Plasma was set as the dependent variable, using an unstructured covariance pattern for the random effect to account for repeated measurements. Logarithmic transformation was applied to plasma suPAR data to correct non-normal residuals, as it adequately reduced skewness and improved the approximation to normality, confirmed by diagnostic plots. Back-transformed results were then expressed as percentwise differences in geometric means with 95% CIs. Three models with different fixed effects were evaluated: (I) an unadjusted model that included only blood sample collection time point and group status, plus their interaction, (II) a similar model additionally adjusted for age, sex, BMI, and smoking status, and (III) a fully adjusted model incorporating all potential confounders.

Model assumptions were assessed through diagnostic evaluations, including histograms and QQ-plots for normally distributed residuals, inspection of residuals vs. fitted values plot for variance homogeneity, and residual over time plots for independence of residuals. Variance inflation factors (VIFs) were assessed for multicollinearity, with values < 5 considered acceptable. The Hosmer-Lemeshow test assessed goodness-of-fit in logistic regression models ($p > 0.05$ indicated adequate fit).

Spearman's rho (r_s) was calculated to evaluate correlations between the change in plasma suPAR concentrations from baseline to Week 24, with efficacy measured by the absolute reduction from baseline to weeks 13 through 24 in MMDs, MHDs, moderate-to-severe MHDs, and monthly number of days with aura.

We conducted a subgroup analysis comprising participants with migraine aura. Statistical significance was defined by a two-sided p-value < 0.05. The

Benjamini-Hochberg procedure was applied to control for false discoveries within each analysis cluster [27]. Complete case analysis was used for all models, as missing data on suPAR values and treatment responses were assumed to be missing not at random [28]. For analyses of the primary outcomes, missing covariate data were imputed using multiple imputation via chained equations with a random forest algorithm (20 imputed datasets). We did this to enable comparison of the confounders effects on the association between suPAR and response, with models with imputed covariates being considered the main result, and complete case results reported for comparison. All statistical analysis were performed using R (version 4.3.3) [29].

Ethical considerations

The parental protocol was approved by the relevant ethics committee and data protection agency. Each participant provided written informed consent before the commencement of study-related tasks or procedures. The study was conducted in accordance with the principles of the Declaration of Helsinki, with later revisions [30]. Our reporting adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [31].

Results

From September 2020 to June 2022, 745 participants were enrolled in the REFORM Biochemistry Core, of whom 679 participants were eligible for the present study and received 1st injection with erenumab. Of these, 43 participants were excluded or dropped-out during the 24-week treatment period, while 636 completed the Week 24 visit (end of treatment). Of the 636 that completed Week 24, 180 were excluded or dropped-out during weeks 24 to 48, while 456 reached Week 48 and completed the full study. Figure 1 outlines the study flow and samples included in the final analyses (detailed reasons for exclusion or drop-out are available in the Supplementary Figure S1).

In total, 623 (91.8%; [623 of 679]) participants with migraine and 154 HCs had at least one plasma suPAR measurement eligible for inclusion. Among these 623 participants, 622 had samples at baseline, 586 at Week 24, and 233 at Week 48, with 225 participants having suPAR measured at all three timepoints.

Response to erenumab could be classified in 541 participants with migraine with at least one blood sample, of whom 282 (52.1%, [282 of 541]) were treatment responders, and 259 (47.9%, [259 of 541]) were non-responders. Of these, eligible plasma suPAR measurements were available from 540 participants at baseline, 522 at Week 24, and 215 at Week 48, with 208 participants having suPAR measured at all three timepoints.

Plasma SuPAR concentrations

The mean baseline plasma suPAR concentration was 2.59 ± 0.69 ng/mL in the entire migraine population, 2.70 ± 0.76 ng/mL in the migraine aura subgroup, and 2.44 ± 0.56 ng/mL in HCs (see Table 1). Supplementary Tables S3 (total population) and S4 (migraine with aura

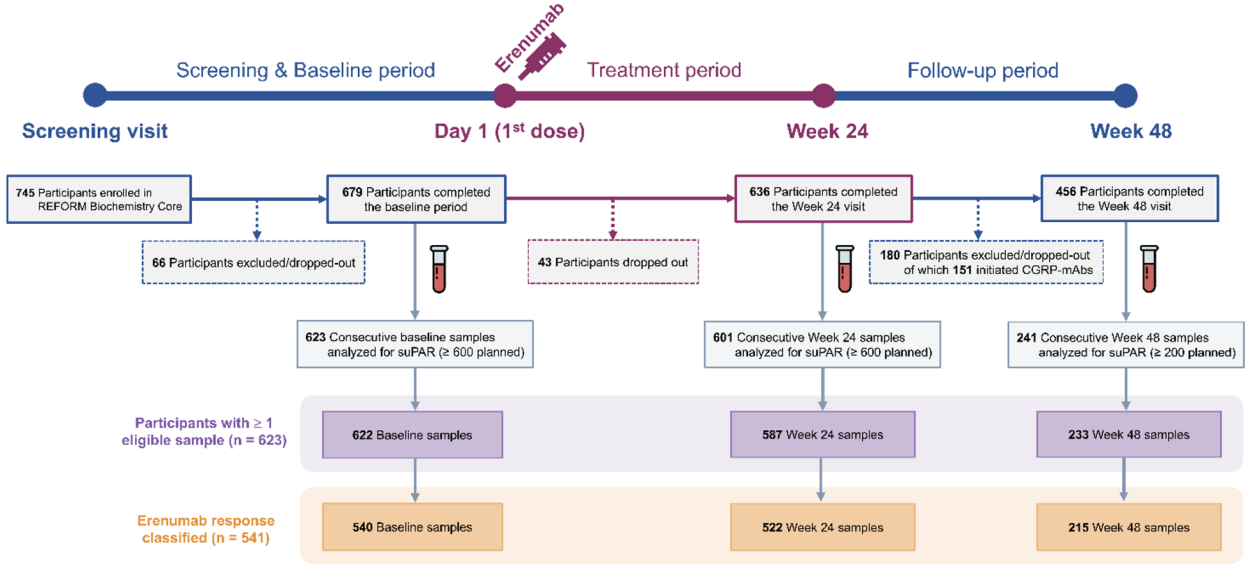

Fig. 1 Study flow. legend: Blood samples analyzed for plasma soluble urokinase-type plasminogen activator receptor (suPAR) were collected at baseline (Screening visit during weeks −5 to -6), Week 24 (± 2), and Week 48 (± 4). Treatment with erenumab was administered every 28th day from day 1 (1st injection) to Week 24. Detailed reasons for exclusion are available in Supplementary Figure S1. Abbreviations: CGRP-mAbs, calcitonin gene-related peptide monoclonal antibodies; MMDs, monthly migraine days; suPAR, soluble urokinase-type plasminogen activator receptor

Table 1 Mean plasma SuPAR levels at each timepoint

	Baseline		Week 24		Week 48	
	n	Mean (SD), ng/mL	n	Mean (SD), ng/mL	n	Mean (SD), ng/mL
Total population						
Migraine (*N* = 623)	622	2.59 (0.69)	587	2.60 (0.67)	233	2.66 (0.65)
Responders* (*n* = 282)	282	2.54 (0.62)	276	2.56 (0.64)	97	2.52 (0.52)‡
Non-responders* (*n* = 259)	258	2.62 (0.72)	246	2.62 (0.65)	118	2.75 (0.69)†,‡
Migraine with aura subgroup						
Migraine (*n* = 183)	182	2.70 (0.76)†	173	2.76 (0.73)†	67	2.85 (0.79)†
Responders* (*n* = 81)	81	2.62 (0.72)	79	2.65 (0.61)	28	2.57 (0.63)
Non-responders* (*n* = 77)	76	2.80 (0.83)†	72	2.76 (0.78)†	36	3.05 (0.82)†
Healthy controls						
Healthy controls (*n* = 154)	154	2.44 (0.56)†	-	-	-	-

Abbreviations: MMDs, monthly migraine days; SD, standard deviation; suPAR, soluble urokinase-type plasminogen activator receptor

Symbols:

*, Responders were participants with a ≥ 50% reduction in MMDs, while non-responders experienced < 50% reduction in MMDs

†, statistically significant difference between participants with migraine and healthy controls ($p < 0.05$)

‡, statistically significant difference between responders and non-responders ($p < 0.05$)

Estimates with 95% confidence intervals and *p*-values are available in Supplementary Table S8 and S14

subgroup) provide median and geometric mean concentrations. No suPAR measurements were below the lower detection and quantification limit (0.4 ng/mL). The time to sample collection was comparable in responders and non-responders at both Week 24 (median [IQR]: 24.0 [24.0–24.4] vs. 24.0 [24.0–24.4]; $p = 0.55$) and Week 48 (47.7 [44.7–48.0] vs. 47.9 [45.7–48.2]; $p = 0.15$).

Overall study population

Baseline characteristics Table 2 summarizes the demographic and clinical characteristics of the overall migraine population. The participants' mean age was 44.1 ± 12.3 years, and 90.4% (563 of 623) were female. The mean BMI was 25.1 ± 4.9 kg/m^2. Among the participants, 29.4% (183 of 623) had migraine with aura, and 65.2% (406 of 623) had chronic migraine. About half of participants with migraine reported current preventive headache medication use (50.4% [314 of 623]). Responders to erenumab had a higher mean age than non-responders (46.1 ± 12.1 vs. 43.4 ± 12.2; $p = 0.010$), as well as lower mean baseline MHDs (17.3 ± 7.3 vs. 19.4 ± 7.0; $p = 0.001$) and MMDs (12.8 ± 6.1 vs. 14.2 ± 6.8; $p = 0.027$). Furthermore, responders less often had chronic migraine (60.6% [171 of 282] vs. 71.0% [184 of 259]; $p = 0.014$) and fewer instances of ≥ 3 failed preventive medications (27.3% [77 of 282] vs. 40.2% [104 of 259]; $p = 0.002$). Medication-overuse was present in 55.4% (345 of 623) of participants and evenly distributed between responders and non-responders (58.2% [164 of 282] vs. 54.1% [140 of 259]; $p = 0.34$). Similarly, other comorbidities were comparable between the groups (Table 2). At the time of baseline blood sampling, responders were less likely to experience migraine headache compared to non-responders (40.7% [114 of 280] vs. 50.4% [130 of 258]; $p = 0.049$), but similarly likely to have used an NSAID within the last 72 h (29.2% [76 of 260] vs. 31.1% [235 of 259]; $p = 0.66$) (Supplementary Table S5). The 154 HCs had a lower mean age compared with the overall migraine population (41.2 ± 11.8 vs. 44.1 ± 12.2; $p = 0.010$). They were otherwise comparable in terms of sex distribution, BMI, and the proportion of current smokers (Table 2).

Baseline plasma suPAR and response to erenumab: In the total migraine population, univariate logistic regression analyses revealed no significant association between baseline suPAR levels and a ≥ 50% reduction in MMDs from baseline to weeks 13 through 24 (OR 0.83, 95% CI 0.64–1.07; $p = 0.14$). An association emerged when adjusting for age alone (OR: 0.76, 95% CI: 0.58–0.99; $p = 0.043$) but disappeared after adjustment for all potential confounders (OR: 0.82, 95% CI: 0.61–1.09; $p = 0.18$), with particularly BMI showing an effect opposite to that of age (see Supplemental Table S6).

Likewise, no significant association was observed between baseline suPAR concentrations and the absolute reduction in MMDs from baseline to weeks 13–24. In the unadjusted model, mean MMD reduction was −0.34 per 1 ng/mL increase in plasma suPAR (95% CI: -1.03 to 0.34; $p = 0.33$). This finding was consistent across models adjusted for baseline MMDs (mean MMD reduction = -0.18, 95% CI: -0.78 to 0.43; $p = 0.57$) and when adjusted for all potential confounders (mean MMD reduction = -0.44, 95% CI: -1.19 to 0.31; $p = 0.25$; see Supplemental Table S7).

Comparisons of plasma SuPAR between groups Figures 2 and 3 illustrate plasma suPAR levels in responders, non-responders, and HCs at baseline, Week 24, and Week 48. The following results stem from models adjusted for age, sex, BMI, and smoking. Including further confounders

Table 2 Baseline demographics and characteristics of the total study population

	Migraine (*N* = 623)	Responders* (*n* = 282)	Non-responders* (*n* = 259)	Healthy controls (*n* = 154)
Demographic characteristics				
Age, mean ± SD, years	44.1 ± 12.3	46.1 ± 12.1	43.4 ± 12.2	41.2 ± 11.8
Female sex, n (%)	563 (90.4%)	252 (89.4%)	238 (91.9%)	132 (85.7%)
BMI, mean ± SD, kg/m^2	25.1 ± 4.9	24.8 ± 4.6	25.6 ± 5.4	24.7 ± 4.0
Current smoking, n (%)†	66 (10.7%)	27 (9.7%)	24 (9.3%)	20 (13.4%)
Clinical characteristics				
Migraine with aura, n (%)	183 (29.4%)	81 (28.7%)	77 (29.7%)	-
Chronic migraine, n (%)	406 (65.2%)	171 (60.6%)	184 (71.0%)	-
Medication-overuse, n (%)	345 (55.4%)	164 (58.2%)	140 (54.1%)	-
Headache frequency (28 days), mean ± SD	-	-	-	-
MHDs	18.4 (7.3)	17.3 (7.3)	19.4 (7.0)	-
MMDs	13.5 (6.5)	12.8 (6.1)	14.2 (6.8)	-
Monthly acute medication days	11.0 (6.0)	11.0 (5.6)	11.2 (6.2)	-
Use of preventive migraine medication, n (%)	314 (50.4%)	145 (51.4%)	124 (47.9%)	-
≥ 3 preventive medication failures, n (%)	208 (33.4%)	77 (27.3%)	104 (40.2%)	-
Comorbidity, n (%)	-	-	-	-
Autoimmune disorders	69 (11.1%)	38 (13.5%)	26 (10.0%)	-
Daily low back pain	61 (9.8%)	29 (10.3%)	21 (8.1%)	-
Daily neck pain	92 (14.8%)	47 (16.7%)	33 (12.7%)	-
Hypertension	67 (10.8%)	26 (9.2%)	33 (12.7%)	-
Other cardiovascular disorders	39 (6.3%)	14 (5.0%)	20 (7.7%)	-
Anxiety	60 (9.6%)	27 (9.6%)	25 (9.7%)	-
Depression	61 (9.8%)	23 (8.2%)	27 (10.4%)	-

Abbreviations: BMI, body mass index; IQR, interquartile range; MHDs, monthly headache days; MMDs, monthly migraine days; NSAIDs, non-steroidal anti-inflammatory drugs; SD, standard deviation

Symbols: *, Responders were defined as participants with a ≥ 50% reduction in MMDs, while non-responders experienced < 50% reduction in MMDs. Data to classify treatment response was missing in 82 (13.2%) of 623 participants.; †, Data on smoking was missing in eight participants with migraine (four responders and zero non-responders) and five healthy controls; -, not applicable

had no substantial effect on the findings (Supplemental Tables S8).

Comparing all participants with migraine with HCs, there were no significant differences in plasma suPAR levels at baseline (3.7% higher; 95% CI -0.7 to 8.2%; $p = 0.10$), Week 24 (4.2%; 95% CI -0.2 to 8.8; $p = 0.090$), or Week 48 (4.6%; 0.0 to 9.3%; $p = 0.090$). In responders, non-responders and HCs, plasma suPAR levels were not statistically different compared to baseline or Week 24 (Supplemental Table S8). At Week 48, plasma non-responders had significantly higher suPAR levels than responders (7.5% higher, 95% CI 3.3 to 11.5%; $p = 0.005$) and to HCs (7.6%, 95% CI 2.5 to 12.9%; $p = 0.015$), while no difference was found between responders and HCs (-0.5%; 95% CI -5.3 to 4.5%; $p = 0.84$).

Longitudinal changes in plasma SuPAR levels Comparisons of plasma suPAR levels across baseline, Week 24, and Week 48 did not reveal significant changes in the total migraine population (All $p > 0.05$; Supplemental Table S8). Furthermore, the change in plasma suPAR between baseline and Week 24 was not correlated with the absolute reduction in MHDs, MMDs, moderate-to-severe MHDs, or monthly days with aura (Supplemental Tables S9).

Migraine with aura subgroup

Baseline characteristics The migraine with aura subgroup ($n = 183$) baseline characteristics were largely comparable to the entire migraine population (Supplemental Table S10). Erenumab responders more frequently had medication-overuse (61.7% [50 of 81] vs. 41.6% [32 of 77]; $p = 0.018$). Conversely, non-responders with aura more often reported ≥ 3 failed preventive medications (41.6% [32 of 77] vs. 24.7% [20 of 81]; $p = 0.037$). There was no significant difference between responders and non-responders with aura in the presence of migraine headache at blood sampling (48.1% [39 of 81] vs. 47.4% [36 of 76]; $p = 0.68$) or recent use of NSAIDs (29.9% [23 of 77] vs. 27.9% [19 of 68]; $p = 0.80$) (Supplementary S11). HCs had a lower mean age than the subgroup with migraine aura (41.2 ± 11.8 vs. 44.6 ± 11.9; $p = 0.010$), but were otherwise comparable in terms of sex distribution, BMI, and the proportion of current smokers (Supplemental Table S10).

Baseline plasma SuPAR and response to erenumab Regression analyses in participants with migraine aura were congruent with those from the entire migraine population, showing no significant associations between baseline

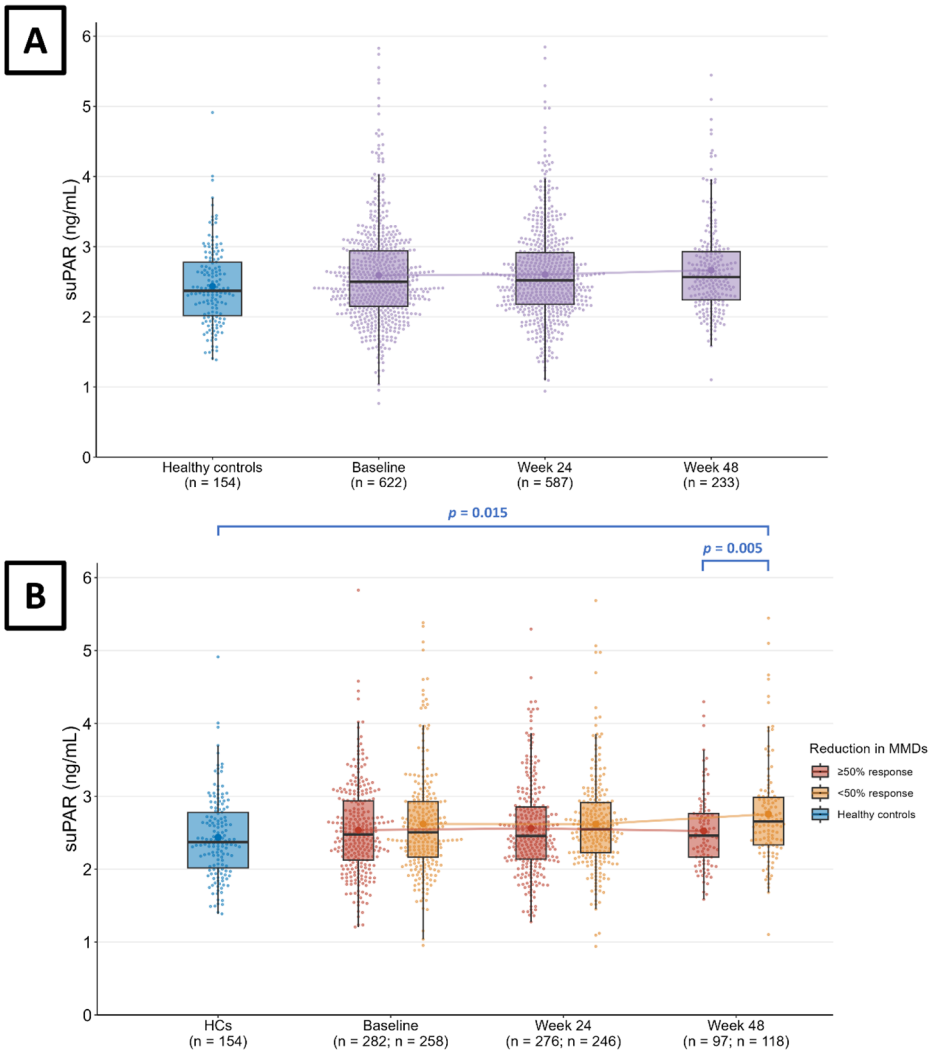

Fig. 2 Plasma suPAR concentrations (total population). legend: Plasma concentrations of soluble urokinase-type plasminogen activator receptor (suPAR) across the study population. Panel (**A**) displays suPAR levels for all participants with at least one eligible blood sample (purple). Panel (**B**) distinguishes between erenumab responders (≥ 50% reduction in monthly migraine days [MMDs], red) and non-responders (< 50% reduction in MMDs, orange). Healthy controls (HCs) are indicated in blue. Box plots represent the median (bold horizontal line) and interquartile range (IQR; top and bottom of the box), with whiskers extending to 1.5 times the IQR. Mean plasma suPAR levels are connected by dots. Statistically significant pairwise differences ($p < 0.05$) are denoted by dark blue brackets (Supplementary Table S8)

plasma suPAR concentrations and erenumab response (All $p > 0.05$; Supplemental Table S12 and S13).

Comparisons of plasma suPAR between groups: In the migraine with aura subgroup, suPAR concentrations were higher in participants with aura compared with HCs at all timepoints: baseline (7.1% higher; 95% CI 1.5 to 12.9; $p = 0.012$), Week 24 (8.7%; 95% CI 3.1 to 14.8; $p = 0.007$), and Week 48 (8.7%; 95% CI 2.6 to 15.2; $p = 0.008$) (Supplementary Table S14). Among non-responders with aura, plasma suPAR levels were significantly higher compared to HCs at baseline (10.1%; 95% CI 3.0 to 17.8%; $p = 0.023$), Week 24 (8.7%; 95% CI 1.6 to 16.2; $p = 0.47$), and Week 48 (12.4%; 95% CI 4.6 to 20.7%; $p = 0.013$). However, no statistically significant differences were observed between responders and non-responders within the aura subgroup (Supplementary Table S14).

Longitudinal changes in plasma SuPAR levels Comparisons of plasma suPAR levels across baseline, Week 24, and Week 48 did not reveal significant changes within the migraine with aura subgroup (All $p > 0.05$; Supplemental Table S14). Furthermore, the change in plasma suPAR between baseline and Week 24 was not correlated with the absolute reduction in MHDs, MMDs, moderate-to-severe MHDs, or monthly days with aura (Supplemental Tables S15).

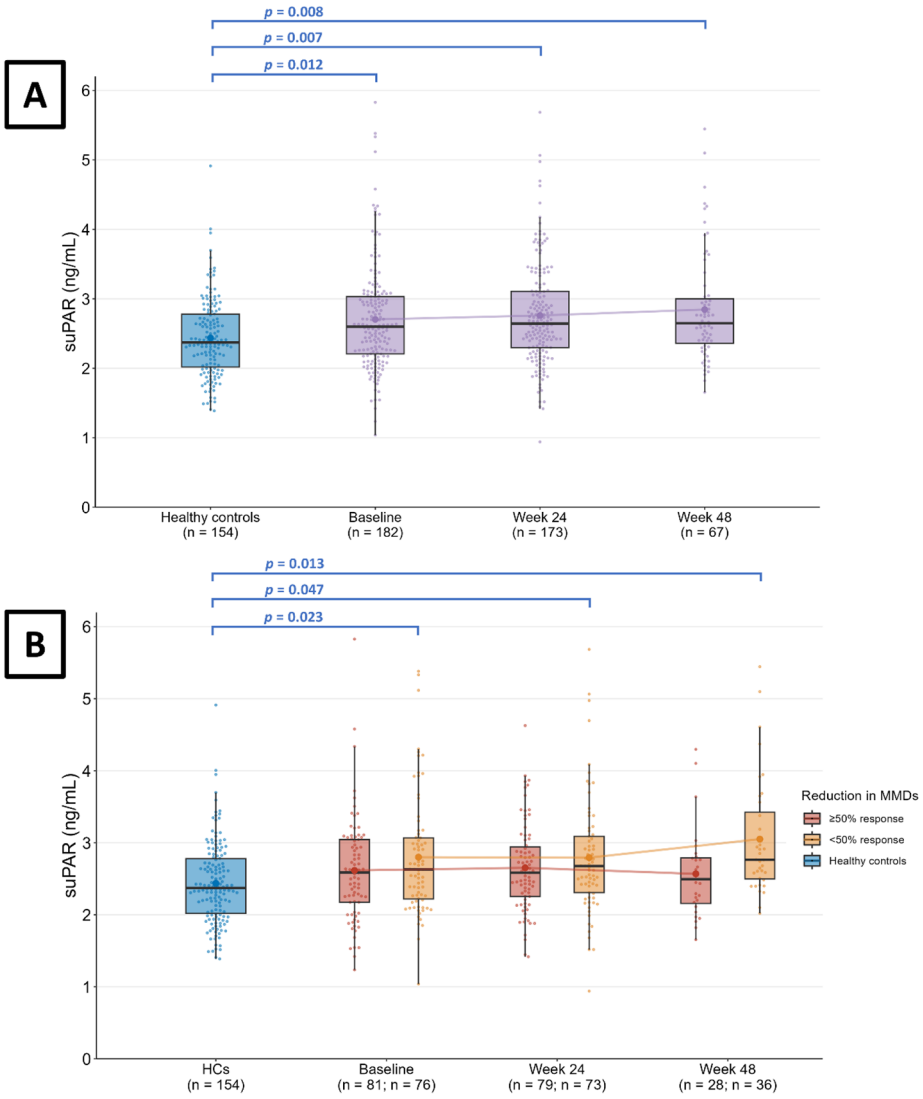

Fig. 3 Plasma suPAR concentrations (migraine with aura subgroup). legend: Plasma concentrations of soluble urokinase-type plasminogen activator receptor (suPAR) across the migraine with aura subpopulation. Panel (**A**) displays suPAR levels for all participants with at least one eligible blood sample (purple). Panel (**B**) distinguishes between erenumab responders (≥ 50% reduction in monthly migraine days [MMDs], red) and non-responders (< 50% reduction in MMDs, orange). Healthy controls (HCs) are indicated in blue. Box plots represent the median (bold horizontal line) and interquartile range (IQR; top and bottom of the box), with whiskers extending to 1.5 times the IQR. Mean plasma suPAR levels are connected by dots. Statistically significant pairwise differences ($p < 0.05$) are denoted by dark blue brackets (Supplementary Table S14)

Discussion

In this longitudinal, prospective study, we found no evidence to suggest that baseline plasma suPAR levels are associated with efficacy of erenumab for migraine prevention. Furthermore, no significant differences in plasma suPAR concentrations were identified between erenumab responders and non-responders at baseline or Week 24, and no longitudinal changes in suPAR levels were detected. However, at Week 48, non-responders had significantly higher plasma suPAR levels than responders. In the migraine with aura subgroup, non-responders consistently exhibited higher plasma suPAR concentrations than HCs at all timepoints (baseline, Week 24, and Week 48). Overall, we did not find any evidence supporting the use of suPAR as a predictive biomarker for therapeutic response to erenumab.

The absence of a clear association between plasma suPAR concentrations and the clinical benefit of erenumab could stem from several factors. First, suPAR might reflect a more generalized, systemic inflammatory burden that is unrelated to the CGRP-driven processes in migraine pathophysiology [32]. Hence, suPAR might capture aspects of chronic inflammation that do not solely dictate the response to CGRP-targeted therapies. Second,

the response to erenumab might depend more on the intricate relationship between CGRP and pro-inflammatory molecules other than suPAR [33]. Future biomarker research in migraine could explore simultaneous profiling of multiple inflammatory markers to delineate the interplay among them, potentially identifying composite signatures more predictive of treatment outcomes.

From a clinical standpoint, our findings suggest that measuring plasma suPAR in routine migraine care to guide erenumab prescription holds no clinical value. Yet, we observed modestly elevated plasma suPAR levels among non-responders with migraine aura, compared with HCs. These data build on our earlier report of the REFORM cohort, which showed higher plasma suPAR concentrations in people with migraine aura than in HCs [10]. Interestingly, plasma suPAR levels did not correlate with headache or aura frequency, nor with ictal versus interictal timing of blood sampling. Such findings suggest a possible relationship between suPAR and the neurobiologic processes that underlie aura, rather than headache frequency or the transient state of being in or out of a migraine attack.

Current concepts in our understanding of migraine aura underscore the importance of glial cells—including microglia—in propagating inflammatory responses in the CNS [34]. In migraine with aura, the defining electrophysiologic event is cortical spreading depression (CSD), a wave of neuronal and glial depolarization that has been tied to pro-inflammatory cascades [35]. When CSD occurs, it leads to transient ionic disequilibrium [36, 37], expression of pro-inflammatory mediators [38–42], and activation of resident microglia and macrophages [37, 43]. These activated glial cells secrete cytokines, such as TNF-α [38, 44], which has been shown to be elevated in migraine with aura in several human studies although the current evidence is inconclusive [45]. In preclinical studies, upregulated urokinase plasminogen activator receptor (uPAR) has been documented in activated microglia and macrophages during acute and chronic inflammation [46]. Because suPAR is the soluble form of uPAR, released into circulation when uPAR is cleaved from the cell membrane [6], increases in suPAR might reflect microglial and macrophage activation triggered by CSD.

Another thread linking elevated suPAR to migraine with aura could be vascular dysfunction [47, 48]. In this respect, elevated plasma suPAR levels might suggest a systemic endothelial phenotype susceptible to migraine aura without necessarily correlating with acute changes in headache frequency or response to therapies for migraine. Activation of endothelial cells by CSD might upregulate uPAR, leading to increased cleavage and release of suPAR into the circulation. Because suPAR also may be able to alter intercellular junctions [49], elevated levels might cause blood-brain barrier dysfunction, facilitating infiltration of immune cells and promoting low-grade inflammation. However, although most preclinical data indicate subtle or regionally localized barrier disruptions [35, 50–52], a human neuroimaging study found no evidence to suggest blood-brain barrier disruption in migraine with aura [53]. Additional neuroimaging studies are required to validate the findings of this study, as subtle or transient permeability changes could not be refuted [53].

Strengths and limitations

To our knowledge, this is the first study to examine inflammatory biomarkers concerning the efficacy of mAbs targeting CGRP signaling. Our study benefits from a large, well-characterized population of adults with migraine and utilizes plasma suPAR, which is highly stable even despite repeated freeze-thaw procedures [54], and shows long-term within-person stability in individuals with minimal susceptibility to immediate fluctuations (e.g., circadian rhythm, day-to-day variation, and fasting status) [6]. We considered the 24-week treatment duration likely to be adequate, as this duration is feasible for identifying the majority of individuals who experience long-term response to CGRP-mAbs [55]. Moreover, to increase data validity and reliability, we excluded participants with current use of immunological treatments and adjusted for potential confounders.

However, several limitations should be acknowledged. First, while most confounders showed no notable impact on the suPAR-response association, age and BMI appeared to have a relevant confounding effect. Sex, BMI, and smoking status were well balanced between participants with migraine and HCs; however, there was a slight age imbalance, with HCs being younger. Residual confounding and potential attrition bias—particularly regarding the elevated plasma suPAR levels in non-responders observed at Week 48—remain as limitations. Although we observed no confounding effect of presence of migraine headache, future studies employing serial measurements in the same individuals during ictal and interictal phases may be ideal to study whether suPAR varies with ictal state. Second, the two-month stable dosing requirement for concomitant preventive medications is shorter than in many randomized controlled trials, particularly for onabotulinumtoxinA. Third, for ethical reasons the REFORM study allowed acute medications, including NSAIDs, and participants were allowed to initiate non-CGRP preventive medications during the follow-up period. While such treatments could theoretically influence suPAR levels, we observed no substantial confounding effects of NSAID use or preventive medications on the suPAR–response association. Fourth, while we chose erenumab for feasibility reasons, we cannot exclude the possibility that treatment with other

CGRP-antagonists may have yielded different results. Although blood measurements are effectively suitable for assessing systemic inflammation, they do not capture localized inflammation of the CNS or meninges. Fifth, it warrants mention that the single-center design and the inclusion of a severely affected study population might not reflect broader migraine populations and thus limit generalizability. Validation in more diverse populations is therefore needed. Lastly, the single-arm design restricts the ability to evaluate predictive biomarkers due to the absence of a comparative placebo group. However, the lack of observed associations suggests this limitation is of limited relevance.

Conclusions

No association was identified between plasma suPAR levels and the therapeutic effects of erenumab for migraine prevention. Furthermore, plasma suPAR concentrations did not change significantly throughout the 24-week treatment period. The slightly higher baseline and follow-up plasma suPAR levels in non-responders with migraine aura, compared to HCs, raise the possibility of an inflammatory component associated with non-response. However, since these levels do not significantly differ from responders with migraine aura, the clinical relevance of this observation remains unclear. Furthermore, confirming this hypothesis would require additional exploration of other biomarkers and their relationship to erenumab's efficacy.

Study highlights

- Baseline plasma soluble urokinase-plasminogen activator receptor (suPAR) was not associated with efficacy of erenumab for migraine.
- Non-responders with aura had consistently higher suPAR levels than healthy controls, but no difference compared to responders.
- Although non-responders had higher suPAR levels than responders 24 weeks after discontinuation of erenumab, overall suPAR levels remained stable, suggesting minimal impact of erenumab on systemic inflammation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s10194-025-02037-9.

Supplementary Material 1

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Author contributions
WKK, MA, and HA conceived and designed the study. WKK, RHC, and HMA performed the procedures and collected the data. WKK, RHC, HMA, MA, and HA contributed to the methods of the study. WKK conducted the statistical analyses. WKK, RHC, HMA, and HA wrote the first draft of the manuscript. All authors interpreted the data and contributed to the writing of the final version of the manuscript. All authors agreed with the results and conclusions of this manuscript and had full access to all the data. HA was responsible for the decision to submit for publication.

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Data availability
Data and materials relevant for the analysis of the study are available upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate
The REFORM study received approval from both the Danish Data Protection Agency and the ethics committee of the Capital Region of Denmark (H-20033264 and H-20047793). All participants were given appropriate time to consider participation and provided written informed consent before undergoing any study-related procedures.

Consent for publication
Not applicable.

Competing interests
WKK and HMA have each received a speaker's honorarium from Pfizer and Lundbeck outside of the submitted work. RHC has received personal fees from Teva, the Lundbeck Foundation and research travel funding from Augustinus Fonden. TK and BNJ have no conflicts to report. OA is named inventor on patents covering suPAR owned by Copenhagen University Hospital Amager and Hvidovre, Hvidovre, Denmark and licensed to ViroGates A/S. MA is a consultant, speaker, or scientific advisor for AbbVie, Amgen, Astra Zeneca, Eli Lilly, GlaxoSmithKline, Lundbeck, Novartis, Pfizer, and Teva; a primary investigator for ongoing AbbVie and Pfizer trials; and is the past president of the International Headache Society. MA is supported through the Lundbeck Foundation Professor Grant (R310-2018-3711) and serves as associate editor of the Journal of Headache and Pain and associate editor of Brain. HA reports personal fees from AbbVie, Lundbeck, Pfizer, and Teva outside of the submitted work.

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