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Genetic predictors of clinical response to erenumab in patients with migraine

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SUMMARY

Abstract

Background: Erenumab is a monoclonal antibody that targets calcitonin gene related peptide (CGRP) receptor and that is used for migraine prophylaxis. Although its efficacy has been demonstrated in both clinical trials and real-world settings, a substantial proportion of patients do not achieve a clinically meaningful response. This study aimed to identify genetic predictors of erenumab response, thereby improving our understanding of the inter-individual variability in treatment outcomes.

Methods: Two independent cohorts of patients with migraine treated with erenumab were included (Lugano cohort, N = 140; BIOMIGA cohort, N = 164). Demographic and clinical characteristics were collected at baseline and the clinical response to erenumab was evaluated based on the reduction of monthly migraine days (Lugano cohort) or monthly headache days (BIOMIGA cohort). Eleven candidate single nucleotide polymorphisms (SNP) within the CGRP-related receptor genes, CALCRL and RAMP1, were selected to evaluate their association with erenumab non-response. Genotypes were determined by real-time polymerase chain reaction. Crude and adjusted logistic regression models were used to identify clinical and genetic predictors for treatment non-response. Lastly, a meta-analysis was performed to combine the genetic results from the two cohorts, estimate the overall effect size for each SNP, and assess between-cohort heterogeneity.

Results: In the Lugano cohort, age of migraine onset (p=0.06), number of failed preventive medications (p=0.01), migraine intensity at baseline (p=0.05), and baseline HIT-6 score (p=0.01) were associated with non-responder status. In the BIOMIGA cohort, only age at study enrollment (p=0.090) showed a potential association with non-response. Regarding the genetic analyses, the CALCRL rs10931283 variant in the Lugano cohort demonstrated a nominally significant association with non-responder status under the dominant model in the crude analysis (OR 0.40, 95% CI 0.18-0.91, p=0.027). This association remained significant after adjustment for clinical confounders (OR 0.32 95% CI 0.13-0.77, p=0.01) and was also observed in the allelic model (allelic model: OR 0.56, 95% CI 0.34-0.92, p=0.019), indicating a lower risk of non-response for the C allele. However, the statistical significance of such associations was lost after Bonferroni's correction for multiple testing. In the BIOMIGA cohort no nominal significant association was found between the analysed polymorphisms and the non-responder status. The meta-analysis of genetic results of the two cohorts showed no significance associations between analysed SNPs and the clinical response to erenumab.

Conclusion: None of the studied SNPs emerged to be associated with the risk of not responding to erenumab. Nevertheless, this study contributes to the growing body of evidence on the pharmacogenetics of anti-CGRP therapies and underscores the need for larger, multicentre studies integrating genomic and other omics approaches to identify reliable biomarkers of treatment response in migraine.

1. INTRODUCTION

1.1 Migraine

Migraine is a debilitating neurological disorder that affects about 14% of population worldwide. According to the Global Burden Disease (GBD) study, migraine constitutes the second among all human diseases, underscoring its impact on public health. It represents one of the leading causes of years lived with disability (YLDs) worldwide and constitutes the first cause of disability among women of childbearing age. The condition is more common in females and is often associated with a more severe clinical presentation compared to males. Furthermore, migraine can unfortunately affect both pregnancy planning and pregnancy itself, with the risk of bad outcome for both the mother and the child.[1]

The I*nternational Classification of Headache Disorders* (ICHD) provides standardized diagnostic criteria for migraine. Considering that headache disorders are heterogeneous and their clinical presentations are highly variable, this classification system has undergone several revisions over time.[2]

1.1.1 Episodic migraine

According to the ICHD-3 beta, episodic migraine is defined as occurring on fewer than 15 headache days per month and can be classified into two subtypes: migraine without aura and migraine with aura. In the first one the recurrent attacks last 4-72hours. Common characteristics are unilateral location, pulsating quality, moderate or severe intensity. Physical activity tends to worsen the symptoms, commonly associated with nausea, and/or photophobia and phonophobia. To receive a diagnosis, patients must have at least five attacks that have the following characteristics: attacks that last 4-72 hours, at least of two among unilateral location, pulsating quality, moderate or severe pain intensity, aggravation by or causing abstention of routine physical activity, at least one between nausea/vomiting or photophobia and phonophobia.

Whereas, migraine with aura is characterized by recurrent attacks, that last minutes, of unilateral or fully reversible visual, sensory or other central nervous system symptoms that onset progressively and are usually succeeded by headache and associated migraine symptoms. To make a diagnosis, patients must satisfy these two criteria: at least two attacks that have one or more of the following aura symptoms: visual, sensory, speech and/or language, motor, brainstem, retinal, and at least three of the following characteristics: at least one aura symptom spreads gradually for more than 5 minutes, two or more aura symptoms occur consecutively, each individual aura symptom last 5-60

minutes, at least one aura symptom is unilateral and positive, the aura is coincided with or followed by headache within 60 minutes. The symptoms must not be described better by another ICHD-3 diagnosis.[3]

1.1.2 Chronic migraine and medication overuse headache

Chronic migraine is defined as a headache that occurs 15 or more days/months for more than 3 months which has the characteristic of migraine headache on, at least, 8 days/months. To make a diagnosis, besides the previous definition, the headache must satisfy these criteria: it has to occur in patients who has had at least five attacks fulfill the criteria of migraine without aura and/or with aura, and on more than 8 days/months for more than 3 months patients have to satisfy any of the criteria for migraine with aura and without aura, and he headache have to be believed by the patient to be migraine at onset be alleviated by a triptan or ergot derivative.

Medication overuse headache is a headache that occurs on 15 or more days/month in a patient with a pre-existing primary headache and that is developed as a consequence of regular overuse of acute or symptomatic headache medication on 10 or more days/month for more than 3 months. In most cases, though not all, it resolves after the overuse has stopped. The diagnostic criteria are: headache that happens on more than 15 days/month in a patient with a pre-existing disorder, regular overuse for more than 3 months of one or more drugs used for acute and/or symptomatic treatment of headache, and it must not be described better by another ICHD-3 diagnosis. This diagnosis is clinically pivotal.[3]

1.1.3 Pathophysiology

Migraine is widely recognized as a multifactorial disorder resulting from the interaction of genetic, environmental, and neurobiological factors. Although genome-wide association studies have identified multiple susceptibility loci[4], no single genetic alteration with a major effect has been consistently identified, except in rare migraine subtype, such as hemiplegic migraine.[2]

From a pathophysiological perspective, a migraine attack can be divided into several phases: premonitory phase, the aura phase, the headache phase, and the postdrome phase, which can be variable and can overlap.

In the premonitory phase, symptoms arise hours before the onset of headache and they may include mood change, irritability, neck pain, polyuria and yawning. Studies of PET and MRI have revealed

changes in hypothalamic function that can be the cause of polyuria, mood change and change in the appetite. In addition, they show an increased activity in the occipital cortex.

A symptom that can start in the premonitory phase and continue through the postdrome phase is neck pain. It is a sign that the upper cervical nerves may be involved in the transmission of migraine pain.[5]

About one third of migraine attack are precede by aura. The ICHD-3 defines migraine with aura as recurrent attacks lasting minutes, characterized by unilateral, fully reversible visual, sensory or other central nervous system symptoms that usually develop gradually and are usually followed by headache and associated migraine symptoms. Aura symptoms usually are visual, but they can also be sensory, speech/language and motor disturbances.[2]

Following the aura phase, the headache one ensues. Migraine headache usually lasts 4-72 hours; pain is unilateral, pulsating, with an intensity ranging from moderate to severe and it can be worsened by physical activity.

The last phase of the migraine attack is the postdrome phase. While it is not formally mentioned in the ICHD-3 beta, recent studies have highlighted its clinical relevance and proved that it is characterized by a variety of symptoms, including fatigue, difficulties in concentrating and neck stiffness.[6]

1.1.4 Risk factors

Epidemiological studies have identified several risk factors and triggers for migraine, as it's shown in figure (1). Factors like insomnia, depression, anxiety, gastric ulcer, and/or gastrointestinal bleeding, angina and epilepsy occur more frequently in patients with migraine than in controls.[7] Moreover, the intensity and the frequency of pain are linked to biological and phycological disorders and to inflammatory processes.[8] Due to the complexity of migraine pathophysiology, distinguish between risk factors, triggers, and outcomes is often challenging.

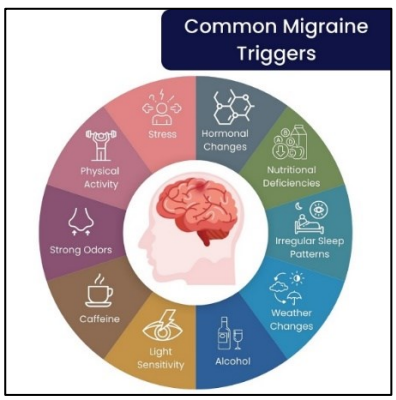
Migraine attacks are also influenced by hormones fluctuations. In particular, migraine is influenced by menstruation, pregnancy, menopause, use of hormonal contraceptive and hormone replacement therapy. Menstrual migraine is a condition that appears when the estrogen levels drop, just before the menstruation.[9,10] Another hormone that is linked with migraine is cortisol, the primary stress hormone. Also, there is a correlation between hypothyroidism and migraine.[11]

Migraine seems to be associated also with metabolic syndrome, which is a group of metabolic disorders comprising obesity, diabetes, dyslipidemia and hypertension.[12] First we have obesity, which is a risk factor for episodic and chronic migraines. This correlation is most often present in women under 55 years old, and it seems to be dependent upon gender and age.[13] Regarding diabetes there is not strong evidence about its correlation with migraine yet. A systematic review has demonstrated the protective role of type I diabetes on patients who suffer from migraine, but it didn't show any significant correlation between migraine and type II diabetes.[14] Another factor is dyslipidemia, which contributes to the development and intensification of migraine attacks and to the increased risk of cardiovascular diseases.[15]

Genetic factors contribute to the development of both migraine with and without aura. Common forms of migraine have a polygenic nature, but rare types of monogenic forms do exist. The polygenic nature of complex traits explains why many genetic loci contribute modest yet additive effects to the pathophysiology of migraine.[16] The combination of the results of 22 Genome-Wide association studies (GWAS) from the International Headache Genetic Consortium (IHGC) has revealed thirty-eight distinct genomic loci, 28 of which had not been previously identified. The expression analysis of these genes has proven two most strongly enriched tissues, which are the aorta and tibial artery in the cardiovascular system and the smooth muscles of the esophagus and the esophageal mucosa in the digestive system.[17]

Studies have also showed that there is an increased risk of addition and substances abuse in migraneous people. For instance, alcohol is one of the first 10 triggers in migraine.[18] Other factors, such as age and low educational status are the most relevant demographic factors for migraine chronification .[19] Migraine is also linked to several psychological factors, such as the tendency towards perfectionism, neuroticism, repressed aggression and melancholic mood.[20]

Figure (1). Migraine triggers.[21]



1.1.5 Therapy

The treatment for migraine is divided into acute (abortive) and preventive (prophylactic) approaches. The aim of acute treatment is to stop migraine progression, which must be treated quickly and with a large single medication dose. The traditional therapy includes non-steroidal inti-inflammatory drugs, antiemetics, triptans, ergot alkaloids. Non-steroidal ant inflammatory drugs are prescribed for mild-to-moderate attacks, without nausea and vomiting.[22] Triptans have been the most effective drugs for the past 25 years, but they are contraindicated for patients with cardiovascular disease, cerebrovascular disease, uncontrolled hypertension or hemiplegic migraine, because they have vasoconstrictive effects on peripheral vessels, comprising cardiac vessels. This vasoconstriction is caused by the non-selective binding to 5-$HT_{1d,}$ 5-HT_{1b} receptors and by a varying affinity to 5-HT_{1f} receptors. In the past few years new drugs have been developed to improve migraine acute therapy, while avoiding vasoconstriction: ditans and gepants. The former binds selectively to 5-$HT_{1f,}$ while the latter is a calcitonin gene related protein (CGRP) antagonist. [5,22, 23,24,25]

On the other hand, the aim of preventive treatment is to reduce migraine frequency, improve responsiveness to the severity and the duration of acute attacks and reduce disability. This kind of treatment is indicated when there are frequent or long-lasting headaches, attacks that cause significant disability and reduce the quality of life, when there are contradictions or failures with acute treatment, when there are side effects from abortive therapies, menstrual headache, hemiplegic migraine, brainstem aura migraine, persistent aura without infraction, migraineurs infraction. Among preventive treatment there are traditional drugs, like beta blockers, tricyclic antidepressants, anticonvulsants, local anesthetics, and calcium channel blockers. These drugs were indicated for other pathologies, and only later they were shown to be effective in migraine. In recent years, more specific drugs have been approved for migraine: onabotulinumtoxinA, gepants and anti-CGRP monoclonal antibodies. Botulinum toxin onabotulinumtoxinA is used for treating chronic migraine. Its mode of action is not fully understood yet, but it has been thought that it can relieve pain, by inhibiting the release of neuropeptides, like CGRP and substance P, which have a role in the initiation of migraine. Gepants are used also for preventive treatment. An important shift in migraine preventive therapy happened with the advent of monoclonal antibodies. They are specific therapies for the prevention of episodic and chronic migraine. They do not cross the blood brain barrier, they are metabolized by the reticuloendothelial system and they are not correlated to liver toxicity. They are administered monthly or quarterly because they have a long half-life and they are given parentally (IV or SC). The first monoclonal antibody approval was erenumab in 2018, both by

FDA and EMA[26]. The second approved drug was fremanezumab, put in the market in some months after the first one[27], the third one is galcanezumab[28] and the last approved drug is eptinezumab, released in the market in 2020. Erenumab differs from the others in terms of pharmacodynamics because it is a CGRP receptor antagonist while the others directly bind to CGRP, thus avoiding its binding with the receptor.[5,22,23,24,29]

In addition to pharmacological treatments, neuromodulation techniques are employed in the management of chronic migraine. These include deep brain stimulation (DBS), sphenopalatine ganglion stimulation (SPGS), vagal nerve stimulation (VNS), transcranial magnetic stimulation (TMS), occipital nerve stimulation (ONS), and supraorbital nerve stimulation (SNS).[30]

However, several pharmacology treatment options can have limited efficacy or they can give severe adverse effects, resulting in patients discontinuing the therapy. So, there is a growing need for safe, non-pharmacological treatment. These interventions are intended to complement, rather than replace, pharmacological therapy, and can enhance overall treatment effectiveness when used in parallel with standard medical care. Lifestyle factors have a crucial role in migraine, and stress is an important trigger for migraine. For this reason, mind-body treatments may be important to manage migraine. Some examples include meditation, mindful meditation yoga, biofeedback, and deep breathing exercises. Even though these practices don't cure migraine, studies have shown that they can decrease migraine frequency and improve migraine-related disabilities.[31]

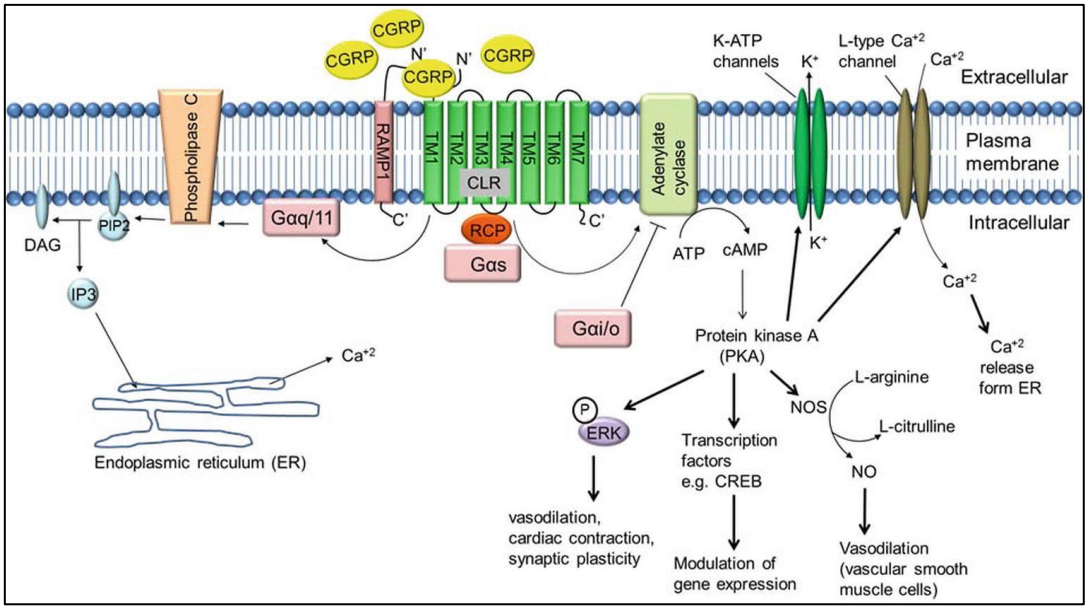
Despite the availability of multiple therapeutic options, a substantial proportion of patients experience inadequate response or poor tolerability, highlighting the need for more effective and personalized treatment strategies. Even with the newest migraine-specific therapies for migraine prevention -monoclonal antibodies against CGRP- approximately 50% of the patients do not have a beneficial effect. To better understand this variability in treatment response, the following chapter will review the role of CGRP in migraine pathophysiology.[5,32]

1.2 Calcitonin Gene-Related Peptide (CGRP)

Calcitonin gene related peptide (CGRP) is a 37-amino acid neuropeptide produced by alternative splicing of calcitonin gene on chromosome 11. It is a transmitter released from thin, perivascular, type A-delta and C sensory nerve fibers after the activation of transient receptor potential vanilloid 1 (TRPV1) membrane receptor. It has two isoforms: alfa and beta. In the nervous system, α-CGRP is the most expressed, while β-CGRP is mainly linked to the enteric sensory system.[6,32]

CGRP acts as two receptor complexes. It can bind to canonical G-protein coupled calcitonin receptor-like-receptor at membrane of vascular smooth muscle and endothelial cells. The receptor forms a heterodimer with another membrane protein, receptor activity modifying protein-1 (RAMP1), which facilitates binding of CGRP, as shown in figure (2). The other receptor is amylin 1 (AMY 1) which is coupled with the calcitonin receptor (CTR) and also for this one RAMP 1 is needed. The result after the activation of CRLR/RAMP1 or AMY1 is the increase of cyclic adenosine monophosphate (cAMP). Studies done in rodents showed that CGRP receptor is widely expressed throughout the brain. A study on human cerebral and middle meningeal arteries has proved the presence of CLR and RAMP1, 2, 3 in the vascular smooth muscle cells. All the three isoforms are expressed, but RAMP1 determines the receptor phenotype. RAMP1 and CALCRL are present in the cytoplasm of trigeminal neurons, at peripheral sites on the intracranial vasculature, in the *dura mater*, and in brainstem. CLR and RAMP1 are not present in most of CGRP-containing neurons, but they are co-expressed on CGRP-negative, large neurons on satellite glial cells, which can be the sign of intra-ganglionic communication.[6,32,33]

Figure (2). CGRP pathway.[34]



CGRP is a potent vasodilator, and it plays a central role in migraine attacks, although the exact triggering mechanisms remain unclear. CGRP can cause relaxation of smooth cells directly with the activation of PKA or indirectly with the generation and release of nitric oxide, which diffuses to smooth muscle cells and increases cyclic guanosine-monophosphate (cGMP).[6,32]

Neuroimaging techniques have allowed researchers to identify several brain regions as midbrain, pons, substantia nigra, red *nucleus*, periaqueductal gray, *nucleus raphe* magnus and *locus coeruleus* that are activated during migraine attack. CGRP is highly expressed in *striatum*, *amygdalae, colliculi, cerebellum* and cortex. The role of CGRP and its receptor in the central nervous system is therefore very complex and multifaceted.

Other clinical trials demonstrated that during migraine attack plasma levels of CGRP are increased. In patients with chronic migraine plasma levels are always high. CGRP causes sensitization of the nociceptors, hyperalgesia, and facilitation of sensing pain. It was thought that migraine may be a consequence of peripheral and central of neurons involved in perception of pain caused by CGRP. It is a relatively large peptide that cannot pass through blood-brain barrier. Consequently, its action is probably limited to the peripheral nervous system or area of the brain without barriers, even though the receptors are present both in central and peripheral nervous system. In the periphery, CGRP is expressed in neurons of the trigeminal ganglion. Probably, CGRP has a positive feedback action on the same neurons from which it is released. Among its various biological roles, sensitization of nerve pathways involved in perception of pain seems to be the most directly implicated in pathophysiology of migraine.

To contrast the effect of CGRP in patients with migraine, several CGRP antagonists have been developed. Gepants are small molecules that block the CGRP receptor and, by doing so, they inhibit vascular nociceptive transmission and thalamic trigeminal nociceptive activation. However, there are still some points that must be considered: two of them- zavegepant and ubrogepant- have a short half-life, so the use as an acute treatment is limited. The other two- atogepant and rimegepant- that have a longer half-life, are limited by the need of daily oral intake. An alternative way to modulate the CGRP activity is represented by the recent development of monoclonal antibodies that target the CGRP ligand or the CGRP receptor. Galcanezumab, fremanzumab and eptinezumab are directed against GCRP itself, whereas erenumab is the only one now available that targets the CGRP receptor. They are studied to be used for preventive treatment of frequent episodic migraine and chronic migraine. Due to their large molecular size, monoclonal antibodies act on peripheral sites, including *dura mater* and in its blood vessels, where they accumulate.[6,24,32]

Moreover, respect to gepants, monoclonal antibodies have a longer half-life, allowing longer dosing intervals. Lastly, they are not eliminated through hepatic, renal or biliary processes, which is linked to a decreased risk of drug-drug interactions.[32]

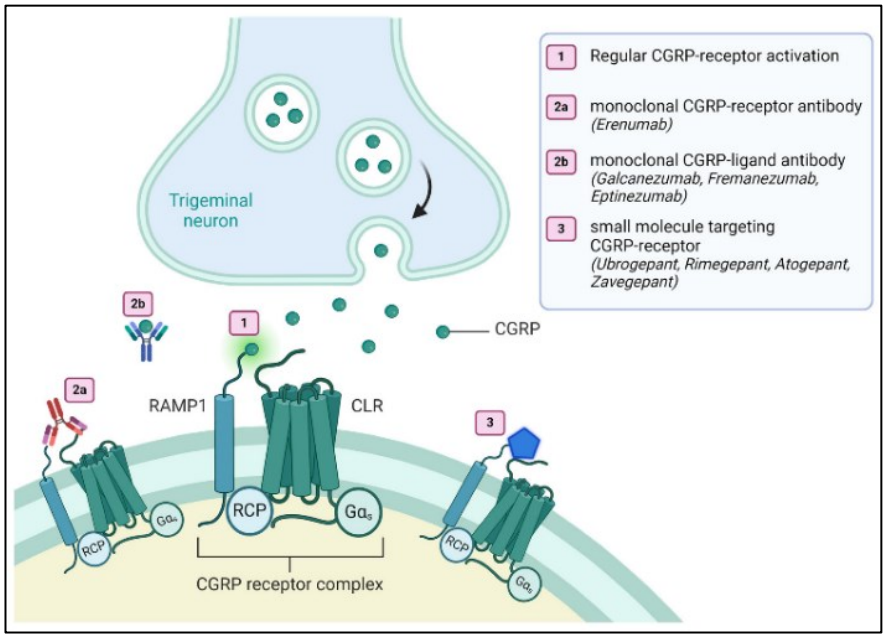
The following chapter provides an in-depth analysis of the pharmaceutical profile of erenumab, the study drug investigated in the pharmacogenetic analysis presented in this thesis.

1.3 Erenumab

Erenumab is a fully human IgG2 monoclonal antibody calcitonin gene-related peptide CGRP receptor antagonist. It has been approved by both "Food and Drug Administration" (FDA) and "European Medicines agency" (EMA) for the prophylaxis of migraine in adults experiencing at least 4 migraine days per months. The recommended dose is 70 mg or 140 mg subcutaneously once a month, and clinical improvement usually is achieved within 3 months of treatment initiation.[36]

From a pharmacodynamic perspective, erenumab competes potently and specifically with CGRP for receptor binding and inhibit its activity at CGRP receptors, which are located in key sites for migraine pathophysiology, as the trigeminal ganglion, as it's shown in figure (3). CGRP is a vasodilator neuropeptide involved in nociceptive signaling. During migraine attacks CGRP levels increase; therefore, its inhibition should attenuate the vasodilatation and pain transmission. [36]

Figure (3). Mechanism of erenumab.[37]



Erenumab was evaluated for prophylaxis of migraine in two studies in chronic[38] and episodic migraine.[39] All the enrolled patients had at least a 12-months history of migraine. In the chronic migraine trial, erenumab produced a significant reduction in monthly migraine days compared with placebo, with evidence of clinical benefit starting from the first week of administration. In the

episodic migraine trial, the frequency of migraine decreased from month 4 to 6 compared to patients received placebo.

Erenumab shows a non-linear kinetics at low concentrations, whereas at therapeutically relevant doses its pharmacokinetic profile is linear. Subcutaneous injections of 140 mg dose or 70 mg dose once a month in healthy volunteers show a C_{max} of 15.8 μg/ml and 6.1 μg/ml, respectively. Median peak serum concentrations were reached within 4 to 6 days, and estimated absolute bioavailability was 82%. The mean (SD) volume distribution during the terminal phase was estimated to be at 3.86 L. Erenumab has a dual elimination phase: at low concentration the elimination occurs through saturable binding to target, while at higher concentration is through a non-specific proteolytic pathway. The effective elimination half-life is 28 days.

Non-clinical data showed no hazard for humans based on studies of safety pharmacology, repeated-dose toxicity, toxicity to reproduction and development. Erenumab has not been tested for carcinogenicity and neither for its possible mutagenic potential. However, monoclonal antibodies are not expected to alter DNA or chromosomes.

The most commonly reported adverse reactions include hypersensitivity reaction (anaphylaxis, angioedema, rash, swelling/edema and urticaria), constipation, oral sores, pruritus, alopecia, rash, muscle spasms, and injection site reaction. During the double-blind treatment phase of clinical studies, the incidence of the development of anti-erenumab antibody was 6.3 % among patients who received 70 mg dose and 2.6 % among patients who received 140 mg dose. There was no impact of anti-erenumab antibody development in the efficacy or safety of erenumab. In clinical studies, there weren't any overdose cases.

Erenumab is contraindicated in patient with cardiovascular disease, and it's not approved for use in adolescents under 18 years old.[40]

Despite its potential, clinical trials have showed that about 50% of patients didn't reach the end point of a reduction in "migraine monthly days" (MMDs) of at least 50%. The treatment response may be correlated by demographic, clinical characteristics of the patients, but also genetics may play an important role. Specifically, some polymorphisms in the two genes that form the CGRP receptor, CALCRL and RAMP1, may change the efficacy of erenumab. So, in this context, pharmacogenetic is useful to predict the success or the failure of this monoclonal antibody.[41]

1.4 Pharmacogenetic

Pharmacogenetic is the study of how genetic variations influence individual responses to pharmacological treatments. Both pharmacogenetic and pharmacogenomic approaches aim to identify genetic predictors of therapeutic efficacy and tolerability, with the ultimate goal of optimizing treatment selection and improving clinical outcomes. [42]

Given that erenumab exerts its effect by targeting the CGRP receptor, genetic variability in the genes encoding its components can be hypothesized to influence drug response. In this context, our research group conducted three multicenter, observational, prospective pharmacogenetic studies enrolling patients aged 18 to 70 who suffered of migraine for at least one year and treated with erenumab in Lugano between 2019 and 2020, enrolling. In the first study, clinical and demographic data of patients were collected and only the patients who responded to the treatment were evaluated. Patients received a baseline evaluation the day they were given the first erenumab 70 mg injection and another evaluation after three months. Then patients were divided according to the response. One group was made of patients who showed a reduction of $\geq$ 50% (50-RESP) in MMDS at month 3 of erenumab treatment and the other group in which there were patients who showed a reduction of $\geq$ 75% (75-RESP). The aim was to find clinical and genomic features (single nucleotide polymorphisms in CALCRL and RAMP1 genes) associated with a reduction of monthly migraine days by at least 50% or 75%.

Regarding clinical factors, our group found that a lower number of failed preventive medications, a higher migraine disability assessment MIDAS score, and an older age at migraine onset were associated with the 75% responder status. In contrast, no significant association was observed between genetic variants and either 75% or 50% -responder status after adjustments for relevant clinical variants. However, univariate logistic regression analysis showed an allele-dose- dependent association between RAMP1 rs7590387G allele and a lower probability of being 75-responder.[41]

In the second study, we investigated the association between clinical and genetic factors (polymorphisms in CALCR and RAMP1 genes) and the response to erenumab, which was determined based on a clinically meaningful improvement on the headache impact test 6 (HIT-6) score. Regarding the genetic factors, the study showed that the presence of the SNPs CALCRL rs6710852G and RAMP1 rs6431564G minor alleles is associated with respectively three- and two-times higher probability of being a HIT-6 responder. In contrast, minor alleles rs13386048A and rs12465864G in RAMP1 gene each decreased the chance of 50%. [43]

In the third and last study that we conducted, the aim was to find clinical and genetic predictors associated to non-responder to erenumab in a larger cohort compared to the ones used in the two previous studies. The main findings were that hypertension, smoking, insomnia treated with medication, and RAMP1 rs6431564 polymorphism were associated to non-responders to erenumab.[44]

No other pharmacogenetic or genomic studies have been published in the field.

2. AIM OF THE THESIS

Although erenumab has demonstrated efficacy in migraine prevention, nearly 50% of patients enrolled in clinical trials failed to achieve a satisfactory therapeutic response. Interindividual variability in erenumab outcomes may be influenced by a combination of several factors, including patients' demographic and clinical characteristics, as well as their genetic background. Identifying predictors of treatment response is therefore crucial to support a more personalized therapeutic approach and to optimize patient management, ultimately reducing both patient burden and healthcare costs.[41,43,44]

A very limited number of genetic variants have been proposed by previous studies as potential predictors of response to anti-CGRP therapies, leaving the pharmacogenetics of erenumab largely unexplored and the available evidence scarce and inconsistent. Therefore, the primary aim of this thesis was to evaluate the association between 11 candidate single nucleotide polymorphisms (SNPs) located in two genes encoding the CGRP receptor and treatment response to erenumab in patients with migraine. Specifically, the study sought to identify genetic variants associated with an increased risk of treatment non-response, with the ultimate goal of contributing to the development of predictive models that may facilitate individualized treatment in clinical practice.

3. MATERIALS AND METHODS

3.1 Approval of Ethics Committees

The involved studies conform with the World Medical Association Declaration of Helsinki and was approved by the local ethics committees. Written informed consent was obtained from all participants.

3.2 Migraine cohorts

This investigation included two cohorts of patients. The first one enrolled consecutive migraine patients started with ERE at two Swiss tertiary headache center (Neurology department, Ospedale Regionale di Lugano; Neurology department, Inselspital Bern) between December 2019 and September 2020. Patients were aged between 18 and 70 years old and suffered from migraine according to *International Classification of Headache Disorders* for at least one year. According to Swiss reimbursement criteria for ERE, patients were required to submit a prospectively maintained diary showing at least 8 days with migraine per months for at least 3 months, with evidence of inadequate response to at least two of all classes of preventive migraine treatment or intolerance/contraindications to all there, comprising anticonvulsants, β blockers or calcium channel antagonists. Main exclusion criteria were migraine onset over 50 years of age, a history of hemiplegic migraine or other primary headache disorders other than migraine, botulinum injection within four months prior to inclusion or having had a neck trauma within the past six months. Patients who did not complete follow up were excluded as well. Patients were evaluated the day they received the first ERE 70 mg, and then there was a follow up evaluation after 3 months according to routine clinical practice. During all this time, patients kept filling up a headache diary. At baseline, socio-demographic characteristics, like age, sex, body mass index, working status, lifestyle habits (alcohol, smoking, physical activity, sleep habits) and migraine history, like age at migraine onset, migraine type, presence of aura, number of failed preventive medications, number of first-degree relatives affected by migraine, lifetime presence and type of comorbidities and concomitant medications were gathered. In this period, blood samples were collected for genetic tests. The monthly number of days with triptans/non-steroidal analgesic use during the 3 months before and after ERE start (MMDs) was found in the patient's headache diary. Presence overused was evaluated according to the definition reported in the *International Classification of Headache Disorder.* Mean pain intensity-assessed via a numerical rating scale scored from 1 (no pain) and 10 (unbearable pain)- and mean attack duration were additionally recorded at baseline and at 3-month

follow-up after ERE initiation. Migraine related disability and its impact on daily life were studied through two validated questionnaires, the Migraine Disability Assessment (MIDAS) scale (score 1-5: little or no disability, 6-10: mild disability, 11-20: moderate disability, >21: severe disability) and Headache Impact test (HIT) (score <49: little or no impact, 50-55: some impact, 56-59: substantial impact), filled out by patients. Adverse events spontaneously reported by patients throughout the study period as well as those elicited by the neurologists at 3-month evaluations were recorded and classified according to the severity (mild, moderate, severe) and seriousness (if requiring/prolonging hospitalization or causing permanent disability or death). Patients were categorized as responders to ERE according to two definitions that are commonly used in clinical trials: 50% responders (50-RESP) were those patients who showed a reduction of ≥50% in MMDs at month 3 of ERE treatment compared to the baseline MMDs, which is 3 months before ERE start, while non-responder were those patients that showed a reduction of ≤50% in MMDs, after 3 months.[41,44]

The second cohort is called BIOMIGA (i.e., BIOmarkers of MIGrAine), which involved 164 patients enrolled across three headache centres: the Fondazione IRCCS Mondino (Italy), the Vall d'Hebron University Hospital (Spain) and the University of Hamburg (Germany). The inclusion criteria were: adults, both males and women, between 25 and 55 years of age, patients who suffer from high frequency migraine (HFM; i.e. patients having 8 or more migraine days per month), or chronic migraine with or without aura (i.e. patients having more than 15 headache days migraine, 8 of which have migraine characteristics, in accordance with the third edition of International Classification of Headache Disorders), postmenopausal women for at least one year, or surgical sterile or unable to conceive, or women who use a contraceptive. For the clinical population the exclusion criteria were having headache on more than 25 days per month in the last 3 months, and reporting medication overuse in accordance of ICHD-3 criteria. While for the entire population the exclusion criteria were: having other relevant pathologies, like neurological disorder, severe psychiatric disorders or cardiovascular disease, addiction or abuse of alcohol (>100 g/week), of smoking or of drugs within 12 months before V1, disclosed by medical reports or by patients, pregnant or breastfeeding women, and women who can conceive and who are not on birth control, and people who use also other migraine preventive drug together with erenumab. They received 3 consecutive cycles of treatment with erenumab for 3 months, one each month. Patients have been put into two classes based on the percentage of reduction of migraine days: responders (R) if a reduction of 50% of monthly headache days (MHD) was obtained in the last months of treatment with erenumab, compared to the initial control month; Non-responders (NR) if a reduction of at least half of the

attacks was not achieved. Participants were also stratified according to sex, and migraine subtype: chronic migraine (CM), and episodic migraine (EM).[46,47]

3.3 DNA extraction

A whole blood sample was collected from each patient in EDTA tubes labelled with a unique alphanumeric code. Regarding the Lugano cohort, whole blood samples were directly sent to our laboratory, and genomic DNA was extracted by laboratory staff using a commercial extraction kit (ReliaPrep Blood gDNA Kit, Promega), according to the manufacturer's instructions. In cases where the available DNA quantity was insufficient for genotyping analyses, genomic DNA was re-extracted from blood samples following the manufacturer's protocol. For the BIOMIGA cohort, genomic DNA was extracted from blood samples by hospital personnel and subsequently sent to our laboratory for single nucleotide polymorphism (SNP) genotyping analyses.

3.4 Genotyping

A total of 11 common single nucleotide polymorphisms (SNP) of CALCRL and RAMP1 genes (table 1) were selected after a bioinformatic analysis using GTEx portal (https://www.gtexportal.org/home/, accessed September 2025). Single tissue expression quantitative trait loci (eQTL) data were obtained for the CALCRL (Ensembl ID: ENSG00000064989) and RAMP1 gene (ENSG00000132329). Then, polymorphisms significantly associated with CALCRL and RAMP1 gene expression regulation were selected based on their nominal p-values across tissues. Specifically, genetic variants showing the strongest association with CALCRL or RAMP1 expression in the tibial artery were prioritized, as this vascular tissue was considered the closest available surrogate for the trigeminal vasculature, which is of particular relevance to migraine pathophysiology.

Lastly, SNPs were selected ensuring the absence of linkage disequilibrium between variants, applying a threshold of $r^2<0.1$ and $D'=0$. Linkage disequilibrium refers to the non-random association between alleles at different genomic loci. Some haplotypes occur more or less often than expected under independence. It doesn't necessarily require proximity. To measure it, three metrics are required: D, which represents the raw difference between observed and expected haplotype frequencies, D' which normalizes D against its theoretical maximum, producing a standardized value ranging from 0 to 1 and r^2, which expresses the squared correlation coefficient between alleles at two loci, providing a measure of how reliably one variant can predict another.[48]

Table (1). General information of the selected SNP.

Gene	SNP	SNP Type	TaqMan assay	VIC/FAM	Position	p-value of genetic expression
RAMP1	rs10199956	Transversion substitution	C__30171509_10	G/T	chr2:237907946 (GRCh38.p14)	$6,9*10^{-6}$
CALCRL	rs17705966	Transition substitution	C__32777752_10	A/G	chr2:187300439 (GRCh38.p14)	$2,5*10^{-84}$
	rs62173500	Transversion substitution	C__88435198_10	A/C	chr2:186991816 (GRCh38.p14)	$7,8*10^{-40}$
	rs13384908	Transition substitution	C__30901318_10	G/A	chr2:187325748 (GRCh38.p14)	$4,1*10^{-75}$
	rs7589163	Transition substitution	C__30005849_10	C/T	chr2:187372092 (GRCh38.p14)	$3*10^{-26}$
	rs13418253	Transversion substitution	C___2671079_10	A/T	chr2:187457707 (GRCh38.p14)	$1,7*10^{-30}$
	rs8176583	Transversion substitution	C___2671094_10	G/T	chr2:187469674 (GRCh38.p14)	$1,7*10^{-30}$
	rs3821184	Transversion substitution	C__30901279_10	C/G	chr2:187384693 (GRCh38.p14)	$8,9*10^{-27}$
	rs10207431	Transversion substitution	C__30456136_10	A/C	chr2:187286460 (GRCh38.p14)	$2,1*10^{-78}$
	rs10931283	Transition substitution	C__26197319_10	C/T	chr2:187346716 (GRCh38.p14)	$2,1*10^{-22}$
	rs78925680	Transition substitution	C__99898522_10	A/G	chr2:187610251 (GRCh38.p14)	$6*10^{-20}$

Abbreviations: SNP, single nucleotide polymorphism.

Genotyping of CALCRL and RAMP1 were performed by Real Time Polymerase Chain Reaction using Applied Biosystem TaqMan pre-designed SNP genotyping assay (figure 4). Real-time PCR is a technique which allows amplification of a target nucleic acid sequence in a biological sample by

using specific primers designed to flank the target sequence: they are short DNA sequences that serve as the starting points for DNA synthesis by the PCR enzyme (DNA polymerase). For a given SNP of interest, the TaqMan discrimination allelic assay includes in the reaction mixture two additional fluorescent oligonucleotide probes, which differ in sequence only at the SNP site.

The TaqMan real time PCR is based on 5' nuclease chemistry, which uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR. Each TaqMan probe is labelled with a fluorescent Reporter (FAM or VIC) at the 5' end, i.e., a high-energy fluorochrome that emits fluorescence, and a Quencher (NFQ) at the 3' end, i.e., a low energy fluorochrome that absorbs the Reporter's fluorescence. As the target sequence is amplified, the reporter molecule emits fluorescence signals that are detected by the thermal cycler detector. When the Reporter is positioned close to the Quencher, the Quencher switches off the effect of the Reporter because the Quencher absorbs the Reporter's photons. Whereas, during the annealing/extension step of the amplification reaction, the probe hybridized to the target, and it is broken by the Taq-polymerase 5'-3' exonuclease activity. The cleavage of the oligonucleotide probe separates the Reporter from the Quencher molecule, resulting in the release of fluorescence. When the probe is intact, the proximity of the quencher dye to the reporter dye suppresses the reporter fluorescence. The real-time PCR instrument measures the fluorescence emitted by the Reporter molecule after each amplification cycle. The fluorescence intensity is directly proportional to the amount of amplified DNA. As the polymerase reaction continues, and thus as the cycles increase, the amplified sequences concentration increases and thus the fluorescence emission generated by the Reporter increases.[49]

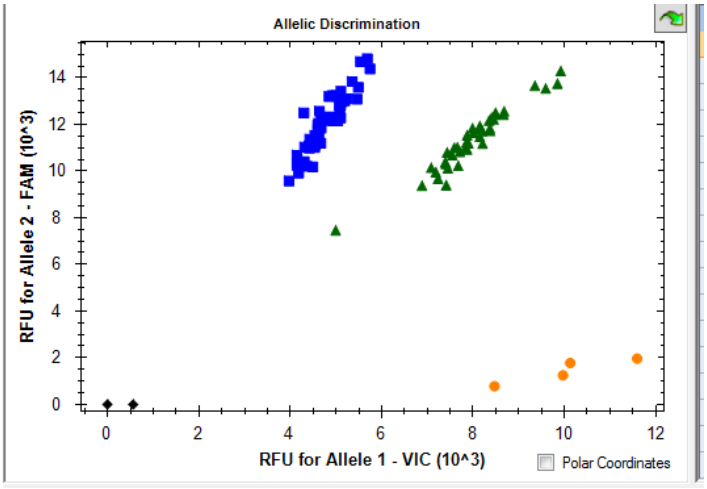
In the present study, SNP genotyping by real-time PCR was performed in 96-well PCR white plates using a CFX Connect Real-Time PCR Detection System (Bio-Rad, Milan, Italy). It was conducted in a final volume of 10 µL, consisting of 5 µL TaqPath ProAmp Multiplex Master Mix (Applied Biosystems), 0.25 µL SNP Assay 40X (containing TaqMan assay and specific primers and probes for the analyzed polymorphism), 3.75 µL H2O and 1 µL of genomic purified DNA sample. For each plate, 3 negative control samples were added, in which DNA was omitted, as well as positive control samples, using known genotype DNA samples.

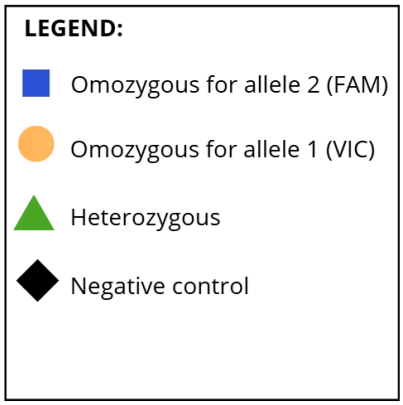
For all the SNPs investigated, PCR amplifications were conducted using the following schedule: 95°C for 10 minutes (initial denaturation phase), followed by 50 cycles of denaturation (92°C for 15 seconds) and annealing/extension (60°C for 90 seconds).

At the end of the real-time PCR, it was possible to assign the genotype via the Bio-Rad CFX Manager Software (Version 3.1) program using the RFU (Relative Fluorescence Unit) mode. Figure X shows the three genotypes groups: firstly, sample group with the FAM signal represents the group of individuals with a homozygous genotype for Allele 2 (y-axis). Secondly, sample group with the VIC signal consists of the individuals with a homozygous genotype for Allele 1 (x-axis). Finally, the third group comprises samples with both VIC and FAM signals, i.e., heterozygous individuals for the variant. Samples with absence of both VIC and FAM signals are the negative controls. As a representative allelic discrimination plot, figure (4) shows the results obtained for the SNP rs78925680 of CALCRL gene.

For validation, about 10% of the samples were re-genotyped the results were reproducible with no discrepancies in genotyping. Genotyped were performed blinded to all clinical data.

Figure (4). Example of Real-Time PCR plot.





3.5 Statistical analysis

3.5.1 Demographic and clinical characteristics

Categorical variables were presented as counts and percentages. Continuous variables were presented as mean ± standard deviation (SD) when normally distributed and as median with interquartile range (IQR) when not normally distributed. Differences between the two patient groups (responders vs non-responders) in the distribution of categorical clinical variables were tested using the chi-square test and the Yates chi-square test when required, while differences between groups in continuous variables were assessed using the t-test for independent samples or the Mann-Whitney U test when appropriate. Statistical analyses on clinical data have been conducted by using CompareGroups, a freely available online software tool (https://www.datarus.eu/aplicacion/comparegroups/, accessed June 2026).

3.5.2 Association analysis between genetic variants and the risk of being not-responder to erenumab

Crude (unadjusted) and adjusted odds ratios (ORs), with corresponding 95% confidence intervals (CIs), were estimated under additive, dominant, and recessive genetic models by comparing genotype distributions between responders and non-responders. Adjusted analyses included clinical variables showing a P value <0.1 in the corresponding univariable logistic regression analyses. Associations with a P value <0.05 were considered nominally significant. To account for multiple testing and reduce the risk of false-positive findings, Bonferroni correction was applied, resulting in a significance threshold of P <0.0045 (0.05/11 SNPs). Genetic association analyses were performed using SNPStats (freely available online at https://www.snpstats.net/start.htm, accessed June 2026). For each polymorphism, the deviation from Hardy-Weinberg equilibrium (HWE) was evaluated using a Chi-squared goodness-of-fit with one degree of freedom, using an online calculator (https://ug.sebc.me/labs/hwe-calculator, accessed up to May 2026). A p-value of <0.05 was used as a threshold for deviation from HWE. The Hardy-Weinberg equilibrium is a mathematical model and it's a pivotal concept in population genetics. It states that, in an ideal population, genotype and allele frequencies will be maintained across generations. It establishes a null model against which deviations in in real-world populations can be evaluated. It's a useful reference point to study the mechanisms that drives or constrains the genetic change within a population. This principle is based on a single biallelic locus where the two alleles are conventionally denoted as A (dominant) and a (recessive) and they respected frequencies are represented by p and q. Since they are the only two alleles, their sum must be 1: p+q=1. Following Hardy-Weinberg equilibrium, the

expected genotype frequencies for homozygous dominant (A/A), heterozygous (A/a), and homozygous recessive (a/a) individuals are p2, 2pq, q2 respectively, leading to the fundamental equation p2+2pq+q2=1.[50]

3.5.3 Meta-analysis of genetic results

Owing to differences in the definition of treatment response to erenumab between the two cohorts, a pooled analysis of individual-level data was not feasible. Therefore, a meta-analysis was performed to combine the effect estimates derived from each cohort while accounting for potential between-study heterogeneity. Odds ratios (ORs) and their corresponding 95% confidence intervals were combined using a random-effects model, which assumes that the true effect size may vary across studies. Effect estimates were weighted using the inverse-variance method. To measure heterogeneity, the chi-square-based Cochran's Q test and heterogeneity index (I^2) were used. I^2 values of 0–25%, 26–50%, 51–75%, and >75% were interpreted as indicating insignificant, low, moderate, and high heterogeneity, respectively. Results were visualized using Forest plot. Meta-analyses were performed using the Meta-Analysis Online software (freely available at https://metaanalysisonline.com/).

4. RESULTS

4.1 Study population

4.1.1 Lugano cohort

A total of 140 patients were included in the analysis. The overall population was predominantly female (85.0%), with a median age of 48.5 years (IQR 37.2–55.0) and a median BMI of 23.3 (IQR 20.9–25.8), indicating a generally normal-weight cohort. Migraine onset occurred early in life, with a median age at onset of 15 years (IQR 12–20). At baseline, 52.9% of patients presented with chronic migraine, while 47.1% had episodic migraine. The burden of disease was substantial, with a median of 15 monthly migraine days (IQR 10–24), a median MIDAS score of 46 (IQR 26–83.5), and a median HIT-6 score of 67 (IQR 64–70), indicating severe impact on daily functioning at baseline. Patients had previously failed a median of three preventive treatments (IQR 2–5), and 69.1% were receiving at least one preventive therapy at baseline. Comorbid conditions were frequent, including insomnia (52.9%), anxiety (49.6%), depression (51.1%), chronic pain (25.2%), and snoring (26.8%), while hypertension was present in 15.9% of cases.

Table (2). Univariate association analysis of clinical variables in the Lugano cohort.

	All patients *N=140*	**Non-Responders** *N=57*	**Responders** *N=83*	**p value**
Gender, n (%):				0.348
Female	119 (85.0%)	46 (80.7%)	73 (88.0%)	
Male	21 (15.0%)	11 (19.3%)	10 (12.0%)	
Age, median (Q1-Q3)	48.5 (37.2-55.0)	47.9 (39.7-53.3)	49.3 (32.4-55.4)	0.991
Age at migraine onset, median (Q1-Q3)	15.0 (12.0-20.0)	14.0 (12.0-18.5)	17.0 (14.0-20.0)	**0.063**
BMI, median (Q1-Q3)	23.3 (20.9-25.8)	23.6 (21.0-27.1)	23.2 (20.9;25.1)	0.231
Migraine form at baseline, n (%):				0.211
Episodic	66 (47.1%)	31 (54.4%)	35 (42.2%)	
Chronic	74 (52.9%)	26 (45.6%)	48 (57.8%)	
Number of failed preventive medication, median (Q1-Q3)	3.00 (2.00-5.00)	4.00 (2.00-6.00)	3.00 (2.00-4.00)	**0.010**

	All patients *N=140*	Non-Responders *N=57*	Responders *N=83*	p value
Number of preventive drugs currently in use, n (%):				0.426
No	43 (30.9%)	15 (26.3%)	28 (34.1%)	
Yes	96 (69.1%)	42 (73.7%)	54 (65.9%)	
Number of monthly migraine days at baseline, median (Q1-Q3)	15.0 (10.0-24.0)	13.0 (10.0-26.0)	15.0 (12.0-22.0)	0.529
Number of days with triptan use at baseline, median (Q1-13)	5.00 (0.00-10.0)	4.00 (0.00;9.00)	5.50 (0.00;12.0)	0.347
Intensity of migraine at baseline, median (Q1-Q3)	8.00 (7.00-9.00)	8.00 (7.00-8.00)	8.00 (8.00-9.88)	**0.056**
MIDAS score at baseline, median (Q1-Q3)	46.0 (26.0-83.5)	46.0 (24.2-75.2)	46.5 (26.5-94.8)	0.450
HIT-6 score at baseline, median (Q1-Q3)	67.0 (64.0-70.0)	66.0 (63.8-68.0)	68.0 (65.0-72.0)	**0.019**
Comorbidities				
Insomnia, n (%):				0.318
No	65 (47.1%)	23 (41.1%)	42 (51.2%)	
Yes	73 (52.9%)	33 (58.9%)	40 (48.8%)	
Anxiety, n (%):				0.918
No	70 (50.4%)	29 (51.8%)	41 (49.4%)	
Yes	69 (49.6%)	27 (48.2%)	42 (50.6%)	
Depression, n (%):				0.512
No	68 (48.9%)	25 (44.6%)	43 (51.8%)	
Yes	71 (51.1%)	31 (55.4%)	40 (48.2%)	
Chronic pain, n (%):				0.577
No	104 (74.8%)	40 (71.4%)	64 (77.1%)	
Yes	35 (25.2%)	16 (28.6%)	19 (22.9%)	
Snoring, n (%):				0.561
No	101 (73.2%)	39 (69.6%)	62 (75.6%)	
Yes	37 (26.8%)	17 (30.4%)	20 (24.4%)	
Hypertension, n (%):				0.456
No	116 (84.1%)	45 (80.4%)	71 (86.6%)	
Yes	22 (15.9%)	11 (19.6%)	11 (13.4%)	

	All patients *N=140*	Non-Responders *N=57*	Responders *N=83*	p value
Other comorbidities, n (%):				0.115
No	59 (42.1%)	19 (33.3%)	40 (48.2%)	
Yes	81 (57.9%)	38 (66.7%)	43 (51.8%)	

ABBREVIATIONS: BMI= Body mass index, MIDAS= Migraine Disability Assessment, HIT-6= Headache Impact test-6. P-value in bold are statistically significant (<0.1).

When stratified according to clinical response, 83 patients (59.3%) were classified as responders and 57 (40.7%) as non-responders. The two groups were broadly comparable in terms of demographic characteristics, with no significant differences in age (p = 0.991), gender distribution (p = 0.348), or BMI (p = 0.231). Similarly, most comorbidities, including insomnia, anxiety, depression, chronic pain, and hypertension, did not differ significantly between groups.

Some clinically relevant differences were observed in disease characteristics. Non-responders had a significantly higher number of previously failed preventive treatments compared with responders (median 4.0 vs 3.0, p = 0.010), suggesting a more treatment-refractory phenotype. In addition, HIT-6 scores were significantly lower in non-responders compared with responders (66 vs 68, p = 0.019), although both groups remained within the range of severe disability. Trends toward differences were also observed for age at migraine onset, with earlier onset in non-responders (p = 0.063), and for migraine intensity at baseline (p = 0.056), while no significant differences were found in monthly migraine days, triptan use, or MIDAS scores.

4.1.2 BIOMIGA cohort

A total of 164 patients were included in the analysis. The overall population was predominantly female (84.1%), with a median age of 42 years (IQR 32.8-49.0) and with a median BMI of 23.3 (IQR 21.0-25.5), which indicates a generally normal-weight cohort. Migraine onset occurred early in life, with a median age of 15 (IQR 11.8-21.2). At baseline, 61.6% of patients had episodic migraine, while 38.4% had chronic migraine, 25.6% of patients had aura and 8.54% suffered from medication overuse headache. The burden of the disease was substantial with a median MIDAS score of 40.5 (IQR 24.0-70.2), and a mean HIT-6 score of 64.8 (4.10%), suggesting severe impact on daily functioning at baseline. 99.4% of patients took prior migraine prophylactic medication: 75.9 % took antidepressants, 68.5% anticonvulsants, 69.8% beta blockers, 37.0 calcium channel blockers, 0.62% CGRP monoclonal antibody, 9.26% angiotensin blockers ACE and ARB, 29.0% botulinum toxin A,

6.17% other drugs. 7.45% of patients was taking an ongoing Migraine Prophylactic Medication. Some patients suffered also form comorbidities, comprising mood disorders (7.93%), anxiety (7.32%), sleep disorders (6.10%), hypertension (6.71%), and other comorbidities (64.0%).

Table 3. Univariate association analysis of clinical variables in the BIOMIGA cohort.

	All patients *N=164*	**Non-responders** *N=68*	**Responders** *N=96*	**p value**
Gender, n (%):				0.322
Female	138 (84.1%)	60 (88.2%)	78 (81.2%)	
Male	26 (15.9%)	8 (11.8%)	18 (18.8%)	
Age, median (Q1-Q3)	42.0 (32.8-49.0)	39.5 (29.0-48.0)	44.0 (35.0;49.0)	**0.090**
Age at migraine onset, median (Q1-Q3)	15.0 (11.8-21.2)	15.0 (10.0-22.0)	15.0 (12.0-20.0)	0.933
BMI, median (Q1-Q3)	23.3 (21.0-25.5)	23.7 (20.9-26.1)	23.3 (21.0-25.2)	0.551
Patient diagnosis, n (%):				0.438
Episodic	101 (61.6%)	39 (57.4%)	62 (64.6%)	
Chronic	63 (38.4%)	29 (42.6%)	34 (35.4%)	
Aura, N (%):				1.000
No	122 (74.4%)	51 (75.0%)	71 (74.0%)	
Yes	42 (25.6%)	17 (25.0%)	25 (26.0%)	
Medication overuse, n (%):				0.863
No	150 (91.5%)	63 (92.6%)	87 (90.6%)	
Yes	14 (8.54%)	5 (7.35%)	9 (9.38%)	
MIDAS Score at baseline, median (Q1-Q3)	40.5 (24.0-70.2)	42.0 (27.0-75.0)	38.0 (23.0-65.0)	0.178
HIT6 score at baseline, n (%)	64.8 (4.10)	64.7 (3.73)	65.0 (4.35)	0.610
Prior migraine prophylactic medication, n (%):				0.414
No	1 (0.62%)	1 (1.49%)	0 (0.00%)	
Yes	161 (99.4%)	66 (98.5%)	95 (100%)	
Antidepressants, n (%):				0.543
No	39 (24.1%)	14 (20.9%)	25 (26.3%)	
Yes	123 (75.9%)	53 (79.1%)	70 (73.7%)	

	All patients	Non-responders	Responders	p value
	N=164	*N=68*	*N=96*	
Anticonvulsants, n (%):				0.839
No	51 (31.5%)	20 (29.9%)	31 (32.6%)	
Yes	111 (68.5%)	47 (70.1%)	64 (67.4%)	
Beta blockers, n (%):				0.337
No	49 (30.2%)	17 (25.4%)	32 (33.7%)	
Yes	113 (69.8%)	50 (74.6%)	63 (66.3%)	
Calcium channel blockers, n (%):				0.223
No	102 (63.0%)	38 (56.7%)	64 (67.4%)	
Yes	60 (37.0%)	29 (43.3%)	31 (32.6%)	
CGRP receptor monoclonal antibodies, n (%):				0.414
No	161 (99.4%)	66 (98.5%)	95 (100%)	
Yes	1 (0.62%)	1 (1.49%)	0 (0.00%)	
Angiotensin blockers, n (%):				0.699
No	147 (90.7%)	62 (92.5%)	85 (89.5%)	
Yes	15 (9.26%)	5 (7.46%)	10 (10.5%)	
Botulinum toxin A, n (%):				0.709
No	115 (71.0%)	46 (68.7%)	69 (72.6%)	
Yes	47 (29.0%)	21 (31.3%)	26 (27.4%)	
Other drug classes, n (%):				0.198
No	152 (93.8%)	65 (97.0%)	87 (91.6%)	
Yes	10 (6.17%)	2 (2.99%)	8 (8.42%)	
Ongoing migraine prophylactic medication, n (%):				1.000
No	149 (92.5%)	61 (92.4%)	88 (92.6%)	
Yes	12 (7.45%)	5 (7.58%)	7 (7.37%)	
Comorbidities				
Mood disorders, n (%):				0.216
No	151 (92.1%)	60 (88.2%)	91 (94.8%)	
Yes	13 (7.93%)	8 (11.8%)	5 (5.21%)	
Anxiety, n (%):				0.556
No	152 (92.7%)	62 (91.2%)	90 (93.8%)	

	All patients N=164	Non-responders N=68	Responders N=96	p value
Yes	12 (7.32%)	6 (8.82%)	6 (6.25%)	
Sleep disorders, n (%):				0.525
No	154 (93.9%)	65 (95.6%)	89 (92.7%)	
Yes	10 (6.10%)	3 (4.41%)	7 (7.29%)	
Hypertension, n (%):				0.365
No	153 (93.3%)	65 (95.6%)	88 (91.7%)	
Yes	11 (6.71%)	3 (4.41%)	8 (8.33%)	
Other comorbidities, n (%):				0.990
No	59 (36.0%)	25 (36.8%)	34 (35.4%)	
Yes	105 (64.0%)	43 (63.2%)	62 (64.6%)	

ABBREVIATIONS: MIDAS= Migraine Disability Assessment, HIT6 score= Headache Impact Test. P-value in bold are statistically significant (<0.1)

When stratified according to clinical response, 96 (58.5%) patients were classified as responders and 68 (41.5%) as non-responders. No statistically significant differences were observed between the two groups for any of the variables analysed. However, there was a trend toward younger age in non-responders (p=0.09).

4.2 Genetic factors associated with the clinical response to erenumab

4.2.1 Lugano cohort

For each polymorphism analyzed, the observed genotype distribution did not deviate from Hardy-Weinberg Equilibrium (RAMP1 rs10199956, p=0.17; CALCRL rs17705966, p=0.24; CALCRL rs62173500, p=0.61; CALCRL rs13384908, p=0.42; CALCRL rs7589163, p= 0.91; CALCRL rs13418253, p=0.21; CALCRL rs8176583, p=0.28; CALCRL rs3821184, p=0.70; CALCRL rs10207431, p= 0.36; CALCRL rs10931283, p=0.05; CALCRL rs78925680, p=0.43).

In the univariate logistic regression analysis, a nominally significant association was found between CALCRL rs10931283 and the non-responder status in the dominant model (OR 0.40, 95% CI 0.18-0.91, p=0.027), suggesting a lower risk for the allele C carriers of being non-responder. This significant nominal association was still found after the adjustment with the potential clinical confounders (i.e., age at migraine onset, number of failed preventive medication, migraine intensity at baseline and HIT-6 score at baseline) in both the dominant and allelic models (dominant model: OR 0.32 95% CI 0.13-0.77, p=0.01; allelic model: OR 0.56, 95% CI 0.34-0.92, p=0.019). However, the

significance was lost when the Bonferroni's correction multiple testing was applied (Bonferroni p-value threshold <0.0045). The other polymorphisms showed no significant association with non-responder status. All the genetic data are shown in table (4).

Table (4). Association analysis of SNPs with the clinical response to erenumab in the Lugano cohort.

SNP	***All patients*** **N= 140** **N (%)**	***R*esponders** ***N= 83*** **N (%)**	***N*on-responders** ***N= 57*** **N (%)**	***Crude OR (95% CI)***	**p *Value***	***Adjusted* OR (95% CI)***	***p value***
RAMP1 rs10199956							
T/T	34 (24%)	19 (23)	15 (28)	A: 0.79 (0.47-1.31)	0.36	A: 0.69 (0.39-1.22)	0.2
T/G	78 (56%)	45 (54%)	33 (58)	D: 0.83 (0.38-1.81)	0.64	D: 0.75 (0.32-1.76)	0.51
G/G	28 (20%)	19 (23%)	9 (16)	R: 0.63(0.26-1.52)	0.3	R: 0.48 (0.17-1.33)	0.15
CALCRL rs17705966							
A/A	85	52 (63)	33 (58)	A: 1.30 (0.76-2.23)	0.33	A: 1.37 (0.76-2.44)	0.29
A/G	45 (32 %)	27 (33)	18 (32)	D: 1.22 (0.61-2.43)	0.57	D: 1.25 (0.59-2.64)	0.56
G/G	10 (7%)	4 (5)	6 (11)	R: 2.32 (0.62-8.64)	0.2	R: 2.76 (0.68-11.18)	0.15
CALCRL rs62173500							
A/A	103 (74)	65 (78)	38 (67)	A: 1.69 (0.84-3.41)	0.14	A:1.85 (0.87-3.91)	0.11
A/C	35 (0.25)	17 (20)	18 (32)	D: 1.81 (0.85-3.86)	0.13	D: 1.93 (0.85-4.40)	0.12
C/C	2 (1)	1 (1)	1 (2)	R: 1.46 (0.09-23.90)	0.79	R: 2.71 (0.16-45.27)	0.5
CALCRL rs13384908							
G/G	82 (59)	51 (61)	31 (54)	A: 1.38 (0.81-2.36)	0.24	A: 1.48 (0.82-2.65)	0.19
G/A	48 (34)	28 (34)	20 (35)	D: 1.34 (0.67-2.65)	0.41	D: 1.41 (0.67-2.98)	0.36
A/A	10 (0.07)	4 (5)	6 (11)	R: 2.32 (0.62-8.64)	0.2	R: 2.76 (0.68-11.18)	0.15
CALCRL rs7589163							
T/T	55 (39)	36 (43)	19 (33)	A: 0.68 (0.41-1.11)	0.12	A: 0.57 (0.33-0.99)	0.043
T/C	65 (46)	38 (46)	27 (0.47)	D: 0.51 (0.20-1.32)	0.16	D: 0.38 (0.13-1.07)	0.065
C/C	20 (14)	9 (11)	11 (19)	R: 0.65 (0.32-1.32)	0.23	R: 0.55 (0.25-1.21)	0.13
CALCRL rs13418253							
T/T	60 (43)	38 (46)	22 (39)	A: 1.15 (0.72-1.85)	0.55	A: 1.18 (0.70-1.97)	0.53
T/A	58 (41)	32 (39)	26 (46)	D: 1.34 (0.68-2.67)	0.4	D: 1.32 (0.62-2.82)	0.47
A/A	22 (16)	13 (16)	9 (16)	R: 1.01 (0.40-2.55)	0.98	R: 1.13 (0.42-3.05)	0.81

SNP	***All patients*** **N= 140** **N (%)**	***R*esponders** ***N= 83*** **N (%)**	***N*on-responders** ***N= 57*** **N (%)**	***Crude OR (95% CI)***	**p *Value***	***Adjusted* OR (95% CI)***	***p value***
CALCRL rs8176583							
T/T	64 (46)	41 (49)	23 (40)	A: 1.30 (0.80-2.10)	0.29	A: 1.39 (0.82-2.35)	0.22
T/G	57 (41)	32 (39)	25 (44)	D: 1.44 (0.73-2.85)	0.29	D: 1.47 (0.70-3.09)	0.31
G/G	19 (14)	10 (12)	9 (16)	R: 1.37 (0.52-3.62)	0.53	R: 1.77 (0.62-5.10)	0.29
CALCRL rs3821184							
C/C	57 (41)	37 (45)	20 (35)	A: 0.69 (0.42-1.12)	0.13	A: 0.60 (0.34-1.03)	0.06
C/G	63 (45)	37 (45)	26 (46)	D: 0.51 (0.20-1.32)	0.16	D: 0.38 (0.13-1.07)	0.065
G/G	20 (14)	9 (11)	11 (19)	R: 0.67 (0.34-1.35)	0.26	R: 0.60 (0.28-1.29)	0.19
CALCRL rs10207431							
A/A	83 (59)	51 (61)	32 (56)	A:1.32 (0.77-2.26)	0.31	A: 1.42 (0.79-2.55)	0.24
A/C	47 (34)	28 (34)	19 (33)	D: 1.25 (0.63-2.47)	0.53	D: 1.33 (0.63-2.81)	0.45
C/C	10 (7)	4 (5)	6 (11)	R: 2.32 (0.62-8.64)	0.2	R: 2.76 (0.68-11.18)	0.15
CALCRL rs10931283							
T/T	52 (37)	34 (41)	18 (32)	A: 0.64 (0.41-1.01)	0.051	**A: 0.56 (0.34-0.92)**	**0.019**
T/C	57 (41)	36 (43)	21 (37)	**D: 0.40 (0.18-0.91)**	**0.027**	**D: 0.32 (0.13-0.77)**	**0.01**
C/C	31 (22)	13 (16)	18 (32)	R: 0.67 (0.33-1.35)	0.26	R: 0.56 (0.26-1.24)	0.15
CALCRL rs78925680							
G/G	71 (51)	42 (51)	29 (51)	A: 0.94 (0.54-1.64)	0.83	A: 1.00 (0.55-1.83)	1
G/A	60 (43)	35 (42)	25 (44)	D: 0.99 (0.50-1.94)	0.97	D: 1.08 (0.52-2.27)	0.83
A/A	9 (6)	6 (7)	3 (5)	R: 0.71 (0.17-2.98)	0.64	R: 0.72 (0.15-3.37)	0.67

In bold, genetic association results for which a nominal significant association was found ($p < 0.05$).

* Logistic regression analysis adjusted by clinical variables found significant ($p<0.1$) in the univariate analysis [i.e., age at migraine onset, number of failed preventive medication, migraine intensity at baseline and HIT-6 at baseline]

ABBREVIATIONS: A=log additive model, which compares the allele 2 (mutated) against the allele 1 (wild type); D= dominant, which pools genotype 1/2 and 2/2 and compared them against 1/1 Genotype. R= recessive model, 2/2 genotype is compared against 1/1 genotype and 1/2 together. CI= confidence intervals; OR= odds ratio; Crude OR refers to the unadjusted odds ratio, while adjusted OR is adjusted for

4.2.2 BIOMIGA cohort

For each polymorphism analyzed, the observed genotype distribution did not deviate from Hardy-Weinberg Equilibrium (RAMP1 rs10199956 p=0.96; CALCRL rs17705966 p=0.28; CALCRL rs62173500 p=0.78; CALCRL rs13384908 p=0.12; CALCRL rs7589163 p=0.96; CALCRL rs13418253 p=0.80; CALCRL rs8176583 p= 0.70; CALCRL rs3821184 p=0.93; CALCRL rs10207431 p=0.09; CALCRL rs10931283 p=0.63; CALCRL rs78925680 p=0.52). None of the eleven analysed polymorphisms showed a nominally significant association with the non-responder status in either the univariate logistic regression analysis or after adjustment for confounders (i.e., age). All the genetic results are shown in table (5).

Table (5). Association analysis of SNP with the clinical response to erenumab in the BIOMIGA cohort.

SNP	***All patients*** **N (%)**	***R N= 94*** **N (%)**	***NR N= 68*** **N (%)**	***Crude OR (95% CI)***	***p. value***	***Adjusted*OR (95% CI)***	***p. value***
RAMP1 rs10199956							
T/T	30 (18)	18 (19)	12 (18)	A: 0.92 (0.59-1.44)	0.73	A:0.92 (0.59-1.44)	0.71
T/G	81 (49)	45 (47)	36 (53)	D: 1.08 (0.48-2.41)	0.86	D:1.02 (0.45-2.31)	0.96
G/G	53 (32)	33 (34)	20 (29)	R: 0.80 (0.41-1.56)	0.5	R:0.81 (0.41-1.60)	0.55
CALCRL rs17705966							
A/A	101 (62)	61 (64)	40 (59)	A: 1.26 (0.72-2.21)	0.42	A:1.27 (0.72-2.23)	0.41
A/G	58 (35)	33 (34)	25 (37)	D: 1.22 (0.65-2.31)	0.54	D:1.20 (0.63-2.28)	0.58
G/G	5 (3)	2 (2)	3 (4)	R: 2.17 (0.35-13.35)	0.4	R: 2.66 (0.42-16.96)	0.29
CALCR rs62173500							
A/A	127 (77)	72 (75)	55 (81)	A: 0.76 (0.38-1.54)	0.44	A:0.74 (0.36-1.51)	0.41
A/C	35 (21)	23 (24)	12 (18)	D: 0.71 (0.33-1.52)	0.37	D: 0.68 (0.32-1.47)	0.33
C/C	2 (1)	1 (1)	1 (1)	R: 1.42 (0.09-23.07)	0.81	R:1.61 (0.09-28.19)	0.75
CALCRL rs13384908							
G/G	100 (61)	61 (64)	39 (57)	A: 1.27 (0.72-2.26)	0.41	A:1.27 (0.71-2.26)	0.42
G/A	60 (37)	33 (34)	27 (40)	D: 1.30 (0.69-2.45)	0.42	D: 1.27 (0.67-2.42)	0.46
A/A	4 (2)	2 (2)	2 (3)	R: 1.42 (0.20-10.37)	0.73	R: 1.63 (0.22-12.28)	0.63
CALCRL rs7589163							
T/T	75 (46)	44 (46)	31 (46)	A: 0.96 (0.60-1.55)	0.88	A: 0.98 (0.60-1.58)	0.92
T/C	72 (44)	42 (44)	30 (44)	D:	0.86	D: 0.86 (0.30-2.46)	0.78
C/C	16 (0.10)	9 (9)	7 (10)	0.91 (0.32-2.58)	0.93	R: 1.01 (0.54-1.91)	0.96
				R: 0.97 (0.52-1.81)			

SNP	***All patients*** **N (%)**	***R N= 94*** **N (%)**	***NR N= 68*** **N (%)**	***Crude OR (95% CI)***	***p. value***	***Adjusted*OR (95% CI)***	***p. value***
CALCRL rs13418253							
T/T	80 (49)	48 (0.51)	32 (47)	A: 1.20 (0.74-1.96)	0.46	A:1.20 (0.73-1.97)	0.47
T/A	70 (43)	41 (43)	29 (43)	D: 1.15 (0.62-2.14)	0.66	D: 1.11 (0.59-2.09)	0.74
A/A	13 (8)	6 (6)	7 (10)	R:1.70 (0.55-5.31)	0.36	R: 1.88 (0.59-5.95)	0.28
CALCRL rs8176583							
T/T	78 (48)	47 (50)	31 (46)	A: 1.23 (0.75-2.01)	0.41	A:1.22 (0.74-2.00)	0.44
T/G	71 (44)	41(44)	30 (44)	D: 1.19 (0.64-2.23)	0.58	D:1.14 (0.61-2.15)	0.68
G/G	13 (8)	6 (6)	7 (10)	R: 1.68 (0.54-5.25)	0.37	R: 1.86 (0.59-5.93)	0.29
CALCRL rs3821184							
C/C	74 (46)	43 (46)	31 (46)	A: 0.98 (0.61-1.58)	0.93	A:1.0 (0.62-1.62	1
C/G	72 (44)	42 (45)	30 (44)	D: 0.92 (0.33-2.61)	0.88	D:0.87 (0.30-2.49)	0.79
G/G	16 (10)	9 (10)	7 (10)	R: 0.99 (0.53-1.86)	0.98	R: 1.05 (0.56-1.98)	0.88
CALCRL rs10207431							
A/A	100 (62)	58 (0.62)	42 (63)	A: 0.97 (0.53-1.76)	0.91	A:0.97 (0.53-1.77)	0.92
A/C	57 (36)	33 (0.35)	24 (36)	D: 0.99 (0.52-1.89)	0.97	D: 0.97 (0.50-1.87)	0.93
C/C	3 (2)	2 (2)	1 (1)	R: 0.69 (0.06-7.76)	0.76	R:0.90 (0.08-10.50	0.93
CALCRL rs10931283							
T/T	60 (38)	36 (39)	24 (36)	A: 0.93 (0.58-1.48)	0.75	A: 0.92 (0.57-1.48)	0.72
T/C	79 (49)	45 (48)	34 (51)	D: 0.95 (0.38-2.41)	0.92	D: 0.88 (0.35-2.26)	0.8
C/C	21 (13)	12 (13)	9 (13)	R: 0.88 (0.46-1.69)	0.71	R: 0.90 (0.47-1.74)	0.76

SNP	*All patients* N (%)	*R N= 94* N (%)	*NR N= 68* N (%)	*Crude OR (95% CI)*	*p. value*	*Adjusted*OR (95% CI)*	*p. value*
CALCRL rs78925680							
G/G	85 (53)	49 (53)	36 (55)	A: 1.01 (0.60-1.69)	0.97	A: 0.99 (0.59-1.67)	0.98
G/A	64 (40)	39 (0.42	25 (38)	D: 0.93 (0.49-1.75)	0.82	D: 0.91 (0.48-1.73)	0.78
A/A	10 (6)	5 (0.05)	5 (8)	R: 1.44 (0.40-5.20)	0.58	R: 1.41 (0.39-5.16)	0.6

In bold, genetic association results for which a nominal significant association was found ($p < 0.05$).

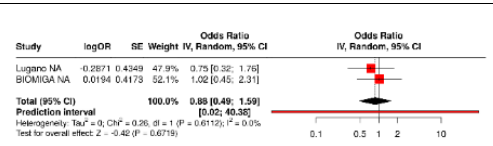
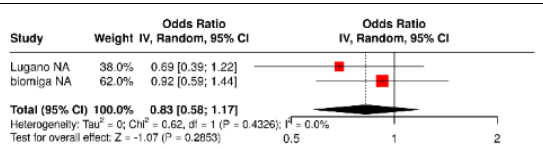
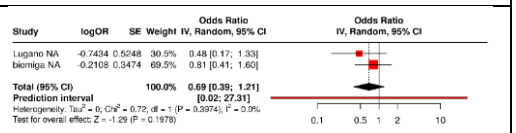
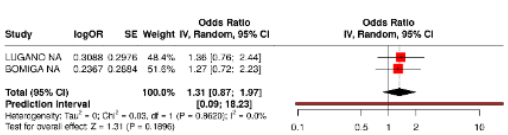
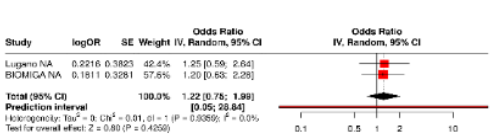
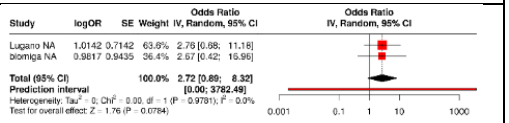
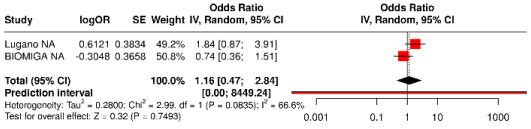
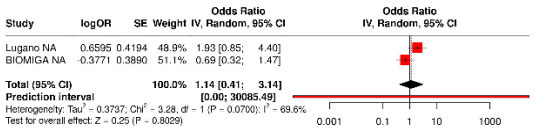
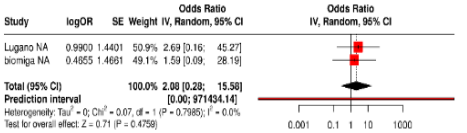
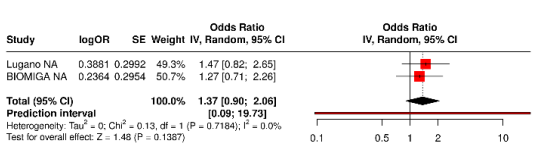
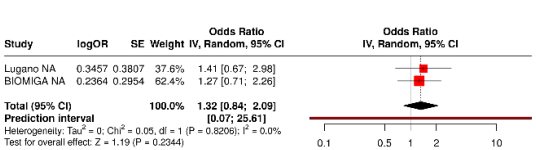
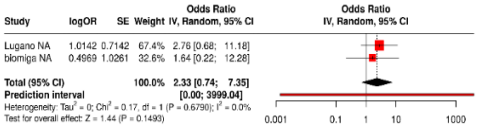
* Logistic regression analysis adjusted by clinical variables found significant ($p<0.1$) in the univariate analysis (i.e. age)

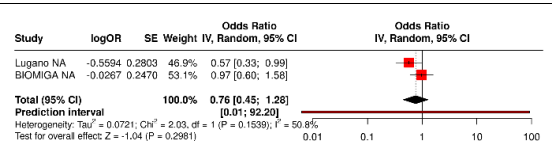
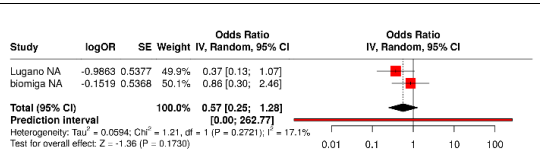
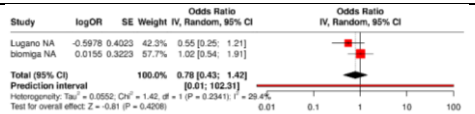
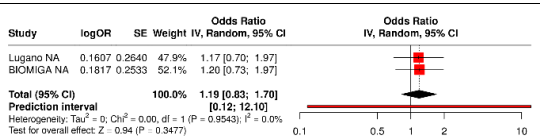
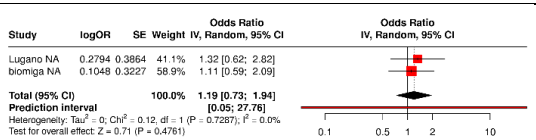
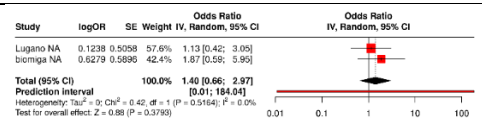
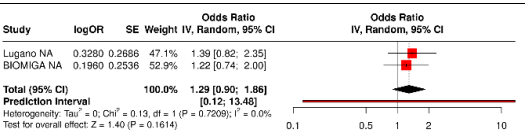
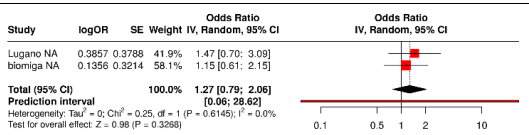
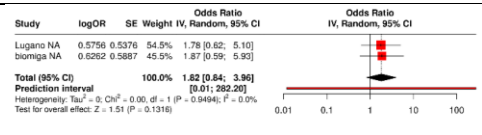
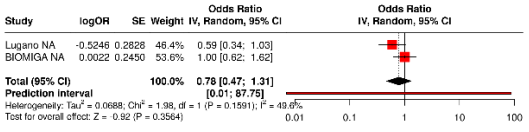
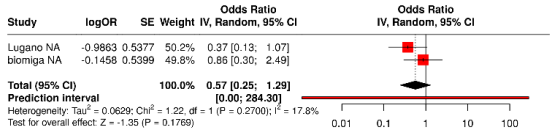
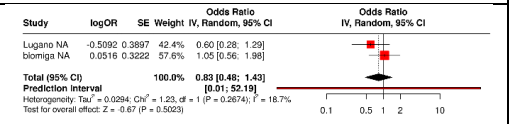
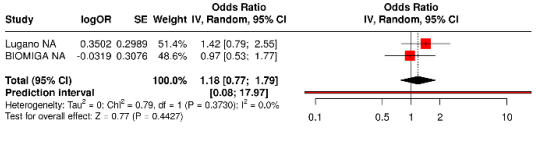
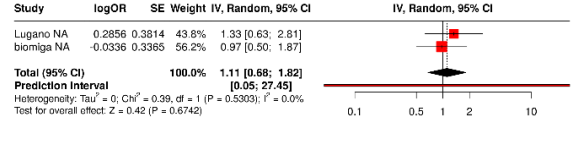
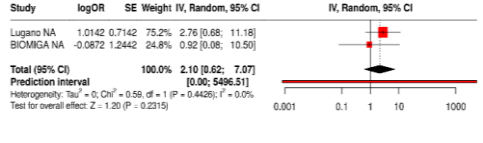
ABBREVIATIONS: CI= confidence intervals: NR=non-responders; OR= odds ratio; R= responders; A=log additive model, which compares the allele 2 (mutated) against the allele 1 (wild type); D= dominant, which pools genotype 1/2 and 2/2 and compared them against 1/1 genotype; R= recessive model, 2/2 genotype is compared against 1/1 genotype and 1/2 together. Association analysis was performed using a log-additive, dominant and a recessive model. Crude OR refers to the unadjusted odds ratio, while adjusted OR is adjusted for age.

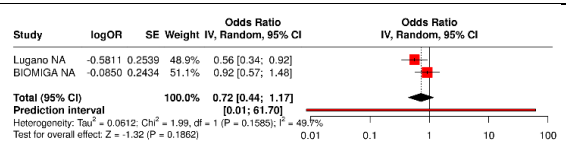
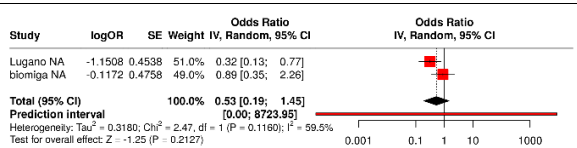
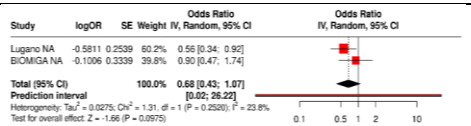
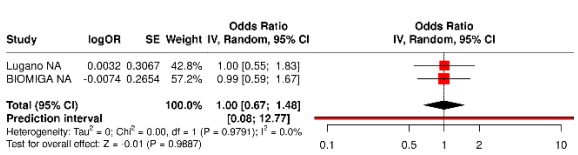
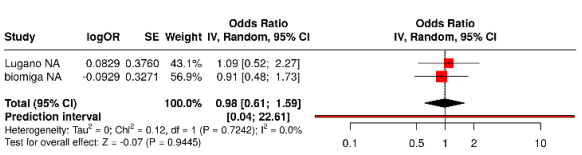
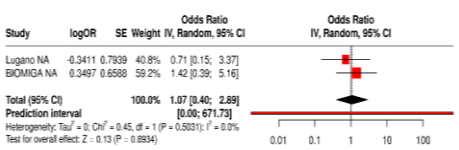
4.3 Meta-analysis of genetic results

To improve the statistical power and assess the consistency of the genetic associations across independent cohorts, a meta-analysis combining the Lugano and BIOMIGA cohorts was performed, including a total of 284 patients. As shown in Table (6), none of the analyzed SNPs was significantly associated with non-responder status under any of the genetic models tested. Notably, the nominal association observed for the CALCRL rs10931283 variant in the Lugano cohort was not replicated in the pooled analysis, indicating that the available evidence does not support a consistent association between this polymorphism and the likelihood of failing to respond to erenumab treatment.

Table 6. Forest plots for the association between SNPs and the non-responder status under allelic, dominant and recessive models of inheritance.

SNP	Allelic model of inheritance		
	Allelic	**Dominant**	**Recessive**
RAMP1 rs10199956			
CALCRL rs17705966			
CALCRL rs62173500			
CALCRL rs13384908			

SNP	Allelic model of inheritance		
	Allelic	**Dominant**	**Recessive**
CALCRL rs7589163			
CALCRL rs13418253			
CALCRL rs8176583			
CALCRL rs3821184			
CALCRL rs10207431			

SNP	Allelic model of inheritance		
	Allelic	**Dominant**	**Recessive**
CALCRL rs10931283			
CALCRL rs78925680			

5. DISCUSSION

The aim of this thesis was to identify genetic variants associated with an increased risk of treatment non-response to erenumab. Knowing predictive factors of non-response to erenumab is pivotal to improve the selection of patients who will likely benefit from the treatment, avoiding exposure to an ineffective therapy. Previous studies have already found some clinical and genetic predictors, but the available evidence is still scarse.[41,43,44]

Regarding the clinical factors, different baseline variables were associated with erenumab non-response across the two cohorts. In the Lugano cohort, age of migraine onset, failed preventive medication, migraine intensity at baseline and HIT-6 score at baseline were significantly associated with non-response (Table 2). In the BIOMIGA cohort, although no clinical variable reached statistical significance, a trend toward younger age was observed among non-responders (Table 3). Previous evidence is only partially consistent with these findings. Karlsson et al. identified the number of failed preventive medications as a predictor of non-response, supporting our observation in the Lugano cohort.[51] In the same study, older age was associated with a better response to erenumab, a finding that is in line with the trend toward younger age among non-responders observed in our BIOMIGA cohort. Overall, these findings suggest that, among those emerged, a history of multiple unsuccessful preventive treatments (that may reflect a more treatment-resistant migraine phenotype) and younger age may represent the most robust clinical predictors of erenumab non-response, whereas the association of other baseline clinical characteristics appears to be less consistent across different patient populations and warrants further investigation.

Regarding the genetic factors, the main finding of this thesis was that in the Lugano cohort a nominal significant association between CALCRL rs10931283 and the non-responder status (table 4) was found. The rs10931283 variant is an intronic SNP that was selected based on bioinformatic evidence from the GTEx Portal, indicating a strong effect on CALCRL gene expression (Table 1). However, to the best of our knowledge, no previous studies have specifically investigated this variant, and there is currently no evidence linking it to migraine, treatment response, or other clinical phenotypes. Therefore, although its selection was biologically supported by its putative regulatory role, the functional significance of rs10931283 remains largely unknown. In our study, we showed that carriers of the mutated allele C seem to have a decreased risk of being non-responder. However, this association did not remain statistically significant after Bonferroni correction for multiple testing. Furthermore, the association was not replicated in the independent BIOMIGA cohort (Table

5), nor was it confirmed by the meta-analysis combining the two cohorts (Table 6). Overall, these findings suggest that the initial association observed in the Lugano cohort may represent a false-positive result. Alternatively, the lack of replication could reflect the limited statistical power of the study or differences in the clinical and demographic characteristics of the two cohorts.

Strengths and limitations of the present study

The present study has several strengths. First, the availability of two independent cohorts allowed the assessment of findings reproducibility. Second, multivariable analyses were performed to adjust for potential confounding factors, ensuring that the observed associations were not driven by extraneous variables. In addition, different genetic models were tested to better characterize the inheritance pattern of each variant. Hardy–Weinberg equilibrium was also evaluated as a quality control measure to exclude potential genotyping errors or population stratification. Finally, a meta-analysis combining the two cohorts was performed, increasing the precision of the effect estimates, reducing the likelihood of chance findings, and allowing the reproducibility of the observed associations to be assessed.

However, our results should be interpreted in light of several limitations which are intrinsic to our study design. First, the sample size of our cohorts was relatively small, and only a limited number of SNPs were investigated, reducing the statistical power to detect modest genetic effects. Despite the central role of the CGRP pathway in the pharmacodynamic mechanism of erenumab, our findings suggest that common germline variants in CALCRL and RAMP1 alone are unlikely to substantially explain the interindividual variability in treatment response. This may reflect insufficient statistical power to detect variants with modest effects, the contribution of rare germline variants that were not investigated in the present study, or the complex polygenic and multifactorial nature of erenumab response, which is likely influenced by multiple genes, biological pathways, and environmental factors. Second, only individuals of Caucasian ancestry were included, limiting the generalizability of the findings to other populations. Finally, despite applying similar inclusion criteria, the two cohorts showed some degree of clinical heterogeneity as highlighted by our meta-analyses, which may have contributed to the differences observed between them. Future studies should include larger, preferably multicentre, cohorts to improve statistical power and facilitate independent replication of genetic associations. From a genetic perspective, targeted sequencing of candidate genes involved in the CGRP pathway could identify rare variants with a potentially greater impact on treatment response, whereas genome-wide association studies (GWAS) may uncover

novel loci associated with erenumab efficacy. Given the complex nature of drug response, future investigations should integrate genetic information with clinical and environmental predictors. Finally, the application of artificial intelligence and machine learning algorithms to integrate these heterogeneous data may improve the prediction of treatment outcomes and contribute to the implementation of precision medicine in migraine management.

CONCLUSIONS

In conclusion, none of the studied SNPs emerged to be robustly associated with the risk of not responding to erenumab as no association remained significant after correction for multiple testing or was replicated in the independent cohort and subsequent meta-analysis. Nevertheless, this study contributes to the growing body of evidence on the pharmacogenetics of anti-CGRP therapies and underscores the need for larger, multicentre studies integrating genomic and other omics approaches to identify reliable biomarkers of treatment response in migraine.

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