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Clinical and genetic predictors of medication-overuse headache and its relapse

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Introduction

In patients with primary headache disorders, such as migraine or tension-type headache (TTH), frequent intake of acute headache medication may increase the frequency and intensity of headache, causing a vicious circle of further intake of medication and increased attack frequency. Unintendedly, the treatment becomes the cause of the disease, known as medication-overuse headache (MOH).

Of note, medication overuse and MOH are two separate conditions: not every patient who frequently uses medication to abort headache attacks will develop MOH.

Studies in several populations have converged on one clinical message: headache induced by analgesics and specific acute treatments for primary headache seems to occur predominantly in patients with a personal or family history of migraine. For example, in a group of patients attending a rheumatology clinic and being treated with regular analgesics, only 10% had chronic daily headache (CDH), and each had a personal or family history of migraine, or both (1). Similarly, in a cohort of patients with bowel motility problems treated with daily opioids, only those with migraine developed CDH (2).

Definition of MOH

It has been known since the 1950s that an excessive use of acute medication can cause headache to worsen and that withdrawal from the overused substances can restore the headache pattern (3, 4). The diagnosis of MOH was also mentioned in the first edition of the International Classification of Headache Disorders published in 1988 (ICHD-1)(5). In this edition, the diagnosis required daily intake of a certain dose of drug and remission of the headache 1 month after withdrawal. In the second edition of the International Classification of Headache Disorders published in 2004 (ICHD-2) (6), the diagnostic criteria were changed to a specified number of days with intake of acute drugs (10 or 15/month, depending on the classes of drug), but still

foresaw headache resolution 2 months after withdrawal. These criteria were shortly afterwards adapted in the appendix criteria of 2006 (7) and confirmed in the third edition of the Classification (ICHD-3β) (8) to be more clinically relevant and easy-to-use in the daily practice. Nowadays therefore the diagnosis of MOH can be given before withdrawal, as it now only requires medication overuse and a pre-existing headache. These criteria can be seen in the box below.

A. Headache present on 15 or more days/month in a patient with a pre-existing headache disorder

B. Regular overuse for more than 3 months of 1 or more drugs that can be taken for acute and/or symptomatic treatment of headache

1. Ergotamine, triptans, opioids or combination analgesics on 10 or more days/month
2. Simple analgesics on 15 or more days/month
3. Any combination of acute/symptomatic drugs on 10 or more days/ month without overuse of any single class alone

C. Not better accounted for by any other ICHD-3 diagnosis

Box. The criteria for Medication Overuse Headache of the International Classification of Headache Disorders (ICHD)-3β Criteria

Clinical characteristics

The characteristics of headache in MOH depend on the primary headache (9). Patients with migraine who overused triptans have reported a migraine-like headache (unilateral, pulsating pain with accompanying symptoms, such as photophobia, phonophobia, nausea and vomiting) to occur daily, or at least a marked increase in migraine frequency. In some patients, the overused medication can suppress symptoms that accompany migraine, meaning the headache has less-typical clinical features and identification of the primary headache diagnosis is difficult until the patient continues to overuse symptomatic medication.

Epidemiology

It is estimated that MOH affects between 1% and 2% of the general population (10, 11), up to 25-50% of the chronic headache population (10, 11), and 30-50% of patients seen in specialized headache centers (12).

MOH is extremely costly both for the patient and the society, because of absenteeism and the burden on the health care services (13). Recently, the Eurolight study estimated the mean per-person annual costs for MOH to €3561, which is 3 times the costs of migraine and more than 10 times the costs of TTH (14). Only 8% of the costs were related to health care and medications, while 92% of the costs were accounted for by absenteeism and reduced productivity (14). The personal and societal gain from curing patients of MOH is thus enormous and should be pursued in all health care systems.

Pathophysiology

The mechanisms behind the development of MOH are largely unknown. Given that patients with migraine and tension-type headache are at higher risk of developing MOH than are individuals without primary headaches (15), the pathophysiological mechanisms of MOH could be related to the migrainous or headache brain itself; that is, a brain could be 'prone to MOH'. This hypothesis is supported by analysis of rare cases in which the same patient suffers from both MOH and cluster headache. Medication overuse is generally not an issue in cluster headache: patients may overuse aborting drugs during their cluster periods, but do not have any problems in self-discontinuation once the cluster period is over. MOH is only reported in patients with cluster headache who also have migraine or a family history of migraine (16). This finding suggests that the pathophysiological mechanisms leading to MOH would be activated specifically in the migraine brain. Yet it is not known why some migraine patients develop MOH while others do not. Beside, it is not known why some patients with MOH relapse after a successful detoxification.

Several studies have provided evidence for the neurophysiological and biochemical changes occurring in MOH patients (17). Yet, they just represent a picture of a condition that has already developed and contribute little information on the modalities of the transformation process itself. What is really missing in the picture is the set of factors that act as the *primum movens* in initiating and maintaining the progression of migraine toward MOH.

Treatment

Withdrawal from the overused substance, either as an abrupt withdrawal or gradual tapering down depending on the drugs overused, is crucial in the treatment for MOH (12). After an abrupt withdrawal, the patients' headache episodes will gradually decrease in frequency over a 4- to 12-week period but in the first 4-10 days after the withdrawal most patients will experience an increase in headache, which gradually declines over time (18). Likewise, most patients will also experience accompanying symptoms in the form of nausea, vomiting, arterial hypotension, tachycardia, sleep disturbances, restlessness, anxiety, and nervousness. It is therefore of the utmost importance to motivate and inform them on the benefits from this treatment in order to optimize patients' compliance.

Studies on MOH patients from the general population have suggested that simple information on MOH and the importance of reducing the use of acute drugs may be effective in a subset of patients to achieve successful withdrawal on their own (18). This however seems true mostly for the so-called 'simple MOH', i.e. subjects with a short history of medication overuse, no or little psychiatric co-morbidity and negative history for previous failures in the withdrawal of overused drugs. Conversely, patients with a duration of MOH over 1 year, overuse of opioids or more than 1 type of prescription medications, coexisting psychiatric disorders, and/or a history of relapse or unsuccessful withdrawal usually show a poor outcome when attempting to withdraw from medication overuse on the basis of advice alone. For these patients a structured detoxification program is recommended. Withdrawal from overused drugs (advice alone or

inpatient detoxification program depending on the clinical features of the patient) is generally effective with a response rate of about 70% of patients. Yet, 20-40% of patients who responded to the detoxification, relapse within 1 year (19).

Hypothesis and Aims of the study

Our central hypothesis is that the transformation of migraine into MOH occurs in predisposed patients in whom the predisposition results from a critical combination of a set of clinical and genetic factors. The secondary hypothesis is that a subset of clinical and genetic factors confers a higher risk to relapse after a successful detoxification program.

Therefore the objectives of the presents study are:-

- The identification of the factors that are predictive for the evolution of migraine into MOH;
- The identification of the factors that are predictive for the relapse into overuse following drug withdrawal.

Achievement of these objectives will allow us to identify a subpopulation of patients who are at high risk of negative outcome. Our findings will also greatly contribute to shed light on the pathophysiology of MOH.

To achieve our objectives, we have analyzed and compared clinical and genetic variables in two groups of migraine patients: i) migraineurs who evolved into MOH (MOH) and ii) patients with stable episodic migraine (MIG). In the MOH group we will further seek differences between i) the subgroup of patients who revert back to overuse between months 2 and 12 following successful detoxification (R-MOH) and ii) the subgroup of patients who do not revert back to overuse between months 2 to 12 following successful detoxification (non-relapsers, NR-MOH).

Subjects and methods

Subjects

This study was conducted at the Headache Science Center (a tertiary referral center) of the National Neurological Institute "C. Mondino" in Pavia, Italy. The enrollment involved consecutive patients with MOH evolved from migraine (MOH) and migraine patients with a stable episodic form (MIG). The study was approved by the Local Ethic Committee (on 5th March 2014) and informed consent was obtained from all the patients. Recruitment started on March 2014 and was completed in July 2016. All consecutive MIG and MOH patients were enrolled and followed-up in a prospective study.

MIG patients

Inclusion criteria for patient with MIG were: i) age >18, <65 years, ii) fulfillment of ICHD-III criteria for migraine without aura, iii) migraine duration $\geq$ 10 years. Exclusion criteria were: i) previous or present history of MOH or any other type of chronic headache (ICHD-III beta), ii) dementia, iii) psychosis, iv) mental retardation.

MIG were seen at T0 and at follow-up after 6 months (T6). At T0 the patient were prescribed with symptomatic medication and, if required, preventive medication, according to the International and National guidelines. They were asked to attend a follow-up (at 6 months, T6), in order to prospectively verify the diagnosis and inclusion/exclusion criteria with particular attention to the absence of medication overuse by checking headache diaries.

MOH patients

Inclusion criteria for patients with MOH were: i) age >18, <65 years, ii) fulfillment of ICHD-III beta criteria for MOH, iii) MOH evolved from migraine. Exclusion criteria were: i) dementia, ii) psychosis, iii) mental retardation, iv) other secondary headaches.

MOH patients were asked to fill in an ad hoc headache diary and were re-evaluated after two months for confirmation of the diagnosis. They subsequently underwent inpatient detoxification (see below) and were seen after 2, 6 and 12 months (respectively T2, T6 and T12) after detoxification.

A standard in-patient withdrawal protocol was performed at the Headache Unit of Mondino National Neurological Institute, Pavia, Italy (20, 21). During hospitalization, which lasted 7 days, the patients discontinued abruptly the overused drug(s) and underwent, daily, a standard i.v. detoxification therapy: a saline solution 250 cm3, delorazepam 0.25mg (this latter dose was gradually eliminated over a period of 3–4 days), cyanocobalamine 2500 mg, folic acid 0.70 mg, nicotinamide 12 mg, ascorbic acid 150 mg, glutathione 600 mg, metoclopramide 5 mg (stopped after 2 days), b.i.d. In the event of butalbital overuse, tapering doses of phenobarbital were added, whereas in cases of narcotics overuse, transdermal clonidine was added. Low-dose benzodiazepines were used just in the initial days of detoxification. In the absence of specific guidelines for the detoxification protocol, we applied the standardized and implemented detoxification procedure that has been in use at our centre for many years ((20, 21)). On day 4 or 5, a preventive treatment was started, personalized according to the presence of comorbidities and the previous prophylactic therapy used by the patient. During hospitalization, each subject was allowed to use a rescue medication (1000 mg of paracetamol e.v.) only in case of severe rebound headache. Upon discharge, we prescribed a symptomatic treatment based on a drug belonging to a different category from that overused, instructing the patient to utilize it only for severe attacks and for no more than 8 days a month. Generally, we chose simple analgesics or triptans.

Successful detoxification was ascertained at T2. Patients were defined **cured** if they were no longer overusing symptomatic medication (according to ICHD-IIIbeta criteria) in the previous 2 months (i.e. first and second month after detoxification) regardless of the pattern of headache.

Relapse into overuse was evaluated over the period 2-12 months. Patients were defined **relapsers** if they reverted back to overuse (according to ICHD-IIIbeta criteria) between months 2 to 12, after stopping overusing during month 1-2.

Procedures

Clinical Study

All the visits have been performed by neurologists with a long-time experience in headache (NEH) At the first visit (T0), the NEH diagnosed the headache type, collect socio-demographical data, lifestyle habits, migraine characteristics and history, accurate history of present and previous use of medications and other substances, medical history, obstetrician and gynecological history, family history. Psychological variables were collected by specific self-compiling questionnaires. We also collected questionnaires on disability: MIDAS, HIT..

At the follow-up visits of both MOH and MIG patients, the NEH verified the characteristics of the headache (e.g. frequency, duration and intensity) and drug intake by means of an ad *hoc diary* that the patients were asked to for the entire length of the study. This allowed validation of the diagnosis of MIG and MOH patients, as well as the occurrence of relapse in the MOH patients.

The NEH used an ad hoc data sheet to collect the following variables, which became part of the database:

1) Socio-demographical variables and lifestyle habits

- gender
- age
- BMI
- marital status
- occupation
- income

- coffee and alcohol intake
- smoking
- physical activity

2) Migraine Characteristic and history

- age of onset
- coexistence of migraine with aura attacks
- headache frequency
- migraine-related disability (MIDAS)
- previous use of migraine preventive medication

(Only for MOH patients)

- age of chronification/overuse
- type(s) of medication overused
- dose and days of medication intake per month
- factors associated with chronification
- previous inpatients detoxification
- previous day hospital detoxification
- migraine symptomatic and preventive medication at discharge
- headache Impact test (HIT)

3) Obstetrician and gynecological history

- age at menarche
- irregular menstrual bleeding
- dysmenorrhea
- current/previous use of combined oral contraceptives (COCs)
- effect of COCS on migraine
- deliveries

- effect of pregnancy on migraine
- voluntary interruption(s) of pregnancy
- miscarriage(s)
- menopause age
- reproductive system comorbidities (Uterine fibroids, uterine polyps, endometriosis, ovarian cysts)

4) Family history

- headache
- arterial hypertension
- depression and/or anxiety
- alcohol dependence
- substance overuse

5) Medical history

- arterial hypertension
- OSAS
- gastrointestinal comorbidities
- depression
- anxiety
- insomnia,
- use of hypnotics
- chronic musculoskeletal pain
- thyroid disease/dysfunction
- traumatic head injury
- traumatic cervical spine injury
- snoring

- bruxism
- constipation

5) Psychological questionnaires

- Toronto Alexithymia Scale (TAS-20) (22). TAS-20 evaluates alexithymia (a personality trait characterized by the inability to identify and express emotions). It uses a five-point Likert response scale and has a three-factor structure consisting of (1) Difficulty in identifying Feelings (2) Difficulty in describing feeling and (3) Externally oriented thinking.
- Leeds Dependence Questionnaire (LDQ) (23, 24), a self-administered questionnaire that measures dependence upon every substance, exploring systematically ICD-10 diagnostic criteria for defining dependence.
- Hospital Anxiety and Depression Scale (HADS) (25, 26), a screening tool for patients in a hospital setting for anxiety and depressive symptoms. The questionnaire consists of 14 items, 7 for measuring anxiety and 7 for depression.
- Childhood Trauma Questionnaire (27, 28), a screening measure for maltreatment histories in both clinical and general population groups, which includes 28 items (25 clinical items and 3 validity items).
- Stressful life-events questionnaire. Patients had to choose from a list of several stressful life events happened to him/her providing a score o severity from 1 to 5. The total score is derived from the sum of the score of single items.

Genetic Study

So far the body of knowledge about genetic predisposition to MOH and tendency to relapse after detoxification is relatively sparse and with contrasting results. In the view of a gene-candidate study several profiles may be associated with MOH susceptibility/pathophysiology or with drug seeking behavior.

We selected SNPs in genes that are relevant for migraine pathophysiology such as CGRP-related genes, TRPV1 and BDNF genes.

TRPV1

TRPV1 gene encodes for the transient receptor potential vanilloid-1 which is a ligand gated Ca2+ permeable non-selective cation channel that is activated by several stimuli, including heat, low Ph, voltage, capsaicin and endogenous lipid substances (29). These ion channels are expressed on a subpopulation of small or medium primary sensory neurons consisting of Aδ and C fiber nociceptors (30). More interestingly, TRPV1 channels are co-localized with CGRP in trigeminal neurons innervating cranial vessels (31). The activation of TRPV1 results in non-selective intracellular influx of cations, which in turn leads to nerve fibers depolarization and to the exocytosis of several bioactive substances, including CGRP. Since CGRP is well known to play a key role in the pathophysiology of migraine attacks (32, 33), a growing interest is arising around the involvement of TRPV1 in the biological mechanisms underlying migraine pathophysiology (31). In support of this hypothesis, the single nucleotide polymorphism TRPV1 rs222741 has emerged to contribute to the genetic susceptibility to migraine(34). Moreover, the observation of an higher expression of TRPV1 channels on nerve fibers innervating scalp arteries in chronic migraineurs compared to healthy subject (35) fosters the plausibility of TRPV1 as a genetic predictor of migraine chronification to MOH too. In the light of this, we selected two functional SNPs in TRPV1 gene, rs8065080 and rs222747, and we tested their role as potential genetic predictors of migraine chronification into MOH. More precisely, rs222747 is known to interfere with protein-protein interaction and homotetramerization of the channel while rs8065080 is supposed to affect the transmembrane domain, which confers responsiveness to capsaicin.

CGRP-related genes

The observation of increased blood levels of CGRP outside migraine attacks in women with chronic migraine compared with women with episodic migraine (36) raises the possibility that genes involved in the signaling pathway of CGRP may modulate the risk of migraine transformation into MOH. Given the unmet need to identify genetic risk factors of MOH, we selected three SNPs in two candidate genes encoding for receptor proteins that are fundamental to mediate CGRP effects. More precisely, the first identified candidate gene was CALCRL, that encodes for the calcitonin receptor-like receptor, an heteromeric receptor composed of a G protein-coupled receptor. The second one was RAMP1, that encodes for a transmembrane protein, named receptor (calcitonin) activity modifying proteins (RAMP1) which is required to transport CALCRL to the membrane (37). Given that the genetic variations CALCRL rs858745 and RAMP1 rs302680 was previously found to be correlated with the risk of migraine chronification (38), we herein aimed to validate their role as genetic predictors of migraine transformation into MOH.

BDNF

The brain-derived neurotrophic factor (BDNF) is the most common neurotrophin in humans which is well known to play a key role in neuronal growth, plasticity and neurogenesis (39). BDNF is expressed in nociceptive sensory neurons and several studies have highlighted its relevant role as pain modulator in adult pain perception and in migraine patophysiology (40). The observation of decreased serum BDNF levels in patients affected by chronic migraine compared to controls (40) supports the role of BDNF as a plausible genetic risk factor for MOH. The most extensively studied BDNF genetic variant is rs6265, consisting in a valine-to-methionine substitution at codon 66 (Val66Met) which in turns leads to impaired intracellular trafficking and activity-dependent secretion of BDNF (41). Since BDNF rs6265 has been also

reported to affect drug consumption in MOH (42), we evaluated the correlation between rs6265 and the susceptibility to MOH.

Methods

For the genetic study about 7 ml of venous whole blood has been collected into one EDTA tube and stored at +4 °C from each patient at T0. DNA has been extracted in the Mondino Experimental Neurobiology Lab (Head: C. Cereda) from each blood sample using a commercial extraction kit and stored at -20 °C. DNA samples has been then transferred to the Center for Pharmacogenetic and Pharmacogenomic Research (CRIFF) of "Università del Piemonte Orientale", Novara (Head: A. Genazzani) for the genetic analysis. Six single nucleotide polymorphisms (SNPs) have been selected in CGRP-related genes (RAMP1 rs7590387 and rs302680, CALCRL rs858745), TRPV1 (rs8065080 and rs222747) and BDNF genes (rs6265). Genotyping was performed by real-time PCR using Applied Biosystems TaqMan Pre-Designed SNP Genotyping assays (RAMP1 rs7590387 assay ID: C_26481962_10; RAMP1 rs302680 assay ID: C___1071215_10; CALCRL rs858745 assay ID: C___8726698_20; TRPV1 rs8065080 assay ID: C__11679656_10; TRPV1 rs222747 assay ID: C___1093688_20; BDNF rs6265 assay ID: C__11592758_10).

Statistical procedures

Clinical Study

Data are presented as means ± standard deviations for continuous data and as n/% for categorical data. The differences between MIG versus MOH patients and R-MOH versus NR-MOH were examined with chi square tests for categorical variables and one-way analysis of variance (ANOVA) for quantitative variables.

Univariate and multivariate logistic regression were applied in order to determine the probability of migraine patients to have MOH and the tendency of MOH patients to relapse into medication overuse after successful detoxification.

For all the variables, odds ratio (OR) was calculated to measure the strength of association with MOH and p value according to Mantel-Haenszel method to measure the level of statistical significance. Confidence intervals (CI) of each OR have been calculated by using standard error from the logistic regression.

The criterion for variable inclusion in the multivariate model was based on statistical significance at the level of $p<0.10$ as obtained by univariate analysis.

Two multivariate analyses were performed. The first one was done within the same group of variables (socio-demographical/lifestyle, migraine characteristics/history, obstetrician-gynecological history (restricted to women), familiar pathological history, medical history, psychological tests). After that, all the variables with statistical significance at the level of $p<0.10$ was included in the second multivariate analysis. Statistical significance of Adjusted (multivaried) ORs was calculated by the likelihood ratio derived from the regression.

All analyses were conducted using Statistical Package for Social Sciences (SPSS) version 24.

Genetic study

For the genetic part each polymorphism was tested for deviation from the Hardy-Weinberg equilibrium (HWE) using the online Finetti's program (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The association between SNPs and the two clinical endpoints (risk of transformation of episodic migraine into MOH and risk of relapse into MOH after withdrawal, respectively) were assessed by logistic regression analysis with adjustment for confounding clinical covariates (cut-off of P value <0.1 from the second multivariate analysis performed on clinical variables). For all the selected polymorphisms, we considered either a log-additive, a dominant, or a recessive mode of inheritance. Corresponding odd ratio (OR) and 95% confidence interval (Cl) were calculated

for each candidate SNP. Statistical significance was set at P<0.0041 according to Bonferroni's correction for multiple testing (0.05/12). Genotype data were managed with SNPStats software (http://bioinfo.iconcologia.net/snpstats/start.htm).

Results

Patient population

Three hundred and eighteen patients were enrolled, of which 158 MIG patients and 163 MOH patients.

MIG patients

At the 6 months follow-up 2 subjects were excluded, one for evidence of chronic migraine/medication overuse headache and one because the revision of the medical chart showed that one inclusion criteria was not fulfilled.

The evaluated population of 156 MIG patients had the following characteristics: 81% were female (n=126), average age was 40.3 ± 10.8 (range: 20–64), the average age of onset of migraine was 15.7 ±7.5 (range: 3– 40). The average monthly frequency of attacks of migraine without aura was 6.0 ± 3.0 (range: 1-14). Twenty patients (12%) had also attacks of migraine with aura. MIDAS score average was 17.1 ± 18.7.

MOH patients

All 163 patients underwent our detoxification program (Figure). One hundred and fifty five completed the follow-up at 2 months while 8 were lost to T2. One patient was excluded because of evidence of a probable secondary headache (headache associated to a chronic infection).

The evaluated population of 162 MOH patients had the following characteristics: 81% were female (n=131), average age was 43.7 ± 9.5 (range: 18-66), the average age of onset of migraine was 13.2 ± 6.2 (range: 3– 40), the average age of onset of medication overuse headache was 37.0 ± 9.0 (range 15-60). Twenty-five patients (15%) suffered also from migraine with aura.

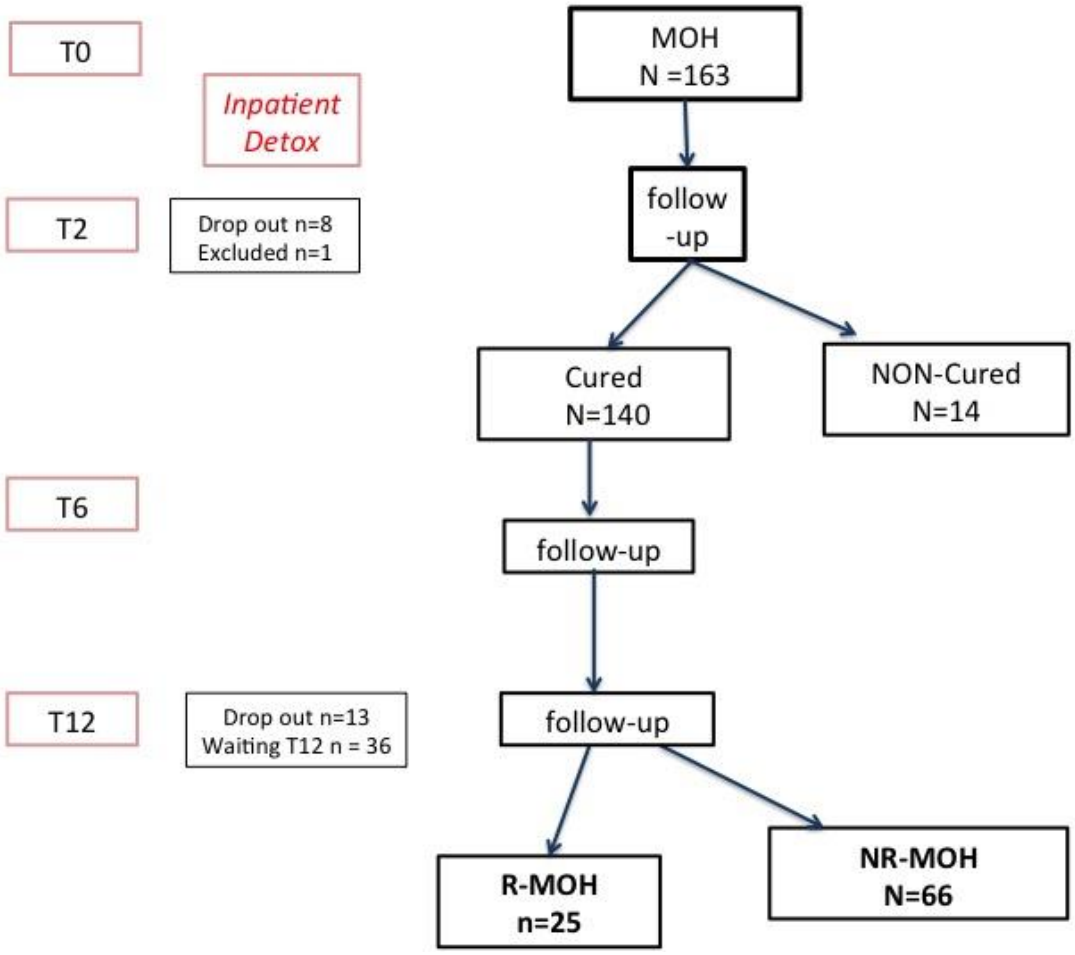

Figure. Flow-chart of the study with respect to MOH patients. R-MOH: relapsers MOH, NR-MOH: non relapsers MOH

The average frequency of headache days per month was 26.1± 4.6 (range 15-30), the average frequency of days of medication intake per month was 23.9 ± 5.6 (range 12-30), the average frequency of doses of medication intake per month was 45.8 ± 37.8 (range 12-30). The overused medication were: triptans in 21 patients (13.0%), simple analgesic in 51 (31.5%), combination-analgesic in 12 (7.4%), and multiple drug classes in 78 (48.1%). None of the patients qualified for ergotamine-overuse headache or opioid-overuse headache. Sixty-two patients (38.3%) previously underwent at least to one hospital detoxification program: 60 underwent inpatient detoxification program(s) (average of 2.1 ± 1.4, range 1-7) and 12 underwent day hospital

detoxification program(s) (average of 1.6 ± 0.7, range 1-3). MIDAS average score was 73.9 ± 52.9, HIT was 66.5 ± 6.1.

Of the 154 subjects who returned for visit T2, 140 (91%) stopped overuse following the detoxification program (cured), while14 patients (9%) failed to stop their drug overuse (non-cured) (Figure). In 112 of the 140 cured subjects (80%) migraine reverted to an episodic form, while in 28 patients (20%) migraine maintained a chronic pattern.

A total of 91 patients belonging to the cured group has completed the observation period and returned to the follow-up visit at T12; 13 patients dropped-out, while 36 patients are still waiting in the observation period from 6 to 12 months. Of those 91, 25 subjects (27%) relapsed into medication overuse (**R-MOH**) in the period between month 2 and month 12, while 66 subjects (63%) did not relapse (**NR-MOH**). In the group of 14 subjects who failed to stop overuse following detoxification: 5 completed the follow-up at T12 (all of them were still suffering from chronic migraine with medication overuse), 5 dropped-out and 4 are still under evaluation.

Table 1 shows the doses of medication per month, the days of medication per month, the headache days per month, HIT and MIDAS scores for R-MOH and NR-MOH groups at T12.

	R-MOH	**NR-MOH**	**Sign**
Doses of medications per month	22.9 (8.1)	7.0 (3.8)	**<0.001**
Days of medication intake per month	19.3 (6.7)	6.5 (3.5)	**<0.001**
Headache days per month	24.0 (5.8)	10.1 (6.3)	**<0.001**
HIT score	63.9 (5.1)	59.4 (7.1)	**0.009**
MIDAS score	75.2 (61.0)	37.8 (44.0)	**0.003**

Table 1. ANOVA for quantitative variables between relapsers and non relapser at T12. R-MOH: relapsers MOH, NR-MOH: non relapsers MOH.

Clinical Study - factors associated to MOH

Table 2 shows the results of the univariate and multivariate analyses (the first one – performed among variables of the same group) applied to the characteristics of MIG and MOH patients. After the second multivariate analysis (Table 3) the factors associated to MOH were age of onset of migraine (earlier), marital status (married or separated/divorced/widowed marital status versus unmarried), physical inactivity, depression, insomnia (insomnia associated to use of hypnotics versus absence of insomnia), traumatic head injuries, snoring, and traumas in childhood. Familiarity for headache was very close to the statistically level of significance.

	MOH n=162	**MIG** n=156	**Sign crude**	**OR 95%CI crude**	**Sign Adj***	**OR 95%CI Adjusted***
SOCIO-DEMOGRAPHICAL VARIABLES AND LIFESTYLE HABITS						
Female	131 (51.0)	126 (49.0)	0.98	1.01 (0.57-1.75)		
Age	43.7 (9.5)	40.3 (10.8)	**0.003**	**1.03 (1.01-1.05)**	0.21	
BMI	25.1 (13.5)	23.9 (13.3)	0.41	1.01 (0.98-1.02)		
High Educational level (> 8 years)	117 (47.8)	128 (52.2)	**0.037**	**0.56 (0.33-0.97)**	0.20	
Marital Status						
- Unmarried	36 (36.2)	60 (63.8)	**0.001**	**ref**	0.12	Ref
- Married	99 (55.3)	80 (44.7)	**0.003**	**2.18 (1.30-3.65)**	0.15	
- Separated/divorced/Widowed	27 (71.1)	11 (28.9)	**<0.001**	**4.33 (1.91-9.81)**	**0.019**	**3.02 (1-19-7.64)**
Occupation: type (n=311)						
- Boss / responsibility	24 (55.8)	19 (44.2)	0.53	Ref		
- Employee	93 (51.7)	87 (48.3)	0.62	0.84 (0.43-1.65)		
- Not working**	42 (49.4)	43 (50.6)	0.49	0.33 (0.77-1.49)		
Occupation: satisfying	32 (60.4)	21 (39.6)	0.16	1.56 (0.83-2.92)		
Occupation: stressful	21 (42.9)	28 (57.1)	0.14	0.62 (0.32-1.17)		
Low Income (<28.000euros/year) (n=311)	100 (52.1)	92 (47.9)	0.77	1.06 (0.67-1.68)		
Coffee (# per day)	2.2 (1.7)	2.2 (1.5)	0.74	0.97 (0.85-1.12)		
Smoking						
- no	124 (50.0)	124 (50.0)	0.69	Ref	0.19	
- 1-10 cigarette(s)/day	17 (42.5)	23 (57.5)	0.38	0.73 (0.37-1.45)	0.37	
- >10 cigarettes/day	21 (70.0)	9 (30.0)	**0.043**	**2.33 (1.02-5.29)**	0.14	
Alcohol daily use (at least one dose per day)	11.9%	8.7%	0.35	1.42 (0.67-2.98)		
Physical Activity (current)	39 (40.6)	57 (59.4)	**0.016**	0.55 (0.33-0.89)	**0.034**	**0.56 (0.33-0.95)**

	MOH n=162	**MIG** n=156	**Sign crude**	**OR 95%CI crude**	**Sign Adj***	**OR 95%CI Adjusted***
MIGRAINE CHARACTERISTICS						
Age of onset	13.2 (6.2)	15.5 (7.5)	**0.004**	**0.95 (0.92-0.98)**	**0.004**	**0.95 (0.92-0.98)**
Duration of disease (primary headache before oveeuse onset for MOH)	24.0 (9.7)	24.6 (10.4)	0.57	0.99 (0.97-1.01)		
Duration of MOH	6.7 (6.2)	n.e.				
Migraine with Aura	25 (55.6)	20 (44.4)	0.50	1.24 (0.65-2.33)		
Use of at least one migraine preventive medication (MIG - whenever, MOH - before overuse): Yes vs no/unable to recall	74 (56.9)	56 (43.1)	**0.07**	**1.50 (0.95-2.35)**	0.07	**1.52 (0.96-2.41)**
OBSTETRICIAN AND GYNECOLOGICAL HISTORY (n=257)						
Menarche (age)	12.3 (1.7)	12.4 (2.1)	0.90	0.99 (0.87-1.12)		
Menstrual cycle: irregular menstrual bleeding	37 (56.1)	29 (43.9)	0.54	1.19 (0.67-2.10)		
Menstrual Cycle: Triggering Migraine	54 (43.0)	98 (57.0)	0.37	1.32 (0.71-2.46)		
Dysmenorrhea	84 (52.8)	75 (47.2)	0.53	0.84 (0.48-1.45)		
COCs: current/previous use	110 (57.3)	82 (42.7)	**0.006**	**2.52 (1.31-4.85)**	**0.022**	**2.19 (1.12-4.27)**
COCs: effect on migraine (n=157)						
- none	54 (60.7)	35 (39.3)	0.39	Ref		
- improved	5 (50.0)	5 (50.0)	0.51	0.64 (0.17-2.40)		
- worsened	36 (62.1)	22 (37.9)	0.86	1.06 (0.53-2.09)		
Deliveries n.	1.13 (0.93)	0.86 (0.94)	**0.022**	**1.37 (1.04-1.79)**	**0.048**	**1.32 (1.00-1.75)**
Effect of Pregnancy on migraine (n=103)						
- none	17 (54.8)	14 (45.2)	0.27	Ref		
- improved	40 (65.6)	21 (34.4)	0.31	1.56 (0.64-3.79)		
- worsened	8 (72.7)	3 (27.3)	0.30	2.19 (0.48-9.87)		
Voluntary interruption(s) of pregnancy	14 (56.0)	11 (44.0)	0.74	1.15 (0.50-2.64)		
Miscarriage(s)	21 (56.8)	16 (43.2)	0.60	1.20 (0.59-2.43)		
Menopause	30 (54.5)	25 (45.5)	0.94	0.98 (0.53-1.79)		
Menopause age (n=55)	44.3 (13.2)	40.3 (17.1)	0.32	1.01 (0.98-1.05)		
Reproductive system comorbidities$	33 (56.9)	25 (43.1)	0.54	1.20 (0.66-2.19)		

	MOH n=162	**MIG** n=156	**Sign crude**	**OR 95%CI crude**	**Sign Adj***	**OR 95%CI Adjusted***
FAMILY HYSTORY						
Headache	145 (55.0)	121 (45.0)	**0.002**	**3.01 (1.51-5.99)**	**0.002**	**3.01 (1.51-5.99)**
Arterial hypertension	103 (55.1)	84 (44.9)	0.17	1.37 (0.87-2.13)		
Depression and/or Anxiety	44 (55.7)	35 (44.3)	0.44	1.22 (0.73-2.04)		
Alcohol Dependence	6 (40.0)	9 (60.0)	0.35	0.61 (0.21-1.75)		
Substance overuse	8 (66.7)	4 (33.3)	0.29	1.92 (0.56-6.51)		
MEDICAL HYSTORY						
Arterial hypertension	31 (63.3)	18 (36.7)	0.06	1.81 (0.96-3.40)		
OSAS	8 (72.7)	3 (23.3§§)	0.14	2.64 (0.69-10.17)		
Gastrointestinal comorbidities (lifetime)	60 (54.5)	50 (45.5)	0.35	1.24 (0.78-1.98)		
Depression (lifetime)	62 (71.3)	25 (28.7)	**<0.001**	**3.24 (1.90-5.53)**	**0.026**	**2.01 (1.08-3.73)**
Anxiety (lifetime)	95 (61.3§)	60 (38.7)	**<0.001**	**2.26 (1.44-3.55)**	0.45	
Insomnia						
- no	74 (43.3)	97 (56.6)	**<0.001**	Ref	**0.010**	**Ref**
- only insomnia	47 (53.4)	41 (46.6)	0.124	1.50	0.68	1.22 (0.64-1.94)
- insomnia + use of hypnotic	39 (81.3)	9 (18.8)	**<0.001**	**5.68**	**0.002**	**3.73 (1.59-8.77)**
Chronic musculoskeletal complaints (lifetime)	52 (60.5)	34 (39.5)	**0.039**	1.69 (1.02-2.80)	0.69	
Thyroid disease/dysfunction	15 (53.6)	13 (46.4)	0.40	1.37 (0.65-2.91)		
Traumatic head injury	40 (64.5)	22 (35.5)	**0.018**	**1.99 (1.12-3.55)**	**0.06**	**1.81 (0.96-3.42)**
Traumatic cervical spine injury	58 (61.7)	36 (38.3)	**0.013**	**1.86 (1.13-3.04)**	0.32	
Snoring	55 (64.7)	30 (35.3)	**0.026**	**1.79 (1.06-3.02)**	**0.015**	**2.00 (1.14-3.49)**
Bruxism	53 (58.2)	38 (41.8)	0.45	1.21 (0.73-1.99)		
Constipation	55 (62.5)	33 (37.5)	**0.044**	**1.68 (1.01-2.79)**	0.10	

	MOH n=162	**MIG** n=156	**Sign crude**	**OR 95%CI crude**	**Sign Adj***	**OR 95%CI Adjusted***
PSYCHOLOGICAL VARIABLES						
HADS_D score (Depression)	6.5 (4.4)	5.1 (3.6)	**0.003**	**1.09 (1.03-1.15)**	0.29	
HADS_A score (Anxiety)	7.6 (4.3)	7.0 (4.0)	0.17	1.03 (0.98-1.09)		
TAS-20 Total score (20-100)	45.8 (11.5)	44.0 (12.5)	0.19	1.01 (0.99-1.03)		
F1-TAS_20	15.9 (6.6)	14.1 (6.5)	**0.017**	**1.04 (1.01-1.08)**	0.43	
F2-TAS-20	12.1 (4.6)	11.7 (5.0)	0.47	1.01 (0.97-1.06)		
F3-TAS-20	17.6 (4.6)	17.1 (5.5)	0.42	1.01 (0.97-1.06)		
Traumatic events in childhood	1.3 (1.1)	0.8 (1.1)	**0.003**	**1.42 (1.11-1.82)**	**0.045**	**1.33 (1.01-1.77)**
Stressful life events (total score)	16.7 (12.7)	13.4 (11.3)	**0.037**	**1.02 (1.01-1.04)**	0.47	

Table 2. Clinical Study. Univariate and multivariate logistic regression equations performed for predicting the tendency to be a MOH versus MIG patient. Data are presented as means ± standard deviations for continuous data and as n/% for frequency data (percentages refer to the rows). * adjusted for variables of the same group with $p<0.10$ at the univariate. **not working: housewife, retired, unemployed, students. $ Uterine fibroids, uterine polyps, endometriosis, ovarian cysts. (no significant differences were found of each of these pathologies between MIG and MOH). HADS: Hospital Anxiety and Depression Scale. TAS: Toronto Alexithymia Scale. F1-TAS20: Difficulty identifying feelings subscale. F2-TAS20: Difficulty describing feelings subscale. F3-TAS20: Externally oriented thinking subscale

	Sign	OR 95% CI
Age of onset	0.016	0.94 (0.89-0.98)
Marital Status		
- **Single**	0.005	Ref
- **Married**	0.002	3.65 (1.63-8.19)
- **Separated/divorced/Widowed**	0.031	4.19 (1.13-15.47)
Physical Activity	0.029	0.42 (0.19-0.91)
Snoring	0.036	2.24 (1.05-4.79)
Depression	0.012	2.91 (1.25-6.73)
Insomnia		
- **no**	0.018	Ref
- **only insomnia**	0.94	1.02 (0.47-2.20)
- **insomnia + use of hypnotic drugs**	0.006	5.59 (1.65-18.93)
Familiarity for headache	0.082	2.63 (0.88-7.86)
Childhood Trauma Questionnaire total score	0.012	1.48 (1.09-2.02)
Use of at least one migraine preventive medication (MIG - whenever, MOH - before overuse): Yes vs no/unable to recall	0.014	2.36 (1.18-4.71)

Table 3. Clinical Study. Second multivariate logistic regression equations performed for predicting the tendency to be a MOH versus MIG patient. The two variables with a $p<0.10$ in the multivariate analysis in obstetrical/gynecologycal history were tested in this model only within the female population without reaching the significance level of $p<0.05$.

Clinical study - factors associated to relapse

Table 4 shows the results of the univariate and multivariate analyses applied to the characteristics of patients who relapse after a successful detoxification and those who did not relapse. After the second multivariate analysis (table 5) the factors associated to relapse were MIDAS score, depression (lifetime) and overuse of combination of analgesics or multiple drug classes.

	R-MOH (n=25)	NR-MOH (n=66)	Sign	OR (CI 95%) crude	Sign*	OR (CI 95%) adjusted*
SOCIO-DEMOGRAPHICAL VARIABLES AND LIFESTYLE HABITS						
Female	20 (29.9)	47 (70.1)	0.39	1.61 (0.53-4.93)		
Age	46.6 (6.2)	44.0 (9.8)	0.24	1.03 (0.97-1.08)		
BMI	24.7 (3.9)	23.5 (5.5)	0.35	1.04 (0.95-1.13)		
High Educational level (> 8 years)	19 (26.8)	52 (73.2)	0.77	0.85 (0.28-2.53)		
Marital Status - Unmarried - Married - Separated/divorced/Widowed	 6 (30.0) 14 (25.5) 5 (31.3)	 14 (70.0) 41 (74.5) 11 (68.8)	 0.86 0.69 0.93	 REF 0.79 (0.25-2.47) 1.06 (0.25-4.41)		
Occupation: type - Boss - Employee - Not working**	 6 (40.0) 16 (28.1) 3 (15.8)	 9 (60.0) 71 (71.9) 16 (84.2)	 0.30 0.37 0.12	 REF 0.58 (0.17-1.91) 0.28 (0.05-1.40)		
Occupation: satisfying	6 (37.5)	10 (62.5)	0.21	1.38 (0.43-4.46)		
Occupation: stressful	3 (25.0)	9 (75.0)	0.20	0.66 (0.16-2.75)		
Low Income (<28.000euros/year) (n=311)	17 (30.4)	39 (69.6)	0.43	1.47 (0.55-3.89)		
Coffee (# per day)	2.3 (1.5)	2.4 (2.0)	0.79	0.96 (0.75-1.23)		
Smoking	5 (20.8)	19 (79.2)	0.37			
Alcohol daily use (at least one dose per day)	3 (20.0)	12 (80.0)	0.44	0.61 (0.15-2.38)		
Physical Activity (current)	6 (26.1)	17 (73.9)	0.86	0.91 (0.31-2.65)		
MIGRAINE CHARACTERISTICS						
Age of onset (years)	14.0 (7.3)	12.4 (5.2)	0.28	1.04 (0.96-1.13)		
Duration of migraine before MOH onset (years)	27.1 (9.6)	24.8 (10.3)	0.32	1.02 (0.97-1.07)		
Duration of overuse (years)	6.4 (6.2)	6.9 (5.3)	0.71	0.98 (0.90-1.07)		

Duration of entire illness (migraine and MOH)	32.5 (7.6)	31.5 (11.0)	0.70	1.00 (0.96-1.05)		
Medication overused: Triptan or Analgesic vs others	7 (16.3)	36 (83.7)	**0.027**	**0.32 (0.11-0.88)**	**0.071**	**0.36 (0.11-1.08)**
Doses of medications per month	59.0 (47.0)	40.4 (26.7)	**0.037**	**1.01 (1.00-1.02)**	0.92	
Days of medication intake per month	26.0 (4.9)	23.0 (5.4)	**0.019**	**1.11 (1.01-1.22)**	0.67	
Headache days per month	27.6 (3.5)	25.1 (4.9)	**0.028**	**1.14 (1.01-1.28)**	0.49	
HIT score	67.5 (4.4)	66.1 (4.8)	0.22	1.06 (0.96-1.18)		
MIDAS score	90.4 (63.6)	62.7 (47.0)	**0.037**	**1.01 (1.00-1.01)**	**0.049**	**1.01 (1.00-1.02)**
Factors associated with chronification:						
- none	13 (32.5)	27 (67.5)	0.77	REF		
- stress	8 (23.5)	26 (76.5)	0.59	0.75 (0.27-2.10)		
- others	4 (33.3)	8 (66.7)	0.76	1.23 (0.31-4.80)		
Use of at least one migraine preventive medication before MOH onset	10 (22.2)	35 (77.8)	0.50	0.54 (0.19-1.50)		
Migraine preventive medications used: total number	4.3 (2.5)	4.4 (3.0)	0.91	0.99 (0.84-1.16)		
Migraine preventive medications: at least one effective	12 (24.5)	37 (75.5)	0.45	0.68 (0.25-1.84)		
Migraine with Aura	2 (16.7)	10 (83.3)	0.37	0.48 (0.09-2.39)		
Previous detoxification (inpatient or DH): total number	1.0 (1.7)	0.8 (1.4)	0.64	1.07 (0.80-1.43)		
Symptomatic medications prescribed at discharge:						
- Triptans	9 (26.5)	25 (73.5)	0.80	REF		
- Simple analgesics	14 (29.8)	33 (70.2)	0.74	1.17(0.44-3.15)		
- Analgesic + caffeine	2 (20.0)	8 (80.0)	0.67	0.69 (0.12-3.90)		
Preventive medication prescribed at discharge:						
- Calcium agonist	2 (16.7)	10 (83.3)	0.60	REF		
- TCA	11 (25.0)	33 (75.0)	0.54	1.66 (0.31-8.80)		
- AED	8 (32.0)	17 (68.0)	0.33	2.35 (0.41-13.34)		
- Others	4 (40.0)	6 (60.0)	0.23	3.33(0.46-24.05)		

	R-MOH (n=25)	NR-MOH (n=66)	Sign	OR (CI 95%) crude	Sign*	OR (CI 95%) adjusted*
OBSTETRICIAN AND GYNECOLOGICAL HISTORY (n=67)						
Menarche	12.1 (3.0)	12.5 (1.3)	0.47	0.91 (0.70-1.17)		
Menstrual Cycle: irregular periodism	7 (33.3)	14 (66.7)	0.71	1.23 (0.40-3.74)		
Menstrual Cycle: Triggering Migraine	17 (32.7)	35 (67.3)	0.28	2.42 (0.47-12.33)		
Dysmenorrhea	11 (27.5)	29 (72.5)	0.54	0.71 (0.24-2.07)		
COCs: current/previous use	15 (26.8)	41 (73.2)	0.28	0.45 (0.10-1.93)		
COC worsened migraine	4 (21.1)	15 (78.9)	0.43	0.58 (0.15-2.22)		
Deliveries #						
Pregnancy improved migraine	6 (25.0)	18 (75.0)	0.92	0.93 (0.23-3.69)		
Voluntary interruption(s) of pregnancy	2 (33.3)	4 (66.7)	0.86	1.16 (0.19-6.95)		
Miscarriage(s)	3 (37.5)	5 (62.5)	0.63	1.44 (0.31-6.74)		
Menopause	5 (31.3)	11 (68.8)	1.00	1.00 (0.29-3.38)		
Menopause age	44.5 (5.9)	44.7 (14.4)	0.97	0.99 (0.91-1.09)		
FAMILIAR HYSTORY						
Headache	22 (27.2)	59 (72.8)	0.69	0.74(0.17-3.24)		
Arterial hypertension	16 (28.1)	41 (71.9)	0.93	1.04 (0.39-2.71)		
Depression and/or Anxiety	5 (26.3)	14 (73.7)	0.84	0.89 (0.28-2.80)		
Alcohol Dependence	0 (0.0)	1 (100.0)	1.00	n.e.		
Substance overuse	1 (50.0)	1 (50.0)	0.49	2.66 (0.16-44.34)		

	R-MOH (n=25)	**NR-MOH** (n=66)	**Sign**	**OR (CI 95%) crude**	**Sign***	**OR (CI 95%) adjusted***
MEDICAL HYSTORY						
Arterial hypertension	9 (42.9)	12 (57.1)	**0.07**	**2.53 (0.90-7.08)**	0.15	
OSAS	3 (50.0)	3 (50.0)	0.21	2.86 (0.53-15.24)		
Gastrointestinal comorbidities	12 (35.3)	22 (64.7)	0.20	1.84 (0.72-4.71)		
Depression	16 (41.0)	23 (59.0)	**0.014**	**3.32 (1.27-8.68)**	**0.025**	**3.05 (1.065-10.71)**
Anxiety	16 (30.8)	36 (69.2)	0.41	1.48 (0.57-3.82)		
Chronic musculoskeletal complaints	7 (24.1)	22 (75.9)	0.62	0.77 (0.28-2.14)		
Thyroid disease/dysfunction	5 (41.7)	7 (58.3)	0.24	2.10 (0.60-7.38)		
Traumatic head injury	9 (37.5)	15 (62.5)	0.20	1.91 (0.70-5.19)		
Traumatic cervical spine injury	10 (31.3)	22 (68.8)	0.55	1.33 (0.51-3.44)		
Insomnia - no - only insomnia - insomnia + use of hypnotics	 11 (26.2) 5 (21.7) 9 (34.6)	 31 (73.8) 18 (78.3) 17 (65.4)	 0.58 0.69 0.46	 REF 0.78 (0.23-2.61) 1.49 (0.51-4.31)		
Snoring	10 (31.3)	22 (68.8)	0.62	1.27 (0.49-3.29)		
Bruxism	11 (39.3)	17 (60.7)	0.11	2.17 (0.82-5.70)		
Constipation	8 (28.6)	20 (71.4)	0.94	1.03 (0.38-2.79)		
PSYCHOLOGICAL VARIABLES						
HADS_D score (Depression)	7.2 (4.8)	6.2 (4.4)	0.369	1.04 (0.94-1.16)		
HADS_A score (Anxiety)	7.6 (5.4)	7.0 (3.9)	0.546	1.03 (0.93-1.14)		
TAS-20 Total score (20-100)	43.4 (11.6)	45.6 (11.9)	0.439	0.98 (0.94-1.02)		
F1-TAS_20	16.0 (7.8)	15.6 (6.7)	0.834	1.00 (0.94-1.07)		
F2-TAS-20	10.9 (4.3)	12.0 (4.9)	0.319	0.95 (0.85-1.05)		
F3-TAS-20	16.4 (4.1)	17.8 (4.2)	0.188	0.92 (0.82-1.03)		
LEEDS Dependency Scale	9.0 (6.3)	7.7 (4.8)	0.318	1.04 (0.95-1.13)		
Traumatic events in childhood	1.0 (1.2)	1.3 (1.2)	0.596	0.84 (0.45-1.56)		
Stressful life events (total score)	16.4 (11.1)	16.4 (10.0)	0.982	0.99 (0.95-1.04)		

Table 4. Clinical Study. Univariate and multivariate logistic regression equations performed for predicting the tendency to relapse after a successful detoxification. R-MOH: relapsers MOH, NR-MOH: non relapsers MOH**;** * adjusted for variables of the same group with p<0.10 at the univariate. **not working: housewife, retired, unemployed, student; n.e.: not evaluable. $ Uterine fibroids, uterine polyps, endometriosis, ovarian cysts. (no significant differences were found of each of these pathologies between two groups of patients). TAS: Toronto Alexithymia Scale. F1-TAS20: Difficulty identifying feelings subscale. F2-TAS20: Difficulty describing feelings subscale. F3-TAS20: Externally oriented thinking subscale.

	Sign	**OR 95% CI**
MIDAS	0.035	1.01 (1.001-1.021)
Depression	0.017	3.82 (1.277-11.472)
Medication overused: Triptan or Analgesic vs others	0.021	0.25 (0.081-0.814)

Table 5. Overall multivariate analysis for predicting the risk of relapse after a successful detoxification.

Genetic Study - factors associated to MOH

After a multivariate analysis adjusted for clinical factors with a $p<0.1$ in the second multivariate, none of the genetic polymorphisms was found to be associated to MOH when compared to MIG (Table 6).

Genetic Study - factors associated to relapse

After a multivariate analysis adjusted for clinical factors with a $p<0.1$ in the second multivariate, RAMP1 rs7590387 polymorphisms showed an association with relapse: $p=0.0015$ in the log-additive model of inheritance and $p=0.0001$ in the dominant model of inheritance (Table 7).

SNP	MIG N, (%)	MOH N, (%)	OR (95% CI)*	P value
CALCRL rs858745				
C/C	112 (76.2)	110 (72.8)	A: 1.26 (0.74-2.14)	0.39
C/T	32 (21.8)	37 (24.4)	D: 1.23 (0.66-2.28)	0.51
T/T	3 (2.)	4 (2.6)	R: 2.14 (0.42-10.88)	0.36
RAMP1 rs7590387	40 (29)	55 (37.9)	A: 0.88 (0.59-1.30)	0.51
C/C	71 (51.5)	61 (42.1)	D: 0.64 (0.35-1.15)	0.13
C/G	27 (19.6)	29 (20)	R: 1.24 (0.62-2.50)	0.55
G/G				
RAMP1 rs302680				
A/A	96 (66.7)	108 (72.5)	A: 0.92 (0.55-1.55)	0.76
A/G	45 (31.2)	38 (25.5)	D: 0.86 (0.48-1.54)	0.62
G/G	3 (2.1)	3 (2)	R: 1.52 (0.26-8.95)	0.64
TRPV1 rs8065080				
T/T	41 (29.3)	47 (32.6)	A: 0.98 (0.66-1.47)	0.93
T/C	74 (52.9)	71 (49.3)	D: 1.08 (0.59-1.95)	0.81
C/C	25 (17.9)	26 (18.1)	R: 0.84 (0.41-1.76)	0.65
TRPV1 rs222747				
G/G	72 (50)	86 (57)	A: 0.89 (0.58-1.38)	0.61
G/C	57 (39.6)	58 (38.4)	D: 1.07 (0.62-1.86)	0.81
C/C	15 (10.4)	7 (4.6)	R: 0.38 (0.13-1.16)	0.085
BDNF rs6265				
G/G	96 (69.1)	101 (67.3)	A: 1.02 (0.61-1.69)	0.94
G/A	39 (28.1)	45 (30)	D: 1.08 (0.61-1.94)	0.78
A/A	4 (2.9)	4 (2.7)	R: 0.66 (0.14-3.25)	0.61

Table 6: Association of SNPs in CALCRL, RAMP1 and TRPV1 and BDNF genes with the risk of episodic migraine transformation into MOH

SNP	Outcome at 1 year		OR (95% CI)*	P value
	NR-MOH N, (%)	R-MOH N, (%)		
CALCRL rs858745				
C/C	44 (75.9)	17 (81)	A: 1.31 (0.40-4.33)	0.66
C/T	13 (22.4)	3 (14.3)	D: 0.93 (0.24-3.62)	0.92
T/T	1 (1.7)	1 (4.8)	R: 18.13 (0.78-420.49)	0.092
RAMP1 rs7590387				
C/C	28 (51.9)	3 (13.6)	**A: 3.83 (1.55-9.47)**	**0.0015**
C/G	19 (35.2)	14 (63.6)	**D: 13.93 (2.75-70.59)**	**0.0001**
G/G	7 (13)	5 (22.7)	R: 2.70 (0.61-12.01)	0.19
RAMP1 rs302680				
A/A	48 (81.4)	15 (75)	A: 1.40 (0.43-4.55)	0.58
A/G	9 (15.2)	5 (25)	D: 1.66 (0.44-6.17)	0.46
G/G	2 (3.4)	0 (0)	R: NC	
TRPV1 rs8065080				
T/T	18 (34)	5 (23.8)	A: 1.32 (0.57-3.06)	0.51
T/C	25 (47.2)	14 (66.7)	D: 3.14 (0.80-12.25)	0.083
C/C	10 (18.9)	2 (9.5)	R: 0.41 (0.06-2.54)	0.31
TRPV1 rs222747				
G/G	35 (61.4)	10 (45.5)	A: 2.49 (0.84-7.42)	0.096
G/C	20 (35.1)	12 (54.5)	D: 2.83 (0.88-9.03)	0.073
C/C	2 (3.5)	0 (0)	R: NC	
BDNF rs6265				
G/G	40 (67.8)	13 (65)	A: 0.76 (0.26-2.22)	0.61
G/A	16 (27.1)	7 (35)	D: 0.98 (0.29-3.28)	0.97
A/A	3 (5.1)	0 (0)	R: NC	

Table 7: Association of SNPs in CALCRL, RAMP1, TRPV1 and BDNF genes with the risk of relapse into MOH after successful

Discussion

MOH is frequent and it represents the most costly chronic headache with a high socio-economical burden. The precise mechanisms involved in the progression from migraine to MOH are elusive. Moreover it is unknown why some patients relapse into MOH after a successful detoxification treatment.

Here we report data from a study involving 318 long-term migraine sufferers of which 163 developed MOH while 156 did not. We followed those patients over a period of 6 months (MIG group) and 14 months (MOH group).

The first finding of our study that is worth attention, because it strengthens our results, is that the duration of migraine (duration of migraine before MOH onset in the case of MOH) was not significantly different between the two groups. This result was likely facilitated by the inclusion criteria we chose for MIG group, which required patients suffering from migraine from > 10 years. We believe this aspect has been important as it reassures us about the fact that MIG patients should have had enough time to develop a medication overuse (in case they would have been prone to it). Unfortunately this particular care was not used in other cross sectional studies (43).

Clinical factors associated to MOH

Multivariate analysis allowed the identification of some factors associated to MOH (Table 3). These are: age of onset of migraine (earlier), marital status (married or separated/divorced/widowed marital status versus single), physical activity (less physical activity), depression, insomnia, traumatic head injury, snoring, traumas in childhood, familiarity of headache (this last one just below of level of significance). The observational design of the

study does not warrant the possibility to identify which of these factors has indeed a role in the development of MOH. Yet, the findings allow some important considerations about the fact that some of these factors are likely to be a consequence of MOH (or of the process of development already started) instead of a cause, while others are more likely to paly a causal role.

Migraine preventive medications

One of the variables that are certainly a consequence of the process leading to MOH is the previous use of migraine preventive medications. Baseline headache frequency has been demonstrated to be the strongest risk factor for the development of MOH (44). Therefore patients who will develop MOH suffer from moderate-high frequency of migraine attacks. Such clinical picture has probably prompted the prescription of preventive medications by the physicians, which explains the higher number of preventative medications prescribed to MOH with respect to MIG group. It is interesting to note that although about half of MOH patients tried preventive medication before developing overuse, but they developed medication overuse nonetheless. This also introduces the possibility that MOH patients responded less than MIG to preventive therapy. Indeed we tried to collect this information by asking patients whether at least one preventive medication was effective in the past. Unfortunately we had 50% of missing data as i) some patients did not used any preventive medication, ii) some patients was not able to recall the efficacy of the treatment, iii) some patients did use the preventive medication with proper dosage, duration of treatment and compliance. It is likely that the issue of poor responsiveness to medications (preventive and/or symptomatic (45)) may be in some direction linked to medication overuse.

Physical Activity

Also the low rate of physical activity (PA) could be a consequence of MOH, as it is quite difficult to practice PA in a chronic disabling condition. On the other hand PA is effective in

reducing frequency of migraine attacks (46) and overall it has been demonstrated in a prospective study that physical inactivity doubled the risk of MOH after 11 years (44). In our study the "practice of physical activity" was referred to the recent period. In view of a next observational study it would be better to know: i) if the patients have practiced physical activity in the past and in positive case the chronological aspects in relation with migraine history (migraine onset, chronification, medication overuse) ii) which type of physical activity, frequency and intensity of training.

Age of onset and familiarity for headache

Our results showed that MOH patients had a lower age of onset of migraine with respect to MIG patients (2.3 years, p=0.016). In order to better contextualize this finding, it is important to note that the earlier onset is not associated to the fact that patients had more time to develop MOH as, at least in our sample, the duration of primary headache (migraine) was similar in the two groups (24 years, p=0.57). Therefore an interpretation could be that MOH patients are more prone to manifest migraine, which starts earlier in their life and with more frequent attacks (44). In this scenario, patients are driven to more frequent use of symptomatic medications and hence to a higher risk to develop medication overuse.

The finding regarding a higher rate of familiarity for headache also seems to speak in favor of a greater predisposition, with a bigger possibility of having received a greater load of "migraine genes" from the previous generations.

Marital Status

Married or separated/divorced/widowed versus unmarried marital status is more common in MOH (3 to 4 fold). In the univariate analysis, MOH patients showed a higher age (3 years), yet the variable *age* loose statistical significance after multivariate analysis. Therefore the marital status itself should enclose particular clues linked to MOH. This can be due to the fact that

marriage and the creation of a family brings with itself a higher number of responsibilities, obligation and concerns that can increase the daily stress load - which is known to be one of the strongest and common trigger factors of migraine attacks (47, 48). Moreover a family life brings several duties, which require a minimum level of functioning. Therefore, those patients can be driven to use more frequently symptomatic medication.

Depression

Our data shows that depression is associated with a 3-fold risk of being a MOH patient. The risk of developing MOH was reported in two prospective, population-based study, one in USA (49) and one in Norway (44). The reproduction of the same finding in large prospective studies seems to remove the hypothesis that depression is, in the majority of cases, a consequence of MOH. Indeed symptoms of depression may influence pain-coping abilities and encourage resort to frequent use of analgesics. In the latter case depression represents a causal factor of MOH, yet it is also possible that depression and MOH share a common biological background. In fact, neurotransmitter disturbances are present in depression (50), and these may play a role in the development of MOH. If so, this may indicate shared susceptibility genes. Although so far, no shared genes between MOH and psychiatric disorders have convincingly been demonstrated, in a previous study our group (51) we showed that a subgroup of MOH patients who did not respond to detoxification carried a specific catechol-O-methyltransferase gene polymorphism. This finding seems particularly relevant when considering that the same polymorphism is associated with major depression disorder (MDD) (52). In other two previous studies, we demonstrated in a population of MOH that specific polymorphisms of the serotonin 5HT2A receptor and serotonin transporter gene SLC6A4 are associated respectively to a higher number of symptomatic drug doses (and therefore possibly to a drug-seeking behavior in these patients) (53) and to a higher number of monthly days of headache (and therefore with a more severe

disease) (54). Also in this case, 5-HT-related gene polymorphisms are known to be related to the pathogenesis of MDD (55).

While in the US study (49) it was evaluated only depression, in the Norwegian one (44) it was evaluated also anxiety, finding a 2-fold risk of developing MOH in patients with anxiety (OR for depression = 2.6) (44). In our study in the univariate analysis we found a significant difference ($p<0.001$) for anxiety (lifetime) between MOH and MIG patients with a crude OR of 2.26 (C.I. 1.44-3.55) toward MOH. This significance disappeared after performing the first multivariate analysis with other variables of medical history ($p=0.45$ OR 1.22 (0.71-2.09)). Indeed Hagen and colleagues adjusted OR of anxiety just for few variables (age, gender, education and headache frequency). When we analyzed our data adjusting the OR of anxiety for the same 4 variables, it remained statistically significant ($p=0.002$, OR 2.0 (1.30-3.29). If we add also depression to anxiety and these 4 variables in the multivariate analysis, anxiety looses statistically significance ($p=0.07$ OR 1.57 (0.96-1.59). Therefore it should be discussed whether the association between anxiety and MOH found by Hagen and coauthors is actually due to the co-occurrence of depression.

Head injury

Our results show that head injury was associated to MOH with an OR of 3.5. Couch and colleagues reported a relationship between head and neck injury (HANI) and chronic daily headache (CDH), which was not limited to injuries proximate to CDH onset (56). Moreover they showed evidence that lifetime risk of CDH increases with increasing number of HANI and that number, but not severity, of HANIs was a risk factor for CDH. Our hypothesis is that HANI could lower the migraine threshold making the patient more prone to develop, also later in life, frequent and severe attacks. From a pathophysiological perspective it has been postulated that central mechanisms included perturbation of pain-related structures such as the periaqueductal gray (57, 58), which are implied in pathophysiology of migraine and MOH.

Insomnia

As regards insomnia, our results shows that only insomnia severe enough to the require use of hypnotics is associated to MOH with an OR of 5.5**.** This finding is in agreement with the results of the prospective study by Hagen and colleagues (44), where they found that only severe insomnia was associated to the risk (OR 1.9) of developing MOH, and with those of another study (59).

Regarding the mechanisms for the relationship between insomnia and MOH, experimental studies have shown that total and selective sleep deprivation (60) as well as disrupted sleep continuity (61) leads to hyperalgesia in healthy subjects, possibly because sleep loss causes a transient disturbance of the descending pain inhibitory control system (62). An alternative theory is that sleep disruption leads to an increase in inflammatory mediators like IL-6, which may sensitize several types of nociceptors (63, 64). Interestingly, behavioral sleep modification proved effective in reducing the frequency and intensity of chronic headache (65).

Snoring

In our sample snoring was also associated to MOH (OR 2.2). Snoring has a high prevalence in chronic headache patients with respect to the episodic forms (48.6% vs 37.2%, respectively) according to the finding on an Italian study (66). Snoring may thus represent an independent risk factor for increasing migraine frequency, thus laying the ground for an increased need of symptomatic medications.

Childhood trauma

Our data shows that childhood traumas are associated to MOH. It is increasingly known that psychosocial variables play key roles in conferring risk for the development of pain, in shaping long-term pain-related adjustment, and in modulating pain treatment outcomes (67). In

particular strong prospective links have been observed between early traumatic experiences and the subsequent development of chronic pain (68-70). It should be noted that many of the traumatic experiences reported are social and interpersonal in nature. It is likely that occurrence of such traumas in genetically predisposed children in an extremely vulnerable period of life are able to change the pain modulating system. Childhood physical, sexual, and psychological abuse are reported to be risk factors for the adult development of pain conditions such as fibromyalgia, irritable bowel syndrome, chronic pelvic pain, and temporomandibular joint disorders (68, 71). Nobody has previously reported the association with MOH, but it is likely that mechanism underlying the occurrence of chronic pain conditions (including MOH) with history of childhood trauma might be shared across chronic pain conditions.

Other factors

Other studies have identified additional factors associated to MOH, which were not confirmed in the present investigation. Hagen and colleagues (44) found an association between MOH and musculoskeletal complaints. We found an association in the univariate analysis with "musculoskeletal chronic pain symptoms", but this was not confirmed in the subsequent multivariate evaluation. It is possible that the discordance is related to dissimilar terms used in the 2 studies: "musculoskeletal chronic pain symptoms" indeed may include less pathological conditions with respect to "musculoskeletal complaints".

The same Norwegian study found smoking as a risk factor for MOH (44). We did not confirm the finding in our study. We do not have an explanation for this discrepancy: smoking may be associated to MOH as a part of the seeking/dependence behavior that some Authors attribute to MOH patients. However it is also true that suffering from MOH may cause quitting tobacco and Hagen and colleagues evaluated this element in a prospective study whereas we did it in a retrospective one. Indeed, MOH is characterized by increasing frequency of migraine attacks, which are accompanied by nausea, vomiting and osmophobia. In the everyday practice many

migraine patients report that they avoid smoking during the attacks and cigarette smoke is one of the smell that can be evaluated in bedside test for osmophobia (72). Thus, the frequent recurrence of those symptoms can be reasonably one cause of quitting smoking in a part of MOH patients.

Low socioeconomic status was found associated to chronic headaches in general (migraine and non-migrainous) headaches (73). The difference in term of diseases with our groups makes it difficult comparing this data with our findings.

In the study by Ferrari and colleagues (43), associations with many variables were found with chronic migraine/MOH versus episodic migraine. Some of those variables were confirmed in our study (marital status, constipation and use of hypnotics – the latter two only in the univariate analysis), whereas others were not (lower levels of educational, unemployment, migraine remission during pregnancy, no use of oral contraceptives, menopause). Indeed, a number of shortcomings in Ferrari's study suggest caution when interpreting their results. First of all, the difference of age between episodic migraine patients and MOH was of 13 years. Data on duration of migraine before overuse in MOH group was not reported, thus it was not possible to compare the duration of primary headache between the two groups. Therefore it is not possible to reasonably rule out that a portion of episodic migraine patients would not have evolved into MOH in the next few years. Moreover a multivariate analysis was not performed.

Clinical factors associated to Relapse

The second part of our study was aimed evaluating prospectively the factors that are associated to relapse into medication overuse after a successful response to detoxification. These factors were: MIDAS score at T0, depression (lifetime), and use of combination of analgesics and/or multiple drugs classes (versus triptan or analgesics).

MIDAS score

Every point adding to the MIDAS score increased the risk of relapse at 1 year by 1%. MIDAS enclosed in itself other variables that were statistically significant at the univariate (doses/days of medication intake per month and headache days per month) but lost significance at the multivariate analysis. MIDAS includes not just the days in which the patients has migraine or uses medication but overall days in which headaches have an impact on his/her life. It makes sense that the subpopulations of MOH with a higher load of illness-related disability turned out to have a more severe disease with more tendencies to relapse. This data was already reported in a previous study with a one-year follow-up (74). Other studies, where MIDAS was not evaluated, reported that the headache days (75) and the monthly intake of drugs predict a higher risk of recurrence (76).

Depression

Our results showed that depression is predictive of relapse (4-fold risk factor). In a previous study of our group on MOH patient followed-up for 3 years after detoxification (77), depression as tested by the MMPI-2 Depression scale score predicted a higher risk of at least one episode of drug overuse during the 3-year follow-up (depression and MIDAS were the only predictors of poor outcome in that study). The population sizes in our previous study and in the present one were similar (in the previous study slightly higher - 137 versus 91) and the relapse rate was identical (27%). In a more recent study (78), we have also evaluated prospectively the factors involved in the failure of detoxification treatment in 248 patients with MOH (98% of which were migraineurs). The analysis showed that depression was one of the risk factors for failure of detoxification (OR 1.071; p=0.05).

The fact that depression is associated not just to the susceptibility to develop MOH but also to a lower rate of response to detoxification and a higher rate of relapses indicates that the presence of depression in the patient's medical history it is linked to a more severe subtype of MOH.

Interestingly, in our study we did not find any statistically significant difference in terms of HADS depression score at T0 between R-MOH and NR-MOH (p=0.36). This would support the hypothesis that there is a common genetic/biological background between depression and MOH, and the more is the amount of element shared, the more MOH is severe and tends to relapse. On the other hand, if depression had brought to a more severe form of MOH by causing problems of coping with disabling attacks, we would have found differences in HADS depression score before detoxification between R-MOH and NR-MOH groups.

Medications overused

We found that overuse of triptans or analgesics are protective with respect to relapse, whereas the use of combination of analgesics or of multiple drugs classes are risk factors. This in partial agreement with the study by Sances et al. (Sances 2012) where the use of triptans was a protective factor for relapse, but only in the univariate analysis. The finding was not confirmed in the multivariate analysis. Other studies aimed at evaluating the outcome of MOH after detoxification showed that the prognosis was better for patients who overused triptans than for analgesics overusers (79, 80). Yet in these studies it is not specified which specific medications were included in the term "analgesics" (there were not any other group with except for ergotamines, i.e. combination of analgesics or multiple classes drugs). Moreover the populations included also patients with tension-type headache (TTH), which typically is treated with analgesics. Indeed in those studies TTH had a poorer outcome in term of relapse. Other studies reported a higher rate of relapses in MOH subjects overusing codeine-containing drugs (81) and of ergotamine (74).

Genetic factors associated to MOH and its Relapse

None of the genetic polymorphisms was found to be associated to the susceptibility to develop MOH. We acknowledge that our study is underpowered to detect small genetic main effects; however, it has sufficient power to detect medium-large effect sizes of clinical relevance. While our results exclude a clinically relevant impact of the SNPs tested susceptibility to develop MOH, our findings suggest that RAMP1 rs7590387 may have a role in predisposing MOH patients toward an more severe endophenotype, which tends to relapse after a successful detoxification.

This finding need to be reconfirmed in future independent study before conclusive interpretation.

Although no *in vitro* or *in vivo* expression/functional data exist regarding RAMP1 rs7590387, it should be noted that it is localized 1.4kb downstream of the RAMP1 gene. Thus, rs7590387 is not expected to be the true causal variant, and the association here reported may be due to linkage disequilibrium of rs7590387 with an unknown functional polymorphism that might be the actual determinant factor for relapse. Therefore, further studies are warranted, based on haplotype analysis of tightly linked SNPs in RAMP1 gene, to provide more conclusive evidence of association with MOH relapse.

Limitations of the study

The major limitation of this study is the cross-sectional observational design in the evaluation of susceptibility of migraine patients toward MOH. A prospective design would have yielded more conclusive findings, but prospective studies are very onerous in terms of economical and human resources and, in the case of MOH require an observation times of several years in order to collect the same population size evaluated in the present study. The only prospective study conducted so far (44) was very valuable for the long follow-up, but was hindered by the fact

that the data collected were based on questionnaires, rather than on face-to-face visits or physician-checked headache diaries. Furthermore, Authors evaluated a limited number of variables whose OR was adjusted just for 3 socio-demographical data and headache frequency. A multivariate logistic regression including all variables was not performed.

Another possible limitation of the present study is the limited level of detail used in the collection of some variables (i.e. the previous practice of physical activity, type, time relationship with different phases of MOH development). Yet, we were aware of the fact that "the more you ask the less you get". The patients in this study at each visit had to: fulfill questionnaires for about 40 minutes, undergo an accurate clinical evaluation and review the diaries. At baseline they also underwent for the collection of blood sample and informed consent explanation and signature. With relation to this aspect, another limitation of this study is that we did not collect (and then analyzed) the characteristics of MIG patients who refused to be enrolled.

With respect to the genetic study, the main limitation is the lack of a validation cohort.

Conclusion

Medication overuse headache (MOH) is the most costly type of chronic headache. MOH is the result of the progressive worsening of a primary headache (mostly migraine) in association with the increasing use of symptomatic medications. Mechanisms behind the development of MOH are unknown. Detoxification is the treatment of choice, yet, a 20-40% of the MOH patient who respond to detoxification, relapse after 1 year.

In this study we evaluated a high number of variables and identified socio-demographical factors, as well as medical, psychological and genetics ones that are associated to the susceptibility to develop MOH and to the risk of relapse into medication overuse after detoxification.

As MOH derives from migraine in the majority of cases, the identification of migraine patients at risk to develop MOH, or MOH patients that poorly respond to detoxification therapy, via clinical features allow to develop specific training programs, follow-up programs and prevention strategies to minimize the risk of MOH, and therefore contain its social and economic burden. Identifying variables involved into the progression to MOH or its relapse after a detoxification, such as the genetic ones, will also allow the research to investigate new, more effective and specific treatments for the disorder.

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