Biomarkers of migraine: Epigenetics
## Disclosures

**Research grants:** AGAUR, ERANet Neuron, FEDER RISC3CAT, la Caixa foundation, Instituto Investigación Carlos III, Novartis, PERIS

**Medical Education:** Allergan/AbbVie, Almirall, Chiesi, Corpus, Eli Lilly, Medscape, Neurodiem, Novartis, PeerVoice, Teva

**Consulting fees:** Allergan/AbbVie, Almirall, Biohaven, Chiesi, Eli Lilly, Medscape, Novartis, Teva

**Participated as a PI in phase III clinical trials:** Janssen Cilag (topiramate), Merck (MK-0974-011, MK-0974-012), Boehringer Ingelheim (1246.4), Colucid (COLMIG-202), Eli-Lilly (I5-MC-CGAL, I5-MC-CGAM, I5-MC-CGAH, I5-MC-CGAI, I5-MC-CGAR, I5-MC-CGAW), Amgen-Novartis (334 20120298 – ARISE-, LIBERTY), Alder (PROMISE2), Teva (FOCUS), Electrocore

Past Coordinator of the Headache Study Group of the Spanish Headache Society
Member of the International Headache Society Board of Trustees
Member of the Council of the European Headache Federation
Founder of website in Spanish: [www.midolordecabeza.org](http://www.midolordecabeza.org)
If you change the way you look at things, the things you look at, change
Epigenetics in Migraine

#1

Why do we need biomarkers in migraine?
Biomarkers in migraine

#1: define migraine predisposition

#2: increase our knowledge on the biological condition of migraine

#3: predict disease evolution

#4: find new drug targets

#5: predict response to treatment

#6: understand complexity & comorbidities
PERSONALIZED medicine

reduce anxiety

modulate attack
PATRICIA POZO ROSICH

PRECISION medicine

personalized & predictive participative
Types of data

- Structural
- Functional
- Physiological
- Biochemical
- Behavioural
- Biophysical
- Ictal
- Interictal
- Diagnosis
- Prognosis
- Therapeutic

Migraine
Individual
Environmental
Migrainomics... how much data is needed?

IDEAL BIOMARKER

- Increased expression in the disease
- Quantifiable & accessible
- Correlated with change (progression)
- Individualized
- Consistant, reliable and “quick”
- Reasonable cost
Epigenetics in Migraine

#2

Why epigenetics?
Why me?
Why me?

Genomics
Epigenetics

Eising et al, Cephalalgia 2013
Epigenetics

Specific of each cell & tissue: dynamic

3D structure of chromatin

Histone modifications

DNA methylation

Non-codifying RNAs

Nucleosome position

Adapted image Telese et al, Neuron 2013
Epigenetics in Migraine

#3

Epigenetics & migraine
DNA methylation

2011 – 2014
Cell lines & rat tissues

Patricia POZO ROSICH

Epigenetic regulation of the calcitonin gene-related peptide gene in trigeminal glia

Ki-Youb Park, Joshua R Fletcher, Ann C Raddant and Andrew F Russo

2015 - 2021
Human samples

Human samples

2015 - 2021
Human samples
DNA methylation could account for both rat CALCA and human CALCA gene silencing.

Hypermethylation at the 18-bp CpG island is correlated with CALCA gene silencing.

- Rat (CA77 & Rat2) and Human (TT & NCI-H460) cell lines
- CA77 and TT express CALCA gene, Rat2 and NCI-H460 don’t
DNA methylation

Research of methylated genes in migraine (CGRP, estrogen receptors, endothelial NOS, MTHFR).

Methylation
- Calca
- Ramp1
- Crbp
- Calcr1
- Usf2
- Esr1
- Gper1
- Nos3
- Mthfr

Labruijere et al, Plos One 2014
Epigenetics

- No effects related with estradiol
- Differences in methylation depend of gene and tissue
- High degree of concordance between human and rat DNA methylation in leukocytes

Labruijere et al, Plos One 2014
DNA methylation

Saline + CSD VS Valproate + CSD VS topiramate

Saline + CSD VS Topiramate + CSD

Sham-operated VS Saline + CSD

CSD effects
No region significantly associated with headache chronification

Genomic study with white blood cells: $p \leq 1.15 \times 10^{-7}$
DNA methylation

- Epigenome-wide association study, quantifying genome-wide patterns of DNA methylation
- **67 migraine** cases and **67 controls** with a matching age and sex distribution
- Sliding window approach to combine adjacent migraine-methylation association P values, we identified **62 independent** differentially methylated regions (DMRs) underlying migraine (false discovery rate < 0.05).

Migraine-associated DMRs were enriched in regulatory elements of the genome and were in close proximity to genes codifying for solute transportation and hemostasis

Gerring et al. BMC Genomics 2018
Common genes and pathways might be associated with PTSD and migraine

Balnomugisa, et al. Frontiers in Neuroscience 2021
microRNAs in migraine

2015-2021

MicroRNA profiling in migraine without aura: Pilot study
Emmanuele Tafuri, Donato Santoro, Valeria de Nardi, Pamela Marsaneto, Carmilla Pugnani, Giampalda Aiello, Marco Bucchi, Andrea Mezzetti, Maria Adele Gambardella & Francesco Cipollone

Serum MicroRNA Signatures in Migraineurs During Attacks and in Pain-Free Periods
Hjalte H. Andersen, Mie Darsøe & Parisa Guerani

Hsa-miR-34a-5p and hsa-miR-375 as Biomarkers for Monitoring the Effects of Drug Treatment for Migraine Pain in Children and Adolescents: A Pilot Study
Luca Gallelli, Erika Cione, Francesco Peltonne, Serena Saviglia, Antonio Veyano, Domenico Chichiluca, Stefania Zampoppi, Vincenzo Guidetti, Luca Sammartino, Angelo Montana, Maria Cristina Cambio, Giovanni Battista De Sarro & Giuliano Di Mizio

MiR-30a relieves migraine by degrading CALCA
Y. ZHAI, Y.-Y. ZHU

Plasma levels of CGRP and expression of specific microRNAs in blood cells of episodic and chronic migraine subjects: towards the identification of a panel of peripheral biomarkers of migraine?
Beatrice Gessi, Roberto De Abajo, Igli, Maria Zorzi, Anna Maria Demonica, Ana Tomelleri, Davide Saraceni, Maria Alma & Cristina Tazza
miRNAs in migraine without aura

Human: **15 female patients vs. 13 controls** (PBMCs)

- miR-27b levels were significantly higher in migraine
- miR-181a, let-7b and miR-22 were significantly lower in migraine

miRNAs profile is associated with migraine without aura

miRNAs are known to be modulated in the setting of atherosclerosis and stroke
miRNAs in migraine during attack

Cohort 1: 8 migraine (in attack phase and in pain-free) patients vs. 8 HC
Cohort 2: 12 migraine vs. 12 HC (PBMCs)

miR-382-5p not only exhibited an upregulation during attack but also proved to be a biomarker for migraine when comparing migraineurs in pain-free periods to the healthy control group.

This implicates serum miRNA alterations as a potential pathogenic feature of migraine and to evaluate their biomarker potentials in attack condition.

miRNAs in migraine

**Endothelial specific miRNA**
30 migraine + 30 controls (PBMCs)

- miR-155, miR-126, and let-7g levels were 2- to 7-fold higher in the interictal migraine patients than in controls
- No miR expression level correlated with migraine patients’ headache features
- miR-155 and miR-126 were associated with syncope frequency

Circulating levels of endothelial-specific miRNAs appear to be elevated in migraine patients and may be associated with syncope comorbidity

*Cheng, et al. Cephalalgia 2018*
miRNAs in migraine

**Pediatric:** 12 mig acute treated vs. 12 mig untreated vs. 12 HC

- Higher expression of hsa-miR-34a-5p and hsa-miR-375 was detected in saliva of untreated MWAs compared to healthy subjects
- qRT-PCR revealed comparable levels of hsa-miRs tested in both blood and saliva significant decrease of about 50% for hsa-miR-34a-5p, hsa-miR-375, in both and saliva of treated patients compared to untreated patients

miRNAs in migraine

Plasma levels of calcitonin gene-related peptide (CGRP) and the expression of miR-34a-5p and miR-382-5p in peripheral blood mononuclear cells of subjects with 27 EM or 28 CM-MO

Subjects in the CM-MO group were also tested 2 months after an in-hospital detoxification protocol

- miR-382-5p & miR-34a-5p in PBMCs was higher in the CM-MO group when compared to EM
- CGRP plasma levels positively correlated with miR-382-5p & miR-34a-5p
- After detoxification (CM-MO), CGRP levels as well as expression of miR-382-5p, and miR-34a-5p decreased significantly in the overall population

Potential panel of peripheral markers associated with migraine subtypes and disease severity
CGRP levels as well as miRNAs expression were influenced by MO, and modulated by detoxification in subjects with CM-MO
The **microMIG** study

2016

**Exploratory study**

Differential gene expression analysis

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>EM</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous population</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Age-sex matched cohorts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No preventive medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Control vs. Migraine</th>
</tr>
</thead>
<tbody>
<tr>
<td>upregulated</td>
<td>33</td>
</tr>
<tr>
<td>downregulated</td>
<td>8</td>
</tr>
<tr>
<td>Total miRNAs</td>
<td>41</td>
</tr>
</tbody>
</table>

**Evidence of differential miRNA expression**

Can we replicate it in **all migraine spectrum**?
The **microMIG** study

2016

Exploratory study

- Differential gene expression analysis

Epigenetic study

- Differential gene expression analysis
- Internal validation
- Functional enrichment analysis

20 HC
20 EM
20 CM

50 HC
50 EM
50 CM

Homogeneous population
Heterogeneous population

2 miRNA signature validated
The microMIG study

Figure 1. Principal component analysis of study samples. Sample were corrected by frozen time. CM (Chronic Migraine); EM (Episodic Migraine); HC (Healthy Controls).
The *microMIG* study

Control vs. Migraine

BDI Score
miRNA_00185
miRNA_07564

AUC: 0.867
p-value: 0.012

Control vs. EM

miRNA_10548
miRNA_08955
miRNA_04678

AUC: 0.885
p-value: 0.012

Control vs. CM

BDI Score
miRNA_00185
miRNA_22595
miRNA_04663

AUC: 0.880
p-value: 0.001

7 miRNA signature
The **microMIG** study

- **2016**
  - **Exploratory study**
    - Differential gene expression analysis

- **2020**
  - **Epigenetic study**
    - Differential gene expression analysis
  - **Validation (RT-qPCR)**
    - Internal validation
    - Functional enrichment analysis
    - 7 miRNA signature

| 2016 HC | 50 HC | 50 HC |
| 20 EM | 50 EM | 50 EM |
| 20 CM | 50 CM | 50 CM |

Homogeneous population

Heterogeneous population

Heterogeneous population
**The microMIG study**

**Figure 4.** miRNA\_07564 and miRNA\_00185 expression (ΔCt RT-qPCR) in the reproducibility phase (41-HC and 100-MIG). Both miRNA candidates presented statistically significant lower expression in migraine patients than healthy controls. Ct levels are inversely proportional to the amount of target nucleic acid in the sample (the lower the Ct level the greater the amount of target nucleic acid in the sample). HC: healthy controls; MIG: migraine patients; CM: chronic migraine; ΔCt: change from housekeeping values in the cycle thresholds levels. Statistical significance assessed by Mann-Whitney U test.

2 miRNA signature validated
What is migraine?

Migraine soup

- Headache Frequency
- Polymorphisms
- Comorbidities
- Chronification
- Habits
- Preventive Therapy
- Triggers
- Medication Overuse

Just triggers...?
Why omics migraine?