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New Insights into Diagnostic Biomarkers of Migraine: Biological, Genetic and Radiological

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Abstract

The diagnosis of migraine is clinical. However, in recent years a great effort has been made to find biomarkers of migraine. Research has focused on finding biological biomarkers (calcitonin gene-related peptide-CGRP, vasointestinal peptide-VIP, pituitary adenylate cyclase-activating polypeptide-PACAP…), genetic biomarkers (glutamate, LR1, MEFD2, TGFBR2, PHATCTR1, ASTN2 genes) and radiological biomarkers (alterations in the analysis of white matter anisotropy; cortical morphometry, and connectivity; iron deposits...). These potential biomarkers can be diagnostic (they confirm migraine) or evolutionary (they change with the evolution of migraine, indicating, for example, the effect of a treatment). The early results of these experiences are promising and suggest that there will be biomarkers of migraine in a few years to confirm the clinical diagnosis and to verify the effect of migraine therapies.

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List of Abbreviations

ICHD: International Classification of Headache Disorders
PACAP: Pituitary Adenylate Cyclase-activating Polypeptide
VIP: Vasointestinal peptide
CGRP: Calcitonin Gene-related Peptide
MRI: Magnetic Resonance Imaging

Introduction

The diagnosis of migraine is clinical, based on the third edition (version Beta) of the International Classification of Headaches Disorders (ICHD 3 beta) of the International Headache Society (IHS) [1] (Table 1). However, in recent years a great effort has been made to find diagnostic biomarkers of migraine. Research has focused on finding biological and genetic biomarkers in blood, and radiological biomarkers by means of brain MRI post-processing applications.

A disease biomarker can be static (it presents only diagnostic significance) or dynamic (it changes as the disease improves or worsens). Both biomarkers must fulfil several premises: validity, reproducibility with high consistency and, above all, reliability [1,2].

A static biomarker of migraine can be useful as a confirmatory test of migraine in clinical practice in patients with clinical diagnostic doubts because they do not meet the ICHD 3 beta [3] criteria. But finding dynamic biomarkers that may change depending on the improvement or worsening of migraine during a treatment can be also very useful. At this moment in time, these are the main variables recommended by the IHS guidelines [4] to ascertain the effectiveness of a preventive drug in migraine: to reduce the number of migraine days a month and/or number of headache days a month. These variables are collected months before starting a preventive treatment in a clinical trial and three months after. Finally, the treatment is considered effective if it achieves a reduction greater than 50% in any of these two variables [2]. However, the value of these variables is provided by the patient noting the days of pain on a migraine calendar at home. This method, while accepted, is rather unscientific. In migraine, as in other diseases, a non-subjective and measurable parameter is needed to provide accurate information about disease evolution. Examples of dynamic biomarkers in other diseases abound, such as plasma levels of thyroidal hormones in the thyroid gland diseases or spirometric values in the respiratory diseases.

Thus, we are presenting an updated review on new insights into biological, genetic and radiological biomarkers of migraine. To this end, a search was made in the Cochrane Database and the PUBMED Database from 2001 to 2015. The search terms were "migraine", "CGRP", "VIP", "PACAP38", "genetics" "genomics", "GWAS", "MRI", "morphometry" and "fractional anisotropy". We located more than 200 English abstracts, and 58 were considered relevant articles.

New Insights into Biological Biomarkers of Migraine

The trigeminovascular system is activated during a migraine attack, and its presynaptic nerve terminals release vasoactive neuropeptides: calcitonin gene-related peptide (CGRP), vasointestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP), peptide histidine methionine (PHM), substance P, neuropeptide Y and neurokinin A. Some of them might become biological biomarkers of migraine: CGRP, VIP and PACAP (Table 2).

CGRP: This neurotransmitter is a 37 amino-acid base polypeptide and induces vasodilation and neurogenic inflammation in leptomeningeal and extracranial vessels inducing migraine pain [5-8]. As a demonstration of this role in the physiopathology of migraine, the infusion of intravenous CGRP induces migraine attacks in up to 75% of migraine patients and in 30% of healthy controls; and the gepants (CGRP antagonists) are effective in migraine attack therapy [8-14].

Three studies analysed CGRP levels in jugular venous blood. Two of them reported an increase of CGRP levels during the migraine attack [5, 15] but the third study did not find this increase [16], although its methodology has been criticised [17].

However, several studies reported an increase of CGRP in peripheral venous blood [19-21], in saliva samples [3,22] and in cerebrospinal fluid [23].

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Episodic Migraine without Aura
A. At least five attacks fulfilling criteria B–D.
B. Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated).
C. Headache has at least two of the following four characteristics:
1. Unilateral location.
2. Ulsating quality.
3. Moderate or severe pain intensity.
4. Aggravation by or causing avoidance of routine physical activity (e.g. walking or climbing stairs).
D. During headache at least one of the following:
1. Nausea and/or vomiting.
2. Photophobia and phonophobia.
E. Not better accounted for by another ICHD-3 diagnosis.
Episodic migraine with aura
A. At least two attacks fulfilling criteria B and C.
B. One or more of the following fully reversible aura symptoms:
1. Visual.
2. Sensory.
3. Speech and/or language.
4. Motor.
5. Brainstem.
6. Retinal.
C. At least two of the following four characteristics:
1. At least one aura symptom spreads gradually over ≥5 minutes, and/or two or more symptoms occur in succession.
2. Each individual aura symptom lasts 5-60 minutes.
3. At least one aura symptom is unilateral.
4. The aura is accompanied, or followed within 60 minutes, by headache.
D. Not better accounted for by another ICHD-3 diagnosis, and transient ischaemic attack has been excluded
Chronic Migraine
A. Headache (tension-type-like and/or migraine-like) on ≥15 days per month for >3 months and fulfilling criteria B and C
B. Occurring in a patient who has had at least five attacks fulfilling criteria B-D for 1.1 Migraine without aura and/or criteria B and C for 1.2 Migraine with aura
C. On ≥8 days per month for >3 months, fulfilling any of the following:
1. Criteria C and D for 1.1 Migraine without aura
2. Criteria B and C for 1.2 Migraine with aura
3. Believed by the patient to be migraine at onset and relieved by a triptan or ergot derivative
D. Not better accounted for by another ICHD-3 diagnosis.

Table 1: Criteria of the Episodic Migraine without Aura, Episodic Migraine with aura and Chronic Migraine of the International Classification of Headache Disorders 3rd edition (Beta version) [1].

CGRP
- CGRP plasma levels are increased during migraine attack, mainly migraine attack with aura, regarding healthy controls
- CGRP plasma levels are more increased during chronic migraine than during episodic migraine.
- The higher the CGRP plasma levels, the better the response to onabotulinumtoxin therapy in chronic migraine patients.

VIP
- VIP plasma levels do not show differences between migraine attack versus healthy controls
- Inter-attack VIP plasma levels during chronic migraine and in inter-ictal periods in episodic migraine do not show significant differences
- The higher the VIP plasma levels, the better the response to onabotulinumtoxin therapy in chronic migraine patients.

PACAP-38
- Inter-attack PACAP-38 plasma levels are lower than the level of healthy controls.
- PACAP-38 levels are elevated during migraine attacks

Table 2: Conclusions of the studies that analysed possible biological biomarkers of migraine in blood samples.

In a recent study [24], CGRP plasma levels were significantly increased in 103 patients with chronic migraine (74 pg/mL) compared to 43 patients with episodic migraine (46 pg/mL), 14 patients with episodic cluster headache (45 pg/mL) and 31 healthy controls (33 pg/mL). Moreover, CGRP levels were significantly increased in patients with a history of migraine with aura as opposed to patients with migraine without aura. The authors conclude that CGRP could be a biomarker of chronic migraine. This same group [25] analysed CGRP plasma levels in 81 patients with chronic migraine and 33 healthy controls, reporting again that chronic migraine patients present increased CGRP levels versus the controls, and that the chronic migraine patients with higher levels of CGRP were the best responders to onabotulinumtoxinA therapy. More specifically, chronic patients with more than 72 pg/mL presented a 28-fold higher probability of becoming a responder than patients below this threshold. Therefore, CGRP plasma levels may not be just a biomarker of migraine, but also a predictor of treatment success. In the same patients, Cernuda-Morollón et al. have shown that onabotulinumtoxinA significantly decreases CGRP levels in peripheral blood samples between attacks measured one month after injections. This confirms the inhibition of CGRP release by the activated trigeminovascular system as the mechanism of action of onabotulinumtoxinA therapy in chronic migraine and supports CGRP levels as a true biomarker for this condition [26].

VIP: This neurotransmitter is a 28 amino-acid base polypeptide that exerts a vasodilator effect in human cerebral parasympathetic perivascular nerve fibres and in the sphenopalatine ganglion. Few studies have analysed VIP in migraine and the results are controversial. Plasma VIP levels show no differences between migraine patients during the migraine attack and healthy controls in a study [5]. However, some studies have demonstrated a reduction in jugular [13] and salivary [27] VIP levels after triptan therapy during a migraine attack. Additionally, intravenous VIP infusion induces only a minimal headache in healthy volunteers [28] and does not induce migraine attacks in migraine sufferers [29].

A recent study [25] analysed VIP plasma levels in 119 women with chronic migraine, 51 episodic migraine patients, 33 healthy controls and 18 patients with cluster headache. VIP levels were significantly higher in chronic migraine patients than in the controls and the cluster headache patients. Nevertheless, the investigators did not find any differences between episodic and chronic migraine.

A second study [30] of the same group confirmed the significant differences in VIP plasma levels between chronic migraine patients and healthy controls and the better response to onabotulinumtoxinA therapy in chronic migraine in the group of patients with higher VIP levels. However, this good response in the group of patients with higher VIP levels was lower than the patients with the higher CGRP levels.

PACAP38: This neurotransmitter presents a very similar structure to VIP and has two forms: a 38 amino acid form (PACAP-38) and a C-truncated 27 amino acid form (PACAP-27).

Several experimental studies have shown that PACAP is released in the activated trigemino-vascular system [31-36]. On the other hand, intravenous PACAP-38 infusion induces headache in healthy controls and migraine attacks in patients with migraine [37-39].

Recently, a study [40] analysed PACAP-38 in the blood samples of 87 migraine patients and 40 healthy controls, both in the period

between attacks and during the attack. Elevated PACAP-38 levels were detected during the migraine attack as opposed to the attack-free period. Moreover, a negative correlation was observed between inter-attack PACAP-38 levels and attack duration. However, levels of PACAP-38 were significantly lower in the inter-attack period of the migraine patients compared to healthy controls. Finally, possible interactions between CGRP and PACAP in the pathophysiology of migraine have been proposed [36,40].

Other possible candidates: As occurs with CGRP, VIP or PACAP, the administration of prostaglandins, histamine and glyceryl trinitrate can also induce migraine attack in patients with migraine and headaches in healthy controls. These human challenge experiments allow us to learn more details about migraine pathophysiology and help to identify possible new therapeutic targets. However, the results of these studies are still controversial.

New Insights into Genetic Biomarkers of Migraine

Migraine is considered a chronic disease with episodic attacks. Genetic studies in migraine began with twin studies, and subsequently expanded with the study of the "syndromic" migraine, particularly familial hemiplegic migraine (FHM).

In FHM, patients have migraine attacks with long-term aura, alteration of awareness, confusion and symptoms of hemiparesis, often self-limiting after several hours/days. In addition, in a percentage of families, patients present permanent cerebellar symptoms such as ataxia and nystagmus. Over the past few years, three genetic variations associated with FHM have been found: FHM1, FHM2 and FHM3.

- FHM1 (47%)[41]: The genetic alteration is located in chromosome 19p13 which encodes the α-subunit of the P/Q-type calcium channel (CACNA1A) in the neuronal membrane. This mutation induces a gain of pre-synaptic function (increase of presynaptic glutamate) through an increase in neuronal calcium reuptake with smaller depolarisation. A cerebellar ataxia is present in 50% of patients, and the onset of symptoms is frequent after cranial traumatism.
- FHM2 (23%)[42]: The genetic alteration is located in the chromosome 1q32 which encodes the Na^+ /K^+ - ATPase gene (ATP1A2). The mutation induces an increase in extra-cellular potassium, reducing or inactivating the glutamate astrocytic transporters and increasing the synaptic glutamate.
- FHM3 (1%)[43]: The genetic alteration is located in the chromosome 2q24 and causes a non-sense mutation (Q1489K) Gln1489Lys in the gene of the α-subunit of the sodium channel, SCN1A, provoking a loss of Na^+ channel function and depolarisation with release of glutamate (fast inactivation).
- Moreover, there are typical clinical cases of FHM not related to the three known mutations. Some cases reported could be associated with a hypothetical FHM type 4 due to alterations in chromosome 14 or a possible FHM type 5 (SLC4A4) and FHM type 6 (SLC1A3) [44].

Animal and cellular models reproducing FHM mutations have been designed with a view to performing functional studies with these mutations. In summary, patients with FHM mutations have higher levels of glutamate and potassium in the synaptic space, inducing a lower threshold of depolarisation to develop one of the most important phenomena of migraine, cortical spreading depression. However, atrigeminovascular dysfunction is also possible, and moreover there is no direct relationship with the common forms of migraine.

Other migraine syndromes are:

- MELAS (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke). It presents a mitochondrial DNA alteration (MTTL1) that cyclically induces migraine attacks with recurrent, focal neurological deficits, vomiting and seizures. Stroke-like lesions have been seen in brain MRI.
- CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy). It presents a mutation in the chromosome 19q13.2 (NOTCH3) that provokes migraine attacks with or without aura in 22%-40% of patients. The brain MRI usually presents diffuse hyperintensities in the white matter involving the temporal lobe poles (Figure 1).
- HERNS (Hereditary Endotheliopathy with Retinopathy, Nephropathy, and Stroke). It presents a 3p21.3 mutation (TREX1) inducing migraine attacks in most cases.

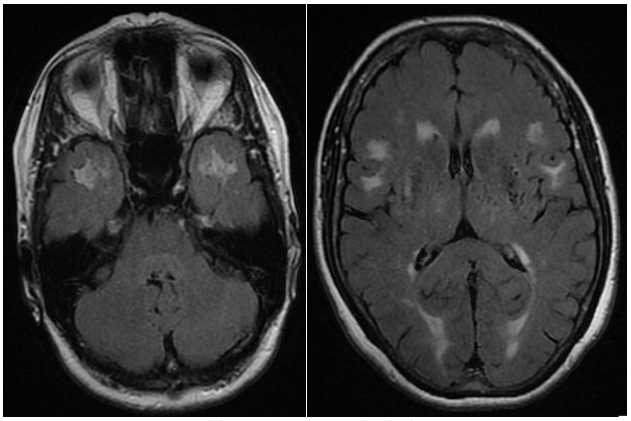

Figure 1: A 52-year-old woman consulted for progressive cognitive impairment. She presented a past medical history of migraines with aura. Her family history revealed multiple cases of migraine, schizophrenia, and stroke at unusual ages. She presented typical radiological findings of CADASIL (atypical white matter hyperintense lesions with polar temporal lobe involvement in MRI-FLAIR), and the mutation c.3062a>G of the exon 19 of the NOTCH3 gene that causes the change p.Tyr1021Cys.

Linkage analyses studies

Linkage analyses allow us to begin to study specific polymorphisms related to a disease. These studies detect alleles with minor effects but with higher frequencies. Although linkage studies have been useful in the study of Mendelian monogenic diseases, their power of detection is minimal when used to explore the genetic basis of complex and multifactorial diseases such as migraine. They are based on generating a hypothesis of alteration in a way that can probably be related to the disease and searching in cases versus controls for differences between them for those specific polymorphisms. On the one hand, these problems reduce the study to a few specific polymorphisms that are not necessarily related to the disease; on the other hand, the studies do not usually include a large number of patients or controls to provide a major statistical validity, taking into account the fact that migraine presents a high prevalence and multiple pathological mechanisms. Therefore, many results tend to be false positives and are not usually replicated in larger cohorts [45].

In recent years, different loci have been identified with linkage studies - we recommend the review by Maher et al. (Table 3) [46].

The absence of a clear disease marker, the role of spouses used as controls in family cases, and the use of different diagnosis methods

(telephone interviews vs clinical history) can also affect the reproducibility of such studies.

Chromosome loci	Phenotype	Methodology
1q22	MO	GWAs
3p24	MO	GWAs
4q21	MO	GWAs
4q24	MA	GWAs
5q21	Pulsating headache	Linkage
10q22-q23	MO/MA	Linkage
Xp22	MA	Linkage
Xq24-q28	MA/MO	Microsatellite marker

Table 3: Loci identified in common forms of migraine.

GWAS (Genome-wide association studies)

GWAS allow us to study millions of polymorphisms (SNPs) at the same time in sufferers versus a control population. These studies have a high power for detecting common variants of a moderate or high effect. For variants with minor effects, their power is reduced to finding variations, mostly for the recessive loci. In addition, these studies should be performed in large populations to ensure a good clinical diagnosis and potent statistics. Moreover, it must be remembered that the results of these studies are variations associated with DNA (changes from a base, i.e., a polymorphism), but we do not know if these changes are functional or not. This is why functional studies must be conducted to assess whether protein synthesis is affected. Thus, in migraine, a disease of the central nervous system (CNS), a twofold challenge is faced: on the one hand, no pathological anatomy has been identified, and on the other, the development of reliable cell lines that reproduce CNS conditions is complicated.

To date, three GWAS have identified genetic markers associated with migraine [47-49]. Some of them are particularly attractive with regard to the pathophysiology of migraine. In the first GWAS: rs1835740, which modulates the homeostasis of glutamate, although it was not replicated in a Spanish population; rs11172113 (in the gene LR1–family of receptors of lipoproteins), which may also have an influence on the glutamate pathways via the interaction with glutamate NMDA receptors; rs10166942, located in the TRPM8, that encodes a sensor required for the nociception of cold.

The last GWAS, related to migraine without aura, reported SNPs related to synaptic activity and glutamatergic excitability, such as MEFD2 (transcription factor of protein that reduces the number of excitatory synapses), TGFBR2 (it encodes threonine kinase, a growth factor related to arterial dissections), PHATCTR1 (phosphatase and actin regulator that controls synaptic activity and synapse morphology through regulation) and ASTN2 (member of the astrotactin family, playing a role in glial migration, which is important for the development of the architecture of the laminar cortical regions of the brain).

It is true that although such studies are becoming economically more affordable, candidate genes in migraine studies still make sense if we analyse them in endophenotypes or we study multiple attractive candidates in combination using pathophysiological hypotheses. They may be useful in studying individual phenotypes. A meta-analysis of the GWAS results is ongoing, as is an exome analysis.

New Insights into Radiological Biomarkers of Migraine

Pathological alterations that differentiate between the brain of migraine patients and healthy controls have not been reported. In other words, no morphological biomarkers of migraine have been proposed.

On the other hand, usual brain MRI sequences (T1, T2, FLAIR, DW...) will hardly detect alterations that can be used as radiological biomarkers in the brain of patients with migraine in the future. Patients with migraine present white matter abnormalities in their brains more often than healthy controls. These findings have been reported by retrospective case-control studies50, even in paediatric migraine patients [51,52], prospective clinic-based studies comparing healthy controls [53-57] and population-based studies also comparing healthy controls [58-61]; but their evolutionary course is controversial [62,63]. Other MRI alterations related to migraine are infarct-like lesions [64-67], although they are more controversial than**.** white matter abnormalities. In any event, these alterations are not pathognomonic of migraine and may appear in other conditions.

Diffusion MRI68 and functional MRI [69,70] sequences can detect transient changes during the migraine attack, although these alterations disappear during the inter-attack period and cannot therefore be considered biomarkers of migraine.

However, MRI post-processing programmes can extract different parameters: coefficient of medium diffusivity-MD, which is the average of the three values of the tensor, and the fractional anisotropy coefficient-FA, which is within the range [0...1]), where 0 is the lowest level of directionality (isotropic diffusion) and 1 the maximum directivity. It is also possible to draw fibres and nerves that pass through a specific region (DTI Tractography) and to study the functional connections between different spatially-distributed networks of the brain (resting-state f-MRI). These programmes can also perform a morphometric analysis and analyse the thickness, density and surface of the cerebral cortex (Figure 2 and Figure 3).

In the last 15 years, several studies examining different ROIs (regions of interest) of the brain have been performed in the inter-attack period comparing migraine patients versus healthy controls (Table 3). These ROIs are the periaqueductal grey matter in the brainstem, the grey matter (some nuclei and cortical areas) and their adjacent white matter areas. It is also interesting to analyse the connectivity between these areas.

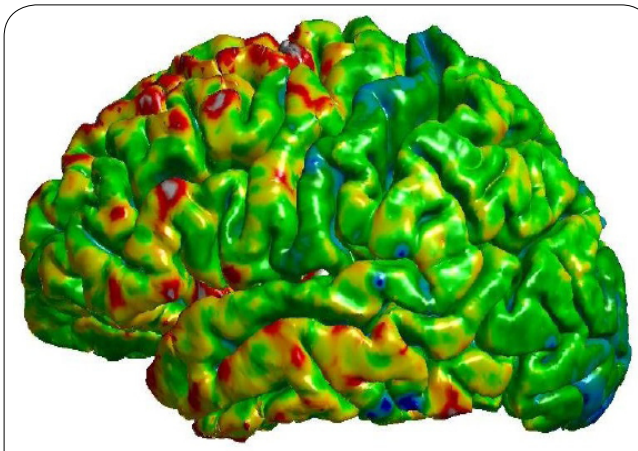

Figure 2. Distribution of the cortical regional thickness in the parametric map obtained after post-processing a brain MRI with the Freesurfer application. Courtesy of Dr. Gracián García of the Biomedical Engineering Department of the Hospital Quirón Valencia.

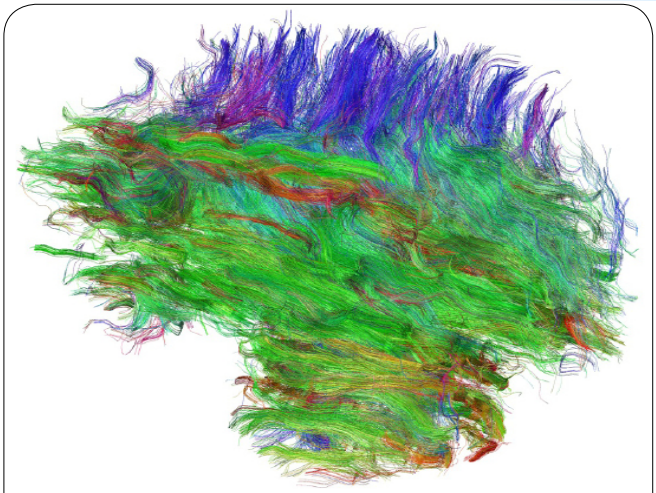

Figure 3: DTI-Tractography images of several white matter tracts obtained after post-processing a brain MRI. Courtesy of Dr. Gracián García of the Biomedical Engineering Department of the Hospital Quirón Valencia.

Investigator	Episodic migraine	Chronic migraine	Healthy controls
KMA Welch[22]	n=17, ferric signals in the PAG	n=17, ferric signals in the PAG, red nucleus and black substance	n=17 No alterations
M A. Rocca[25]	n=16, ferric signal in the PAG and dorsolateral pontine area	/	n=15 No alterations
CAMERA-1[26]	n=138, Ferric signals in the PAG, red nucleus, putamen nucleus and pallid globe.	/	n=75 No alterations

Table 4: Clinical studies (KMA Welch and MA Rocca) and population studies (CAMERA-1) that have analysed alterations in PAG suggesting iron deposits in the inter-attack brain of migraine patients. Only the study by KMA Welch studied PAG as a primary endpoint.

Brainstem: The periaqueductal grey matter (PAG) is the principal ROI in the brainstem. It has been analysed in more than 170 patients versus more than 100 healthy controls in three studies [71-76], and in all of them the PAG presents ferric-like signals more frequently in migraine patients (Table 4). These findings in the PAG have not been reported in other pain diseases. The PAG was regarded as the migraine generator in the past, although we now think that its role is simply to modulate pain. The dorsolateral pontine area is another ROI in the brainstem that is activated in several pain syndromes such as trigeminal pain, provoked pains and even in urine bowel distension, rectal distension or apnoea. However, this area is very heterogeneous and encompasses the trigeminal sensitive nucleus, the reticular dorsolateral pontine nucleus, the parabrachial nucleus, the cuneiform nucleus, the locus coeruleus, the vestibular nucleus and the inferior colliculus.

Brain Grey Matter (cortex and nuclei): The grey matter of the brain has been analysed in more than 460 migraine patients and in more than 270 healthy controls (Table 5). MRI post-processing applications have analysed the brain's volume, density, thickness and surface. Twenty studies [55,74,77-94] have reported more alterations in the grey matter of migraine patients than in healthy controls, and only two [95,96] have not reported differences between them. The ROIs altered in these studies are in Tables 6 and 7.

The White Matter: The white matter was only analysed by eight [80-83,86,87,89] of the twenty studies that analyse the morphology of the cortex and included more than 190 migraine patients and more than 100 healthy controls. They reported a significant reduction in fractional anisotropy in the areas adjacent to the altered cortex (Table 6).

Investigator	N	M0A	MA	CM	Controls	Dominance controlled	Gender	Evolution (years)	Frequency (attacks/ year)
MA Rocca [7,23]	16	16	0	0	17	Yes	Mixed	15	26
NS Matharu [43]	34	28	6	0	11	No	Mixed	13	44
MA Rocca [27-28]	28	17	11	0	17	No	Mixed	/	/
C. Granziera, A Da Silva [29-32]	16	9	7	0	15	No	Mixed	28	20
						No	Mixed	24	
T. Schmidt-Wilcke [33]	24	12	12	0	5	No	Mixed	25	48
									20
									48
JH Kim [34]	35	35	0	0	31	No	Mixed	/	/
	20	15	5	0	30	No	Mixed	9	32
N. Schmitz[35,36]	35	27	8	0	28	No	Women	30	42
W. Valfre[37]	27	21	6	15	27	Yes	Mixed	14	/
R. Datta[44]	56	28	28	0	28	No	Mixed	17	36
N. Szabó[38]	21	18	3	0	17	No	Mixed	17	33
N. Maleki [39-41]	20			0	0	No	Mixed	/	/
	22	11	11	0	22				
R. Mesina[42]	63	31	32	0	18	Yes	Mixed	17	36

Table 5. Sample sizes and composition of samples from studies that have analysed the cortex or white matter of the inter-attack brain of patients with migraine compared to healthy controls.

Investigator	Changes described in patients versus controls
MA Rocca,R. Mesina[7,23,27-28,45]	• Reorganisation of the primary somatosensory cortex in the non-dominant hemisphere and rostral movement of the supplementary motor area • Alteration of the mean diffusion coefficient. • Decrease in density in the temporal, frontal and cingulate cortex bilaterally; and increasing density in the PAG and dorsal pontine area in patients (especially in patients with MA).
C. Granziera and A Da Silva[29-32]	• Bilateral thickening of the cortex in studies of Morphometry in cortical areas V3A and MT+. • Fractional anisotropy decreased in white matter adjacent to the visual cortical areas V3A and MT, superior colliculus, and left lateral geniculate nucleus. • Thickening in the somatosensory cortex (SM1) especially the head and face. • Fractional anisotropy decreased in adjacent white matter and trigemino-thalamic ventral tract in patients with MA and the ventrolateral PAG in patients with M0A.
T. Schmidt-Wilcke[33]	• Decrease in density of grey matter in the right anterior insula and cingulate cortex.
JH Kim[34]	• Reduction in volume of grey matter in the bilateral insula, motor/premotor cortex, prefrontal cortex, cingulate cortex, orbitofrontal cortex, and right parietal cortex.
N. Schmitz[35,36]	• Changes of fractional anisotropy in frontal lobe, cerebellum and brain stem; and reduction in the density of the frontal, parietal, and occipital cortex. • Decrease in density in the cortex of the frontal and parietal lobes
W. Valfre[37]	• Reduction of grey matter of the right superior temporal gyrus, right inferior frontal gyrus, and left pre-central gyrus.
N. Szabo[38]	• Reduction of fractional anisotropy in the right frontal white matter.
N. Maleki [39-41]	• Increased thickness of the area representing the face in the post-central gyrus and reduction of the thickness of the cingulate cortex. • Greater volume of the hippocampus. • Largest increase cortical thickness in women with migraine about men with migraine and healthy controls in the posterior insula and the precuneus cortex.

Table 6: Changes described in the inter-attack brain of patients with migraine at cerebral cortex and white matter level compared to healthy controls.

Supplementary motor cortex (SM).	Insula.
Primary somatosensory cortex (S1).	Amygdala.
Cortical visual area V3A.	Prefrontal cortex.
Cortical visual area MT+.	Thalamus.
Cingulate anterior cortex.	Orbitofrontal cortex.
Posterior parietal cortex.	Secondary sensory cortex (S2).
Periaqueductal grey matter (PAG).	Basal ganglia.
Cerebellum cortex.	

Table 7: ROIs described in the cerebral cortex in the studies that have compared patients with migraine with healthy controls.

Connectivity has been analysed in 19 very recent studies [97-114] that employed DTI tractography or resting-state f-MRI, only one of which [114] reported no connectivity differences between migraine patients and healthy controls. More than 500 migraine patients and more than 400 healthy controls were included in these studies. The tracts and pathways altered in these studies are the ventral trigemino-thalamic tract, the optical radiations and the connectivity of the insula, amygdala and the corpus callosum [97-114].

Conclusion

We will definitely be witnessing the advent of reliable biomarkers of migraine in the next few years, and currently the most promising ones are biological, genetic and radiological.

Biological biomarkers: The detection of reliable biological biomarkers of activated trigeminovascular system in blood during the period between attacks is a method that fulfils the assumptions to be considered a diagnostic biomarker of migraine. It is comfortable for the patient and easily workable and repeatable. Currently, CGRP plasma levels are the most promising candidates for becoming the first biological biomarker of migraine. Firstly, CGRP plasma levels are elevated during migraine attack with regard to healthy controls. We could therefore consider it to be a static diagnostic biomarker. Secondly, CGRP is more increased during chronic migraine than episodic migraine. Therefore, CGRP may be considered a dynamic therapeutic biomarker because it diminishes if we treat a chronic migraine successfully. In addition, CGRP levels could be used to screen candidates that respond better to therapy with onabotulinumtoxinA. VIP and PACAP-38 are the next most promising molecules for becoming biomarkers of migraine after CGRP.

Genetic biomarkers: Regarding the genetics of migraine, the first studies were performed after clinical observations of the transmission of the disease in families, and moreover by means of genetic findings in the "syndromic" migraine such as familial hemiplegic migraine and CADASIL.

In recent years, the development of genomics and bio-computerised data analysis tools has led to a change in the genetics of multigenic and multifactorial diseases such as migraine. Researchers are currently looking for common factors shared by all persons with migraine to have a better understanding of its pathophysiology and identify diagnostic and prognostic biomarkers. They are even investigating ways of making therapeutic progress through pharmacogenomics.

Technical advances in genome sequencing are usually called next generation sequencing. These platforms are capable of generating more sequencing data and are less expensive than other original "capillary" Sanger methods. Moreover, they can analyse structural variations and can handle smaller and more complex genomes, copy-number variations (CNVs) and exomes. This type of technology is

probably more organised around the individual patient and not large populations. The variations in the genes found in GWAS studies or pharmacogenomics studies can even be specifically studied.

Currently, these genetic studies are restricted because of their cost. However, their use will become more widespread in the field of migraine when costs are reduced. We may therefore conclude that we trust that these approaches will help to explain the molecular genetics of migraine and will provide answers to pathophysiological questions. In addition, they will be capable of offering more individualized therapies to groups of patients with similar molecular alterations.

Radiological biomarkers: The results of the initial experiences in the post-processing of MRI images applied to the migraine brain may seem confusing if they are analysed superficially, mainly due to the heterogeneity of the techniques used. In addition, the samples in each one of these studies and the overall sample of patients included are small, hence the statistical potency of the results is limited if we consider that the results must be extrapolated to the 15% of people with migraine in the world. Moreover, most of the studies did not address the question of patient brain dominance; they included patients of both genders and ignored the predominant laterality of migraine attacks. Other limitations are that the synchronization between the last migraine attack and the acquisition of MRI images is very different between studies (from 72 hours to one month) and that the preventive treatment that patients were taking during the MRI was not controlled. Moreover, chronic migraine has not been specifically analysed. Finally, some of these studies use criteria predating the current migraine ICHD3 Beta classification [3].

In addition, grey matter alterations in migraine patients have also been described in more than 30 studies of approximately 15 pathologies that present pain, such as [115,116]: fibromyalgia, tension-type headache, cluster headache, lumbar ache, phantom limb syndrome, neuropathic pain, irritable bowel syndrome, spinal cord lesions, vulvodynia, hip osteoarthritis and regional complex syndrome.

Finally, these brain alterations in migraine patents may have two explanations:

1. They are the cause of migraine: the genetic predisposition of migraine patients induces micro-structural alterations in the brain that are not visible in conventional brain MRI techniques. These alterations must already be present in children with migraine, a fact that was only reported in a recent study.
2. They are the consequence of migraine: the recurrence of migraine attacks in the course of life and their number and complexity/severity induce these alterations. Therefore, these abnormal signals constitute evolutionary brain damage due to neuroplasticity and reorganization in neurons and/or glial cells.

These radiological studies using post-processing image programmes are banishing a longstanding dogma from neurology, since after years of autopsy studies no structural alterations (a biomarker) have ever been reported in the brain of migraine patients allowing differential diagnosis or indicating an evolutionary damage of the brain. These results are currently difficult to understand, but we can categorically state that the inter-attack brain of migraine patients presents micro-structural, and not only functional, differences during the attack, as opposed to people without migraine. It is still unclear whether these MRI alterations are exclusive to migraine or are common to any pathology with recurrent or chronic pain.

Competing Interests

The authors have declared that no competing interests exist.

Author Contributions

Both the author substantially contributed to the study conception and design as well as the acquisition and interpretation of the data and drafting the manuscript.

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