Investigation of Migraine-Related Intracerebral White Matter Lesions

Doctoral (Ph.D.) Thesis

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List of Abbreviations

^{1}H-MRS	-	proton magnetic resonance spectroscopy
2D	-	2-dimensional
3D	-	3-dimensional
3T	-	3.0-Tesla
5-HT	-	serotonin
A-	-	patients without aura (aura negative)
A+	-	patients with aura (aura positive)
AC-PC line	-	anterior and posterior commissural line
ADC	-	apparent diffusion coefficient
ADMA	-	asymmetric dimethylarginine
ANCOVA	-	analysis of covariance
ANOVA	-	analysis of variance
ATP	-	adenosine triphosphate
BOLD	-	blood oxygen level dependent
Ca^{2+}	-	calcium
CGRP	-	calcitonin gene related peptide
Cr	-	creatine and phosphocreatine
CRP	-	C-reactive protein
CSD	-	cortical spreading depression
CSF	-	cerebrospinal fluid
DDAH-2	-	dimethylarginine dimethylaminohydrolase-2

DKT - “Desikan–Killiany–Tourville”

DNA - deoxyribonucleic acid

DWI - diffusion weighted imaging

EDSS - Expanded Disability Status Scale

EEG - electroencephalogram

eNOS - Endothelial nitric oxide (NO) synthase

FHM - familial hemiplegic migraine

FLAIR - fluid attenuated inversion recovery

FLASH - fast low angle shot

fMRI - functional magnetic resonance imaging (MRI)

FOV - field-of-view

HPLC - high-performance liquid chromatography

Hz - Hertz

ICHD - International Classification of Headache Disorders

ICV - intracranial volume

IHS - International Headache Society

K^+ - potassium

L- - lesion not present

L+ - lesion present

LDL - low density lipoprotein

L-NMMA - N-monomethyl-L-arginine

MIDAS - Migraine Disability Assessment

MMP-9 - matrix metalloproteinase 9

MPRAGE - magnetization-prepared rapid gradient-echo

MRI - magnetic resonance imaging

MS - multiple sclerosis

MTHFR - methyltetrahydrofolate

Na^+ - sodium

NAA - N-acetyl-aspartate

NAWM - normal appearing white matter

NKA - neurokinin A

NO - nitric oxide

NOS - nitric oxide synthase

NSAID - nonsteroidal anti-inflammatory drug

PACAP - pituitary adenylate cyclase-activating polypeptide

1 Introduction

1.1 Current Knowledge on Migraine

The International Classification of Headache Disorders (ICHD) categorizes primary headaches into four major groups, as follows:

1. Migraine
2. Tension-type headache
3. Trigeminal autonomic cephalalgias
4. Other primary headache disorders

Migraine is defined by the International Headache Society (IHS) as a recurrent primary headache disorder, usually unilateral and pulsatile in nature with sever to moderate pain, with attacks lasting 4-72 hours in adulthood. (1)

Migraine is further classified in the following subcategories:

1. Migraine

    1.1 Migraine without aura

    1.2 Migraine with aura

        1.2.1 Migraine with typical aura

            1.2.1.1 Typical aura with headache

            1.2.1.2 Typical aura without headache

        1.2.2 Migraine with brainstem aura

        1.2.3 Hemiplegic migraine

            1.2.3.1 Familial hemiplegic migraine (FHM)

                1.2.3.1.1 Familial hemiplegic migraine type 1 (FHM1)

                1.2.3.1.2 Familial hemiplegic migraine type 2 (FHM2)

                1.2.3.1.3 Familial hemiplegic migraine type 3 (FHM3)

                1.2.3.1.4 Familial hemiplegic migraine, other loci

1.2.3.2 Sporadic hemiplegic migraine

1.2.4 Retinal migraine

1.3 Chronic migraine

1.4 Complications of migraine

1.4.1 Status migrainosus

1.4.2 Persistent aura without infarction

1.4.3 Migrainous infarction

1.4.4 Migraine aura-triggered seizure

1.5 Probable migraine

1.5.1 Probable migraine without aura

1.5.2 Probable migraine with aura

1.6 Episodic syndromes that may be associated with migraine

1.6.1 Recurrent gastrointestinal disturbance

1.6.1.1 Cyclical vomiting syndrome

1.6.1.2 Abdominal migraine

1.6.2 Benign paroxysmal vertigo

1.6.3 Benign paroxysmal torticollis

1.1.1 Migraine Prevalence

The global percentage of adults who suffer from headache disorders was estimated to be 46% in 2007, with migraine, one of the primary headache disorders, being the second most common after tension type headache. The worldwide prevalence of migraine is estimated to be 11% which increases to 15% if we look at lifetime prevalence. The majority of the research data available is from Europe and North America where the migraine prevalence is 15% (lifetime 17%) and 13% (lifetime 13%) respectively. When the data is further broken down into subcategories, migraine is more common in adults (11%, lifetime 15%) than children/adolescents (7%, lifetime 5%), with a decreasing prevalence (6%, lifetime 8%) in adults over the age of 60 years. In all categories, migraine is more prevalent in women than in men, with 14% vs 6% in adults, 9% vs

7% in children/adolescents and 8% vs 3% in the elderly (2).

1.1.2 Migraine Disability

Despite the high prevalence of headache in general and migraine specifically, it is often not viewed as a serious condition by the general population and many health care providers, with access to proper medical treatment often being difficult to obtain for many patients, if they seek treatment at all (3,4). If we examine the World Health Organization's (c's) global burden of disease estimates, however, we find that since the year 2000 migraine has been in the top ten causes for years lost due to disability (YLD) with an estimated 18, 538 YLDs in 2012, accounting for 2.5% of YLDs from the top twenty causes, and placing it ahead of common disorders such as osteoarthritis (2.4%) and asthma (1.9%) placed 11th and 13th, respectively (5).

According to the American Migraine Prevalence and Prevention (AMPP) study, which is a longitudinal population-based study of Americans with migraine, 53.7% of migraineurs described severe impairment or need for bed rest during a migraine attack. In addition, 22% scored between moderate and severe migraine related disability on the Migraine Disability Assessment questionnaire (MIDAS), which is a test used to help measure the impact of headache on patients' lives. Chronic migraine comprised 7.7% of total migraine cases but with greater headache-related disability (38%) compared to episodic migraine (9.5%), with a significant increase in rates of depression and anxiety among these patients (6).

1.1.3 Cost of Migraine

The monetary cost of migraine can be viewed in terms of direct cost (which would encompass primary care and specialist visits, emergency department visits, hospitalizations, laboratory and diagnostic testing and medical treatment for the condition and side effects) and

indirect costs (mainly to employers due to absenteeism and loss of productivity) (5). It was estimated that the cost of migraine in the US was between 13 to 17 billion USD annually. Headache was 5th leading cause for emergency room visits in the US in 2009, with "migraine" being the 16th most common over all emergency department diagnosis for women. (6).

Triptans, which are serotonin (5-HT) 1B/1D receptor agonists, are highly effective and used in the acute setting as well as for the short-term prevention of migraine associated with menstruation. (8, 3, 9). Available as oral and subcutaneous preparations as well as a nasal spray, triptans are even more effective when combined with a nonsteroidal anti-inflammatory drug (NSAID) (3,8). In the US, of the 1.5 billion USD annual cost of migraine medication, triptans account for 1.18 billion of the total (7) though these figures are likely to decrease as these estimates were calculated before the generic version of sumatriptan became available in 2008, which is by far the most prescribed anti-migraine drug accounting for 48% of the 7 preparations available (6). It should be duly noted, however, that triptan medications are not interchangeable with individual patient response varying between the preparations, as well as some formulations being more effective than others in certain situations, such as the use of nasal spray triptans in patients with nausea and vomiting (3,7,8).

The indirect costs of migraine can also be staggering. In the UK, with 25 million working days lost due to migraine every year, the estimated cost to the economy is approximately 2 billion GBP in addition to the direct health care costs (3). In the US, after analysing data from Thomson-Medstat's Database from 2002-2003, it was found that the projected annual cost of migraine for US employers was approximately 12 billion USD, mostly due to absenteeism (10).

1.1.4 Characteristics of Migraine Headache

- Recurrent headaches

- Unilateral or bilateral
- Pulsating or throbbing quality
- Moderate to severe intensity
- Nausea, vomiting
- Phonophobia, photophobia
- Episodic vertigo
- Aggravated by routine physical activity
- Aura and autonomic symptoms
- Cutaneous allodynia
- Duration: 4 – 72 hours
- In childhood: rarely unilateral, duration 2 – 24 hours (1)

1.1.5 Trigger Factors of Migraine Headache

Several factors are believed to trigger migraine headache in susceptible patients, and they are as follows (11-20):

- Smoking
- Alcohol (red wine, tannin)
- Cheese (tyramine)
- Brown chocolate (phenylethylamine)
- Caffeine
- Milk, eggs, smoked sausage and ham, fermented food, chicken and beef liver
- Monosodium glutamate (Chinese food, flavour enhancer)
- Meat preserved by glutamate and glutamine, beans, onions, nuts
- Oral contraceptives, menstruation (changes in oestrogen level)
- Climate change, high air humidity, effect of high altitude
- Time zone change
- Stress
- Sleep deprivation or too much sleep (shift workers)
- Reduced water (dehydration) and food intake (hunger)
- Fluorescent light
- Disturbing smells

1.1.6 Clinical Phases of Migraine Headache

Migraine headache is clinically categorized into the following phases: ***1)*** Prodromal (or premonitory phase), ***2)*** Aura phase, ***3)*** Attack phase, ***4)*** Resolution phase and ***5)*** Postdrome (or recovery phase). The premonitory phase symptoms may precede a migraine from hours to even 2 days before headache and can include fatigue, hyperactivity, depression, difficulties in concentration, neck stiffness, light and/or sound sensitivity, nausea, blurred vision, yawning, pallor, dysphoria and changes in appetite. In migraine with aura, after the premonitory phase, the headache is preceded or accompanied by gradually developing, though fully reversible, visual, speech/language, motor, brainstem and/or retinal symptoms, with each individual aura symptom lasting 5-60 minutes. When the aura consists of monocular visual disturbances, such as scintillations, scotoma or blindness, it is subcategorized as retinal migraine and if the aura is associated with motor weakness then it is subcategorized as hemiplegic migraine, which may have a hereditary component, as is the case with the three types of FHM, though sporadic hemiplegic migraine is not genetic. Migraine with brainstem aura has at least two of the following brainstem symptoms: dysarthria, vertigo, tinnitus, hypoacusis, diplopia, ataxia and decreased level of consciousness. The attack phase is the onset of headache, which usually occurs within 60 minutes of aura, though aura can be the stand-alone symptom. In migraine without aura no aura symptoms are reported, and the attack phase follows the prodromal phase, however it is not uncommon for patients to experience both types throughout their lifetime. The attack phase is followed by the resolution phase, which may exhibit the symptoms of the premonitory phase, ending with the postdrome phase during which the patient has no headache but has fatigue and decreased concentration usually for 1 day (1).

Chronic migraine is defined as headache occurring on 15 or more days per month for more

than 3 months which has migraine features on at least 8 days per month (1). The complications of migraine include status migrainosus (a debilitating attack lasting longer than 72 hours), persistent aura (lasting for 1 week or longer) without infarction, migrainous infarction (aura associated with ischemic lesions in the appropriate territory) and migraine aura-triggered seizure (1).

1.2 Migraine Pathophysiology

The early theory of migraine pathophysiology posited that it was a disorder of vascular origin (21). It was observed by Graham and Wolff that the amplitude of pulsations of the external carotid vessels and the intracranial vessels (indirectly measured through measuring the pulsations of cerebrospinal fluid (CSF) in the lumbar subarachnoid space) decreased following the injection of ergotamine, a vasoconstrictor isolated from ergot which was used as treatment of migraine. Since the decrease in amplitude had a close temporal relationship with a decrease in headache pain intensity, it was hypothesized that the stretching of cranial arterial vascular walls was the causative factor for migraine pain. Throughout the first half of the 20th century, ergotamine and derivative dihydroergotamine remained the only medications available for migraine treatment, preparations of which are still used by some migraine sufferers today. A recent magnetic resonance imaging (MRI) angiographic study of spontaneous migraine patients, however, found no extracranial arterial dilatation on the headache pain side compared to the non-pain side and to pain free days and slight intracranial arterial dilatation on the migraine pain side. Though sumatriptan injection caused extracranial arterial constriction it had no effect on the dilated intracranial arteries (22). There is increased consensus that vasoactivity in migraine is consequential rather than causative in migraine (23). The current neurobiological evidence of migraine pathophysiology is discussed below.

1.2.1 The Premonitory Phase and the Hypothalamus: A Key Organ in Migraine

The hypothalamus plays an important role in systemic homeostasis and through its vascular and neural connections to the limbic system, brainstem, pituitary gland, retina and spinal cord regulate numerous physiological functions and behaviours. It receives its blood supply from the circle of Willis and can be subdivided into the lateral, medial and periventricular morphological and functional anatomical subdivisions. The numerous nuclei located in hypothalamus work alone or in concert to help regulate such diverse functions as follows (24):

- Growth regulation: paraventricular and arcuate nuclei
- Reproductive function and sexual behaviour: medial preoptic nucleus
- Uterine and mammillary gland contraction, bonding behaviour: supraoptic and paraventricular nuclei
- Lactation: paraventricular and arcuate nuclei
- Water homeostasis: supraoptic and paraventricular nuclei
- Thermoregulation: anterior hypothalamic nucleus
- Circadian rhythms and sleep: anterior hypothalamic, suprachiasmatic and lateral preoptic nuclei
- Alertness: tuberoinfundibular nucleus
- Metabolism: paraventricular nucleus
- Hunger: dorsomedial, ventromedial and lateral hypothalamic nuclei
- Fear and aggression: ventromedial nucleus
- Emotion, memory: mammillary nuclei

In 2014, positron emission tomography (PET) scans using radioactive water ($H_2{}^{15}O$) of 25 patients with diagnosed migraine without aura were performed during a nitro-glycerine induced migraine headache attack (25). The patients were scanned during the premonitory phase, the attack phase and a week after headache for a baseline comparison. During the early premonitory phase, the researchers found increased blood flow to the posterior and lateral regions of the hypothalamus

and the ventral tegmentum, but not during the late premonitory or attack phases. The researchers postulated that these activations could explain varied premonitory symptoms such as yawning, related to dopaminergic mechanisms, frequent urination and thirst, related to vasopressin, and mood changes, through hypothalamic connections to the limbic system. In the posterior and lateral hypothalamus hypocretin/orexin messenger ribonucleic acid (RNA) are located and projections of the orexinergic systems have been implicated in circadian cycles, feeding behaviour and neuroendocrine and autonomic functions. This could be an explanation for such symptoms as appetite and sleep disturbances.

Other areas of early premonitory activation were the periaqueductal grey matter (PAG), which is involved in pain modulation, the locus coeruleus, which controls intra- and extracerebral vascular tone and modulates cortical excitability, possibly contributing to phonophobia and photophobia, and the medulla, likely contributing to nausea and vomiting. Cortical activation was found first in the occipital cortices (likely involved in photophobia), then in the temporal and prefrontal cortices (25). This was the first study of its kind investigating the premonitory phase of migraine. Taken together, these results indicate that a disturbance in homeostasis occurs during the premonitory phase, through hypothalamic and other regional activations, can be a provoking factor in migraine headache.

1.2.2 Migraine Aura and Cortical Spreading Depression (CSD)

In 1941, Lashley, himself a migraine with aura sufferer, published his observations of the progression of his own scintillating scotoma, beginning at the macula and spreading temporally at a rate of 3 mm/minute, followed by a complete inhibition of activity, then a recovery 5-10 minutes later progressing at the same rate (21). In 1944, the Brazilian neurophysiologist Leão published his

discovery of a self-propagating wave of decreased electrical activity spreading in all directions from the point of stimulation of the rabbit cerebral cortex, followed by a recovery after 5-10 minutes (26). He also noted that this phenomenon was followed by a dilatation of the pial vessels in the affected area (27). Leão called the phenomenon a CSD and while he noted a possible relationship between migraine aura and CSD, the nature of this relationship was not further studied until the later decades of the 20th century.

We now know that CSD is a pathological phenomenon in which a self-propagating wave of depolarization is followed by a complete cessation of electrical activity which spreads at a slow constant rate of approximately 2-5 mm/minute in all directions from the point of origin. Recovery follows in the affected region, spreading at the same rate and directionality, approximately 5-10 minutes later. The electrical activity does not cross sulci and stops at the white matter. There is also a contemporaneous vessel dilation and hyperaemia followed by oligemia lasting up to one hour, though this oligemia normally does not reach ischemic levels (28-35).

On the cellular level, CSD is characterized by a massive potassium (K^+) efflux, raising the extracellular K^+ concentration ($[K^+]_e$) to 40-50 mM. K^+ efflux is accompanied by sodium (Na^+) and water influx into neuronal cells, leading to cellular swelling and a decrease in the extracellular space. It is believed that the high $[K^+]_e$ depolarizes adjacent neuronal tissue and the self-propagation is further aided by concurrent glutamate efflux and calcium (Ca^{2+}) influx (29). Neuronal depolarization is thought to also remove the voltage sensitive magnesium (Mg^{2+}), block of N-methyl-D-aspartate (NMDA) receptors (-R), sensitizing them to interstitial glutamate and to further release of K^+ and glutamate and adding to the feedback loop (32,34). This hyperpolarization leads to a complete cessation of recordable spontaneous and stimulated electrical activity and the recovery from this transient phenomenon reverses itself, propagating along the same route and at the same rate after 5-10 minutes. It is likely that astrocyte uptake of K^+ aids in recovery as CSD

has been shown to attenuate in astrocyte rich regions and does not spread to cerebral white matter (30,32-34).

CSD is readily reproduced in animal models and can be induced by application of K^+, glutamate, Na^+/K^+ pump inhibitors, and mechanical, electric and thermal stimulation (28,30,33). Familial hemiplegic migraine (FHM), which is a rare autosomal dominant form of migraine with aura including motor weakness, has been used in CSD research with knockout mice carrying the genetic mutations of the three subtypes of the disease. FHM1 has a mutation (CACNA1A on chromosome 19) coding for a calcium channel, FHM2 has mutation (ATP1A2 on chromosome 1) for a Na^+/K^+-adenosine triphosphatase (ATPase) and FHM3 has a mutation (SCN1A on chromosome 2) coding for a Na^+ channel (1,28). In all three forms, mice carrying the genetic mutations show an increased susceptibility for CSD (28). These mutations are not present in other forms of migraine, however (1).

Demonstrating CSD in human migraine has met with difficulty due to the unpredictable nature of migraine and it is also difficult to detect by electroencephalogram (EEG) due to its slow propagation and relatively small area affected (32). The use of subdural EEG electrodes in patients undergoing brain surgery, however, has provided evidence of spreading depression occurring in patients with traumatic brain injury, malignant hemispheric stroke and subarachnoid haemorrhage and in these instances the subsequent decrease in regional blood flow can reach hypoxic levels (32).

Invasive methods of CSD study are not an option in migraine and imaging of cerebral blood flow indicative of CSD has been utilized instead. Intra-arterial injections of xenon-133 was used to study the regional cerebral blood flow (rCBF) in patients with migraine with aura during a developing migraine found a slowly spreading decrease in rCBF, an oligemia, originating in the posterior region of the brain, spreading anteriorly at a slow rate, crossing neurovascular boundaries

and not spreading across sulci (36-38). Perfusion and diffusion weighted functional MRI (fMRI) during spontaneous visual aura showed decreases in rCBF in the occipital cortex contralateral to the affected visual hemifield (39). An important blood oxygen level dependent (BOLD) fMRI study found, that during visual aura, an increase in BOLD signal which developed in the extra-striate cortex, progressed over the occipital cortex at a rate of approximately 3.5 mm/min, and was followed by a BOLD signal decrease progressing over the same area in the same manner (35). Further, retinotopic analysis indicated the BOLD signal changes followed the perceived progression of the visual aura of the patient. Today it is widely accepted that CSD is the likely pathophysiological phenomenon behind migraine aura (1).

While the early xenon 133 studies did not demonstrate regional blood flow changes indicative of CSD in migraine without aura (40), and the presence of CSD in migraine without aura is questioned (1,41) some studies suggest blood flow changes are present in both types. A BOLD-fMRI study of visually triggered migraine headache found similar signal changes in patients both with and without aura, and of the 6 migraine patients with aura, only 2 developed visual symptoms and the other 4 had previous attacks of migraine without aura (42). An oxygen 15 labelled positron emission tomography case study of a spontaneous migraine without aura, in a patient with no history of aura, found bilateral hypoperfusion in the occipital lobes which spread anteriorly (43). A more recent PET study found significant relative posterior cortical hypoperfusion within 4 hours of headache onset in migraine without aura which persisted after pain relief following sumatriptan injection (44).

Abnormalities in brainstem perfusion were found in migraine without aura, as well. A PET study of spontaneous attacks of migraine without aura found significant increased rCBF in median brainstem structures slightly contralateral to the headache side during the attack, as well as increased activation in the inferior antero-caudal cingulate cortex and in the visual and auditory

cortices (45, 46). The increased rCBF persisted in the brainstem after sumatriptan injection, projecting on the PAG, midbrain reticular formation and the locus coeruleus. The findings were replicated in glyceryl trinitrate induced migraine without aura attacks which found activation in the dorsal lateral pons ipsilateral to the side of pain (bilateral in the case of bilateral headache) which also persisted after pain relief with sumatriptan (47). Another study on spontaneous migraine without aura found significant and persistent activation in the hypothalamus, as well (48). Brainstem activation appears to be specific to migraine (46,49) and these studies lend support to the theory that central activation and involvement of the trigeminovascular pathway have a role in migraine headache.

1.2.3 Trigeminovascular nociceptive pathway

The peripheral nociceptive innervation of the intracranial vasculature and meninges originate in the trigeminal ganglion (50,51). These non-myelinated C-fibres and thinly myelinated Aδ-fibres contain vasoactive peptides such as substance P (SP), calcitonin gene related peptide (CGRP), neurokinin A (NKA) and pituitary adenylate cyclase-activating polypeptide (PACAP), reach the dura in large part through cranial nerve V1 (ophthalmic branch of the trigeminal nerve), but also through cranial nerve V2 (maxillary branch of the trigeminal nerve) and cranial nerve V3 (mandibular branch of the trigeminal nerve). Further innervation originates from the upper cervical dorsal root ganglion. The central processes of these sensory afferents enter the brainstem through the trigeminal tract terminating in the spinal trigeminal nucleus (majority in laminae I and II) and the upper cervical spinal cord (C1-C3). From the spinal trigeminal nucleus, second order trigeminovascular neurons project to the parabrachial area, the hypothalamus (anterior, lateral and perifornical areas), the lateral preoptic nucleus, zona incerta and the thalamus (ventral posteromedial, posterior and parafascicular nuclei). From the ventrolateral area of the upper

cervical and medullary dorsal horn, secondary trigeminovascular neurons project to the ventrolateral PAG, rostral trigeminal spinal nuclei, nucleus of the solitary tract, brainstem reticular areas, superior salivatory nuclei and the cuneiform nuclei. From the thalamus, third order neurons project to the cerebral cortices. It is believed that activation of the trigeminovascular neurons leads to signal modulation in the brainstem, with the PAG playing an important role, then higher order processing of pain signals occurs in the thalamus and further in the cortex. Cranial parasympathetic fibres are also activated in the superior salivatory nucleus in the brainstem and postganglionic parasympathetic fibres project to the lacrimal, nasal mucosa and salivary glands, and the craniofacial vasculature, which may induce lacrimation and rhinorrhoea (50,51) (Figure 1).

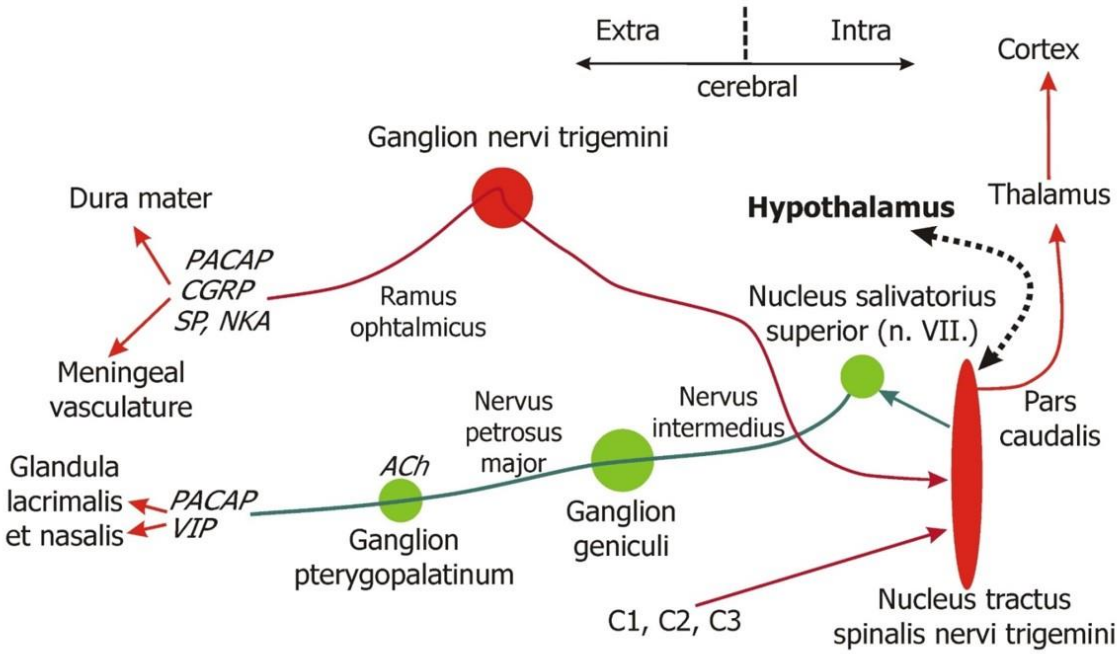

Figure 1. *The trigeminovascular system provides an important pain-transmission link between the vascular (dural and cortical) and neuronal (brainstem and thalamus) regions.*

Activation of the trigeminovascular system was initially indirectly demonstrated by increased c-fos expression in the superficial laminae of the trigeminal nucleus caudalis following CSD (50, 52). More recently, direct evidence has been published of peripheral nociceptor (53) and central trigeminovascular neuronal activation (54) by CSD in the rat brain.

It has been proposed that neuronal activation during CSD causes the release of vasoactive peptides such substance P (SP), calcitonin gene related peptide (CGRP), neurokinin A (NKA), pituitary adenylate cyclase-activating polypeptide (PACAP), and that this causes a local neurogenic sterile inflammation and plasma protein extravasation which activates the trigeminovascular nociceptive system (50,55,56). Plasma protein extravasation has been demonstrated in animal migraine models but clinical trials of medications specific to blockade of neurogenic plasma protein extravasation have been ineffective against migraine (50). CGRP has been shown to be elevated in humans during acute migraine (34), and CGRP antagonists olcegepant and telcagepant showed high efficacy in the treatment of acute migraine, however increased triglycerides after long-term use have made their use in human migraine uncertain for the present (50,57,58). Also, CGRP alone does not promote plasma protein extravasation and the phenomenon's role in migraine is falling out of favor (50), however peripheral trigeminovascular neuron sensitization cannot be completely discounted (59).

5-HT administration was shown early on to abort migraine attacks (21,55). It had been found that interictal 5-HT levels are reduced while ictal levels are increased which led to the development of sumatriptan, a $5\text{-HT}_{1B\text{ cranial vasculature/1D meningeal pain fibers}}$ receptor agonist designed to selectively act on 5-HT_1 receptors of cranial blood vessels (21,60). Developed when the vascular theory of migraine was prevalent, its efficacy was thought to be due to its vasoconstrictive activity while acting on 5-HT_{1B} receptors. Increasingly the focus has shifted to understanding the effects of sumatriptan, and other triptans, on the peripheral (through presynaptic inhibition of neurogenic

inflammation) and the central (through inhibition of pain transmission in the brain stem and spinal cord) trigeminovascular system (56,61). Indeed, the new drug lasmiditan which selectively targets the 5-HT_{1F} receptors in the trigeminal pathway has been proven to be highly potent with no vasoconstrictor effects (62,63) and is currently in phase 3 clinical trials (www.colucid.com/why-lasmiditan). It is posited that low 5-HT levels in migraine patients causes a deficit in the descending pain inhibitory system and facilitates the activation of the trigeminovascular nociceptive system (63,64), that it is rather a central mechanism. It is also likely that pain in migraine stems from a combination of sensitization both peripherally, as in the case of pulsatile pain (51,56), and centrally, as in the case of non-trigeminal innervated cutaneous allodynia (51) and in photophobia (51,65).

1.2.4 Migraine as a Risk Factor

Increasingly evidence points to migraine not being a totally benign headache disorder as previously thought, with vascular structural white matter abnormalities having been observed in migraine both with and without aura.

There is evidence of the brain being "hyperexcitable" in interictal migraineurs (66-69) with the brain showing dishabituation to repetitive stimuli. In normal subjects, in BOLD fMRI studies, a quantifiable hemodynamic refractory effect is observed when exposed to repetitive visual stimulation (70-72). This refractory effect was not observed in interictal migraineurs without aura and interpreted as a lack of habituation to repetitive stimuli in the patients (73). The visual evoked potential (VEP) testing has also shown a lack of habituation in migraine patients (74,75), with one study finding that dishabituation tended to normalize prior to and during migraine attacks (76). Another study found that an increased (BOLD) response in the trigeminal nuclei to nociceptive stimulation during headache free intervals was predictive of a coming migraine attack (77). It could be speculated that hyperexcitability can make the brains of migraine patients more vulnerable to

CSD and migraine attacks, though more evidence is needed.

Migraine has been shown to be a risk factor for ischemic stroke (78-86), with a higher risk in migraine with aura (79-82,85,86) increasing with increased attack frequency (83), women (83-86), users of oral contraceptives (79,85), smokers (85) and in those younger than 45 years (85). There is also an association between both types of migraine and other cardiovascular diseases such as hypertension, dyslipidaemia, myocardial infarction and claudication (81,82,85). Interictal cerebral perfusion abnormalities have been observed in migraineurs, with areas of hypo and hyperperfusion (87,88). One study found a significant increase of subclinical posterior circulation infarcts in migraine with aura patients who did not have concomitant otherwise increased cardiovascular risks (89). L-arginine is a precursor to nitric oxide (NO) and when systemically infused has been found to induce vasodilation (90). A decreased cerebrovascular response to L-arginine infusion was found in the posterior cerebral arteries in migraine patients without comorbidities in one study (91), indicating possible endothelial dysfunction (92). Vascular wall dysfunction was also suggested in a case control study which found a strong association with migraine in patients with cervical artery dissection (93). Further, a study found migraineurs to have an interictal increase in temporal artery diameter and blood flow along with increased brachial artery stiffness compared to controls (94). Decreased peripheral arterial diameter and compliance along with increased central and peripheral blood pressure were also found in young patients with recent onset migraine who did not take vasoactive drugs (95). These studies suggest vascular endothelial dysfunction could be behind the increased cardiovascular risk in migraine.

Migraine has not been associated with cognitive decline (96-98), however brain structural abnormalities have been observed in migraine (99). MRI studies found increased non-hem iron deposition in the PAG (100,101), putamen, globus pallidus and the red nucleus in migraine patients both with and without aura compared to age matched controls and which increased with disease

duration (102). It was originally interpreted as free radical damage due to disturbance in iron homeostasis (100,101) but a more conservative interpretation is that it's likely due to repeated activation of the regions during migraine (102). MRI evidence of increased grey matter density in the PAG was also found in headache free migraine patients, which was more severe in patients with aura who also had increased grey matter intensity in the pons (103), possibly a sign of remodelling due to repetitive attacks. Studies have also found grey matter volume decreases in the frontal, prefrontal, temporal, parietal, occipital and insular cortices, the cingulate cortex, cerebellum and the brain stem of migraine patients (103-107). This is hypothesized to be a complication of frequent migraine attacks (99).

In addition to grey matter abnormalities, intracerebral white matter abnormalities visible on MRI can be observed in migraine both with and without aura (99). These have been termed white matter hyperintensities (WMHs) or white matter lesions (WMLs) and as the focus of our research group will be discussed in concert with our research below.

1.3 Intracerebral White Matter Lesions

1.3.1 Definition of White Matter Lesions

White matter lesions were considered if they were visible as hyperintense areas on T2-weighted and FLAIR images without hypointensity on T1-weighted scans and were larger than 3 mm and appeared in at least two consecutive slices (1.5 mm/slice) (108,109).

1.3.2 Location of White Matter Lesions

WMLs can be located in either cerebral hemisphere, in any of the supratentorial cerebral lobes and in the brainstem and the cerebellum in the infratentorial region. Supratentorially they can be further categorized as being located subcortically with U-fibres, in the deep white matter,

periventricularly or in the corpus callosum (Figure 2) (99,108-112).

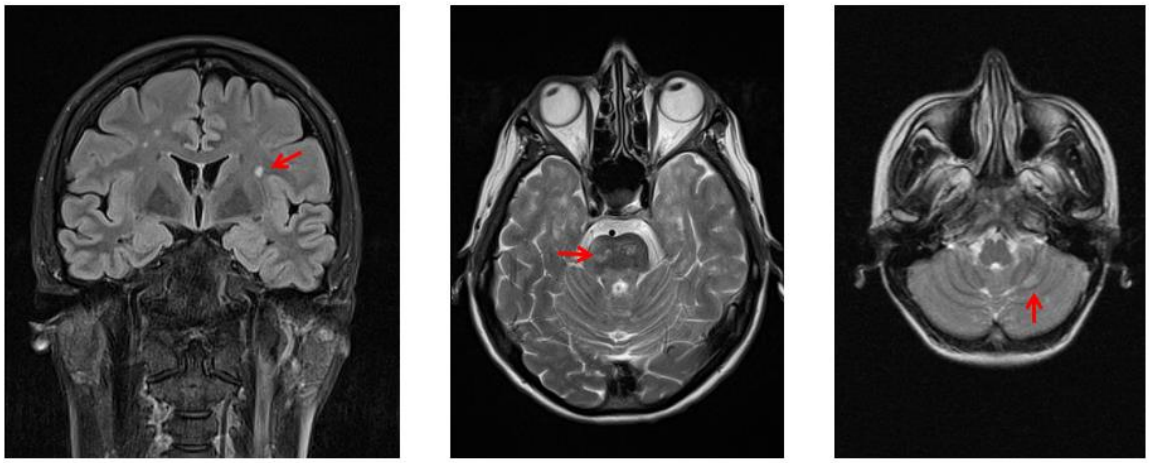

Figure 2. *White matter lesions in different intracerebral locations. Red arrows point to lesions.*

1.3.3 Prevalence of White Matter Lesions

White matter lesions can develop at any age during the active migraine years (99). Among the risk factors for WML are increased age, hypertension, cardiovascular disease, hyperhomocysteinaemia, smoking and migraine (113). These lesions have been found to be an independent predictor of stroke in the elderly (114). Migraine patients both with and without aura have an increased risk for WMLs (110) with an increased prevalence in migraine with aura (112). This risk is increased even in young migraineurs without comorbid cerebrovascular risk (115) and small WMLs have even been found in children and adolescents (age 6-18) with migraine (116). The risk is further increased for women (115,117) and with increased attack frequency and duration (118,119) but the effects of comorbid disease may also contribute to lesion development (119).

1.3.4 Supposed pathophysiology of lesion formation

There have been a number of pathomechanisms proposed for lesion formation. One of the

early suggestions was that the lesions may be due to an immune based white matter demyelination (120). It has also been suggested that they are a consequence of mitochondrial dysfunction with an impairment of the brain oxidative metabolism (121,122). Inflammatory processes have been implicated with findings of increased levels c-reactive protein (CRP) (123), soluble intercellular adhesion molecule-1 (sICAM-1), tumour necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) during migraine attacks (124). It has been proposed that attack related oligemia and focal hypoperfusion are behind lesion formation (111,132), possibly through glutamatergic excitotoxicity, as a consequence of protracted CSD and oligemia, leading to neural death (125). It has also been proposed that CSD alters the blood brain barrier permeability through activation of matrix metalloproteinase-9 (MMP-9) cascade (126) and that repeated sterile vascular inflammation results in endothelial injury (124,127). Endothelial dysfunction with a genetic component has also been suggested, such as the methyltetrahydrofolate reductase (MTHFR) C677T polymorphism which leads to increased circulating homocysteine (128), increased von Willebrand factor antigen and activity (129), and angiotensin converting enzyme insertions/deletions (130). Histopathologic findings of WMHs in elderly patients showed reduced vascular and blood-brain integrity (131) however histopathological investigations of WMHs in migraine are not available and lesion pathogenesis are not yet known.

1.4 Previous Scientific Findings of the Migraine Research Group at the Department of Neurology, University of Pécs

1.4.1 The Risk Factors of Silent Brain WMLs

Our research group examined the risk factors for brain white matter lesions in 186 patients (156 females and 30 males, mean age ± SD: 36.4 ± 8.9 years, age range 18-58 years) (119). The

patients were referred to the Outpatient Headache Department of the Department of Neurology, Faculty of Medicine, University of Pécs between 2006 and 2007. The chosen patients met the criteria of migraine as defined by the IHS (1), 141 patients without aura and 45 patients with aura, of whom 58 (mean age 40.2 years) had WMLs and 128 (mean age 34.9 years) did not. The patients were further screened for other major comorbidities, such as hypertension, cardiac disease, oncological and haematological diseases, infectious diseases (e.g. HIV, hepatitis), central nervous system demyelination and genetically inherited disorders (e.g. CADASIL). The patients were examined by 3T MRI and a qualified neuroradiologist blinded to migraine and clinical diagnosis rated the WMLs.

Upon statistical analysis, we found a statistically significant correlation between the presence of WMLs and increased disease duration, higher attack frequency, elevated serum homocysteine levels and thyroid gland dysfunction. While smoking did not correlate with a statistically significant increase in the presence of WMLs, smokers had an increased headache frequency, and therefore may indirectly cause WMLs. In addition, while not statistically significant, WML were found more frequently in patients with elevated serum cholesterol and uric acid levels. The presence of aura, contrary to previous studies, was not a statistically significant risk for WML formation. This study supported migraine being and independent risk factor for white matter lesion formation and provided additional information on the contribution of comorbid diseases.

1.4.2 Differentiation of migraine and multiple sclerosis lesions

Migraine, a neurovascular disorder, and multiple sclerosis (MS), an inflammatory demyelinating disorder, can both cause white matter lesions which appear similar on conventional MRI. Our research group performed a study which aimed to compare these lesions and find

anatomical biomarkers specific for migraine (133). We enrolled 17 migraine patients (15 females and 2 males, mean age ± SD: 42.6 ± 11.2 years, age range 19 – 65 years) who fulfilled the IHS classification for migraine, 10 with aura and 7 without aura, who had supratentorial WMHs on T2 weighted and FLAIR MRI. They were further screened for lack of comorbidities, same as previously listed above, and other types of headache. Fifteen age matched patients (11 females and 4 males, mean age ± SD: 37.3 ± 9.0 years, age range 23-51 years) with relapsing and remitting MS according to the 2005 modified McDonald criteria (Expanded Disability Status Scale (EDSS) ± SD: 1.6 ± 1.53, EDSS range: 0-4) were enrolled, who had supra- and infratentorial WMHs and no history of migraine or cerebrovascular risk factors. The patients were examined by 3T MRI, image analyses (lesion number, total lesion volume: ml/patient, average lesion size: ml/lesion) were performed using the Slicer 3D 2.6 software and the data was statistically analysed.

We found that migraine-related WMLs affected the characteristic intrahemispheric MS-related lesion locations, mainly in the deep white matter and subcortical U-fibres. These lesions belonged to the anterior circulation, appeared more frequently in the frontal and parietal lobes, showed no difference in average size between lobes, and were smaller and fewer than in MS. Most of the MS WMLs were in the frontal lobe and were the smallest average size, while the fewest WML with the largest size were in the occipital lobe. The pattern of supratentorial WML appearance differs between the two groups; however, accurate differential diagnosis of WML by conventional MRI is probably not possible in individual patients.

Except two migraine patients, the same patient groups were investigated in a diffusion-weighted image (DWI) study (134). The images were acquired on a 3.0-Tesla magnetic resonance imaging system. Diffusion parameters were estimated using monoexponential (0–1000 s/mm2) and biexponential (0–5000 s/mm2) approaches from 15 multiple sclerosis patients, 15 patients with migraine and 15 healthy control subjects. High lesional ADCmono values were detected in both

patient groups without significant differences between the groups. The biexponential measurements showed significantly higher ADCfast, ADCslow, and P (percentage) slow values in the migraine lesions than in the multiple sclerosis lesions. These findings suggested that biexponential diffusion analysis may help to differentiate multiple sclerosis-related white matter lesions from migraine-related ones in individual patients.

1.4.3 Radiological features of migraine lesions

Our research group also examined the same 17 migraineurs as above using advanced MRI techniques, as follows: T1- and T2-weighted and 3D-FLAIR images, diffusion weighted images (DWIs) and perfusion weighted images (PWIs), proton magnetic resonance spectroscopy (^{1}H-MRS) and T1 and T2 relaxation time measurements (135). We compared the lesions with contralateral normal appearing white matter (NAWM) in the same patients, as well as to 17 age matched healthy control subjects in the same brain locations (15 females and 2 males, mean age ± SD: 40.7 ± 11 years, age range 19-65 years). There were no MRI abnormalities in the control group. DWI detects changes in the movement of water across neuronal membranes and we detected significantly increased apparent diffusion coefficient (ADC) values in WMHs compared to normal white matter and controls. This indicates elevated levels of random water molecule motion inside the lesions which can indicate tissue damage (135,136). In vivo T1 and T2 relaxation times also provide information about tissue water environment and the prolonged relaxation times we measured can indicate an increased extracellular water fraction, similar to that seen in damaged tissue in chronic diseases (135). In ^{1}H-MRS high background signals are suppressed to allow the measurement of brain neurotransmitter and metabolite concentrations. We found decreased N-acetyl-aspartate (NAA), which is a neuronal and axonal marker, and decreased creatine and phosphocreatine (Cr), concentrations of which are higher in astrocytes and oligodendrocytes than

in neurons, resonances. Decreased NAA can indicate axonal loss while decreased Cr can reflect tissue degeneration with impaired intracellular energy metabolism (135,137). We also performed PWI, a gadolinium-based fMRI technique which can measure capillary perfusion and found mildly reduced intralesional rCBF and relative cerebral blood volume (rCBV), which can also be indicative of axonal and cellular loss (135,136). These radiological features of these chronic WMLs were similar to those detected in chronic WMLs with ischemic origin (138-140). As stated earlier, histopathology of ischemic white matter lesions showed a reduced vascular and blood brain barrier integrity (132) and our findings indicated that these are microvascular in nature.

1.4.4 Changes of migraine-related white matter lesions after three years

Although the follow-up study mainly showed worsening in the status of brain WMLs, occasional regression of these abnormalities was also observed in some cases. A lower baseline migraine attack frequency was associated with a tendency of decrease in WML number at three-year follow-up, implying a possible long-term relationship between the headaches and these imaging abnormalities. This longitudinal MRI study found clinically silent brain white matter hyperintensities to be predominantly progressive in nature (133).

1.5 Study Aims

Here we report the findings of two clinical migraine research. First, we investigated the oxidative stress in migraine patients with or without WMLs in interictal period. Second, we investigated the cerebral cortical thickness and volume in lesional and non-lesional migraineurs to discover the possible impact of white matter tissue injuries on thickness and volume. Since these studies are differing from each other, we published them separately.

2 Serum L-arginine and Dimethylarginine Levels in Migraine Patients with Brain White Matter Lesions

2.1 Introduction

Migraine is a primary headache disorder with recurrent headache attacks (1). Migraine is an independent risk factor for the development of silent brain white matter lesions (WMLs) and infarcts (2-8). As such, WMLs are more prevalent in migraine patients than in the general population (2,3), and they can develop at any age during the active migraine years (3,9). Migraine, especially with aura, carries an increased risk for cerebro- and cardiovascular diseases that cannot be explained by traditional vascular risk factors (10-12). Although histopathological data are lacking in migraine, the microvascular ischemic injury theory (5,13) was supported by our previous quantitative magnetic resonance imaging (MRI) data demonstrating intralesional tissue damage (14) consistent with previous studies of WMLs of ischemic origin (15,16). In addition, longitudinal assessment of the same migraine patient group showed intralesional and intracerebral progression of WMLs over time (17).

A vascular aetiology, such as impairment of the L-arginine/nitric oxide (NO) pathway with vascular endothelial dysfunction could explain the development and progression of WMLs and could also provide a link between migraine and ischemic stroke or coronary heart disease. Endothelial NO-synthase (eNOS) utilizes L-arginine to generate the strong vasodilator, anti-atherogenic NO (18). Methylated analogues of L-arginine, symmetric and asymmetric dimethylarginine (SDMA and ADMA) are modulators of the L-arginine/NO pathway (19,20). Both ADMA and SDMA levels are associated with an increased cardiovascular risk and mortality (19,21). ADMA has also been found to be a marker of oxidative stress and endothelial dysfunction in migraine (21,22).

Despite the growing evidence of oxidative stress and vascular endothelial activation in migraine (23-27), the abovementioned biomarkers of endothelial dysfunction have not been comprehensively analysed in relation to WMLs. Therefore, our objective in the present study was to quantify the L-arginine, ADMA and SDMA serum concentrations of migraine patients with or without cerebral WMLs in a headache-free period, to detect group differences between lesional and non-lesional migraineurs, to investigate the effect of migraine characteristics, and to determine the differences between migraine patients and controls.

2.2 Patients and Methods

2.2.1 Subjects

Between 2010 and 2013, a total of 177 patients fulfilling the International Headache Society (IHS) classification criteria for migraine with or without aura (1) were screened from the Outpatient Headache Clinic of the Department of Neurology, University of Pécs. A total of 109 migraineurs (93 females, mean age 36.7 ± 10.8 years, age range 19-65 years; Table 1), 82 without and 27 with aura subtype remained and were prospectively enrolled in the study after exclusion of those with: (i) concurrent non-migraine headache types, (ii) focal neurological signs, (iii) abnormal screening blood test findings (e.g., abnormal lipid profile, etc.), (iv) suffering from any chronic disease other than medically treated hyperlipidaemia or hyperuricemia, (v) active smoking habit. Patient selection was not restricted by age, since WMLs can be formed at any age during the active migraine headache years (9,10). Since the migraineurs were either newly diagnosed, or established patients with low headache frequency (≤ 2/month) maintaining adequate control on acute migraine regimens, none of them were on chronic prophylactic therapy during the study period. For acute migraine treatment, eletriptan, sumatriptan, ibuprofen, diclofenac and acetaminophen were

utilized. As controls, 56 subjects were screened, and 46 age-matched healthy subjects (33 females, mean age 37.5 ± 10.5 years, age range 20-65 years) were enrolled. These subjects were previously recruited via bulletin board advertisements throughout the University of Pécs accessible to students and staff. They had no history of any type of headache and passed the above-mentioned exclusion criteria. All participants underwent detailed clinical investigations to uncover vascular risk factors, which themselves may cause elevation of serum dimethylarginine levels and WMLs. All subjects with previously diagnosed hypercholesterolemia were treated with statins, and those with elevated uric acid levels were on allopurinol at the time of blood sampling. The clinical data of patients and controls are presented in Table 1, while migraine characteristics are seen in Table 2.

All included participants underwent brain MRI in a headache free period utilizing the same scanner and acquisition protocol to evaluate the presence of white matter hyperintensities. Given the previous findings of focal tissue damage (14,17), we will refer to these as WMLs later in the text. WMLs were detected in 43 migraineurs, whereas no WMLs or other structural abnormalities were found in the rest of the studied population including controls.

Migraine patients were divided into two subgroups based on presence of WMLs (L+) or absence of those **(**L-). Presence of aura, disease duration in years and monthly attack frequency were investigated, followed by the estimation of lifetime migraine attack number **(**average monthly attack number x 12 x number of migraine disease years to date**)** in all migraineurs.

Serum levels of L-arginine, ADMA and SDMA were quantified from fasting blood samples taken between 8 and 9 a.m. by venepuncture from the antecubital vein in all investigated participants. Patients were headache free for at least 3 days before blood samples were taken. The MR scanning and the taking of blood samples were performed on the same week.

2.2.2 Identification of white matter lesions and MR scanning protocol

Two qualified neuroradiologists who were blinded to migraine diagnosis and clinical data rated the white matter lesions. WMLs were considered if visible as hyperintense areas on T2-weighted and FLAIR images without hypointensity on T1-weighted scans and larger than 3 mm appearing in at least two consecutive slices (2) (Figure 1).

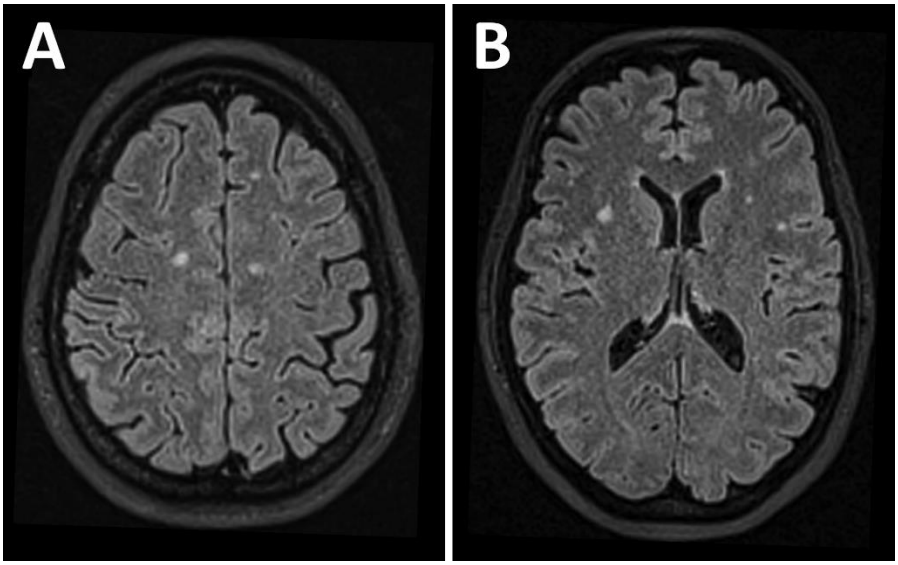

Figure 1. *Axial fluid-attenuated inversion recovery brain MRI images of a migraine patient with aura. The figure shows bifrontal white matter hyperintensities in two different slices (A and B).*

A 3.0-Tesla clinical MRI scanner (Magnetom TIM Trio, Siemens Medical Solutions, Erlangen, Germany) with a 12-channel phased-array head coil was used for MR measurements. Routine T1-, T2-weighted and FLAIR imaging were performed. For standard and accurate axial slice positioning the anterior and posterior commissural line (AC-PC line) was used as a reference for T2-weighted and FLAIR images. Sagittal T1-weighted images were obtained using a fast low angle shot (FLASH) 2-dimensional (2D) sequence: repetition time/echo time (TR/TE) = 300/2.46 ms; flip angle = 88°; 27 slices; slice thickness = 4 mm; distance factor = 30%; field-of-view (FOV) = 220x220 mm^2; matrix size = 256x320; receiver bandwidth = 330 Hertz (Hz)/pixel (px). For T2-

weighted images a turbo spin echo (TSE) sequence was used: TR/TE = 6000/93 ms, 30 slices, slice thickness = 4 mm, distance factor = 20%, FOV = 193x220 mm^2, matrix size = 280×320, bandwidth = 220 Hz/pixel, number of echo trains = 18. A TSE sequence was also used for the FLAIR images: TR/inversion time (TI)/TE = 15710/2750/105 ms, 100 slices, slice thickness = 1.5 mm, distance factor = 0% (no gap), interleaved slice readout with two concatenations, FOV = 220x220 mm^2, matrix size = 192×192, bandwidth = 400 Hz/pixel, number of echo trains = 14.

2.2.3 Measuring serum concentrations of dimethylarginines and L-arginine

The amino acids were extracted from the blood serums via solid phase extraction (SPE) according to the method of Nonaka et al. (24): a mixture of 250 µL serum sample and 700 µL borate buffer (pH = 9.00) was mixed with the internal standard that was L-homoarginine hydrochloride (Aldrich, Germany) and 50 µL of 1000 µmol/L solution was applied. Then the mixture with the internal standard was passed through SPE cartridges (OASIS® MCX 3cc SPE) at 750 mbar in a 12-column manifold (J. T. Baker). The elution was performed by concentrated aqueous ammonia (Reanal, Hungary), water and methanol with a volume ratio of 10/40/50. The solvent was evaporated beginning under a nitrogen atmosphere and finished in a vacuum at 60 °C. The dry residue was dissolved in 200 µL deionized water (Millipore, Milli-Q) and derivatized according to Molnar-Perl et al. (25); at room temperature the reaction time was 10 minutes. The high-performance liquid chromatography (HPLC) analysis was performed with a Waters 2695 Separations Module equipped with a thermostable autosampler (5 °C) and column module (35 °C). Separation was achieved with a Waters Symmetry SB C18 (4.6 x 150 mm, 3.5 µm) column and detected by a Waters 2475 fluorescence detector (Waters Milford, MA, USA) (26). For the measurements, 10 µL was injected from the samples and the gradient elution was applied during the analysis with two mobile phases: A (20 mM $(NH_4)_2CO_3$ in water, pH = 7.50 ± 0.05) and B

(acetonitrile). The gradient program was as follows: 0-16 min: 91% A and 9% B, 16-17 min: linear change to 70 % A and 30 % B and hold this for 5 minutes, 22-23 min: linear change to 91 % A, 9% B and hold this for 12 minutes. The flow rate was constant (1 ml/min). Arginine and homoarginine were detected at λ_{ex} = 337 nm, λ_{em} = 520 nm, but λ_{em} = 454 nm was used for ADMA and SDMA.

2.2.4 Statistical analysis

Statistical analyses were performed utilizing the IBM SPSS Software (version 21, SPSS Inc, Chicago, IL). First, the distribution of each studied variable was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Differences in age between the whole migraine group and healthy controls were assessed by the Mann-Whitney U-test.

Univariate analyses. Differences of continuous variables (e.g., age, BMI, disease duration, migraine attack frequency, total number of migraine attacks, L-arginine/ADMA/SDMA levels) among groups (L+, L-, control) were compared using the Kruskal-Wallis test followed by the Mann-Whitney U test. Differences in binary population characteristics (e.g., gender, smoking, hypercholesterolemia, hyperuricemia, elevated CRP level) among the same groups were assessed utilizing Pearson's chi-square test.

Multivariate analyses. First, we tested predictors of L-arginine/ADMA/SDMA levels using stepwise multiple linear regression analyses. Age, gender, disease duration, migraine attack frequency, total number of migraine attacks and presence of aura were included as independent variables along with the grouping variable (L+ vs. L-), and L-arginine/ADMA/SDMA level as the dependent variable. Similar analyses were performed to assess the L-arginine/ADMA/SDMA level differences between patient and control groups (L+ vs. control and L- vs. control), excluding migraine characteristics (not present in the control group) from the list of independent variables. In the control group, multiple linear regression analyses were performed to assess the potential effects

of age and gender on L-arginine, ADMA, and SDMA concentrations. Serum concentrations served as dependent, while age and gender as independent variables. The assumptions of multiple linear regression were satisfied, as judged by testing for linearity, and normality. In addition, in the patient group, binary logistic regression was performed including the covariates of age, gender, L-arginine/ADMA/SDMA levels, disease duration, frequency of migraine attacks, total number of migraine attacks and presence of aura to assess the likelihood of WML presence. The final covariates were selected using a stepwise method (forward conditional).

Finally, Spearman's correlations were performed to assess the internal dependencies between the three reported lab-variables (L-arginine, ADMA, SDMA) in all subjects, and to investigate the correlation between age and disease duration in the migraine group. The level of statistical significance was set as < 0.05.

2.3 Results

2.3.1 Age

Since the age of controls was matched to the whole migraine group, there was no significant difference in age between them. The Kruskal-Wallis test revealed significant age differences among migraine subgroups and controls ($P < 0.001$) (Table 1). Post-hoc testing indicated significant differences between all possible pairs of the three groups ($P < 0.05$).

2.3.2 Control group

Since the study subgroups were not age-, and gender-matched, the healthy control group was investigated next by multiple linear regression. There were no significant effects of either age or gender on L-arginine, ADMA, or SDMA concentrations; there were no interactions between age and gender (i.e. age*gender).

2.3.3 Categorical data

Regarding the gender of subjects, and the presence of comorbid disorders, no significant differences were found among the groups (Table 1).

Table 1. *Clinical data of migraine patients and healthy controls and differences among groups*

Investigated parameters	Migraine patients with lesions (n = 43)	Migraine patients without lesions (n = 66)	Controls (n = 46)	Differences (P – value)
Age (years)	41.7 (21 - 65)	32.7 (19 - 54)	37.5 (20 - 65)	< 0.001'
Female/male	38/5	55/11	33/13	0.107"
Hypertension	0/43	0/66	0/46	N/A
Diabetes	0/43	0/66	0/46	N/A
Kidney disease	0/43	0/66	0/46	N/A
Hepatopathy	0/43	0/66	0/46	N/A
High LDL cholesterol*	5/43	6/66	2/46	0.886"
Hyperuricemia**	1/43	2/66	3/46	0.660"
Elevated CRP level***	2/43	5/66	2/46	0.228"
Thyroid disorder	0/43	0/66	0/46	N/A
Systemic autoimmune disease	0/43	0/66	0/46	N/A
Smoking****	6/43	8/66	5/46	.445"
Cardiac source of embolism	0/43	0/66	0/46	N/A
BMI (kg/m^2)	23.69 ± 2.21	23.29 ± 2.46	23.65 ± 2.12	0.267'

LDL: low-density lipoprotein; CRP: C-reactive protein; BMI: body mass index; N/A: Not Applicable; Participants were treated with statins* (LDL < 3.4 mmol/l) and allopurinol** (uric acid < 350 mmol/l) at the time of blood sample taking; ***CRP was between 5.0 and 7.0 mg/l in elevated cases; ****History of smoking, no active smoking; 'Kruskal-Wallis test; "Pearson's chi-square test

2.3.4 Migraine characteristics

Compared to lesion-free migraineurs, patients with WMLs had a longer disease duration ($P < 0.001$; Table 2), and a higher number of lifetime headache attacks ($P = 0.005$; Table 2). The attack frequency did not show *a* significant difference between the L+ and L-groups (Table 2).

	White matter lesions						Statistics			
	Lesion+ (*n* = 43)*		Lesion- (*n* = 66)**		Control (*n* = 46)		Mann-Whitney test			Kruskal-Wallis test
	Mean	SD	Mean	SD	Mean	SD	L+ vs. L-	L+ vs. C	L- vs. C	
Disease duration (years)	20.12	11.84	11.98	7.45	-	-	**< 0.001**	-	-	**< 0.001**
Migraine attack frequency/month	3.88	3.24	3.43	3.04	-	-	0.45	-	-	0.45
Total number of migraine attacks	900.3	913.4	428.4	398.5	-	-	**0.005**	-	**-**	**0.005**
L-arginine	173.6	55.6	164.8	53.4	107.7	22.53	0.55	**< 0.001**	**< .001**	**< 0.001**
ADMA	0.781	0.155	0.650	0.159	0.663	0.077	**< 0.001**	**< 0.001**	0.48	**< 0.001**
SDMA	0.559	0.094	0.488	0.106	0.520	0.047	**< 0.001**	0.07	**0.017**	**< 0.001**

ADMA = asymmetric dimethylarginine; SDMA = symmetric dimethylarginine; *13 migraine patients with aura; **14 migraine patients with aura

Table 2. *Results of measured variables and statistical differences between migraine subgroups and controls*

2.3.5 L-arginine levels

Markedly higher blood serum concentrations were measured in the migraine groups (L+ 173.6 ± 55.6, L– 164.8 ± 53.4) than in the control group (107.7 ± 22.53) ($P < 0.001$, in all comparisons; Table 2, Figure 2). Statistically significant differences were not found between the migraine groups (Table 2, Figure 2). Since the multiple linear regression analyses revealed no additional significant predictors (e.g., age, gender), the pattern of significance remained unchanged.

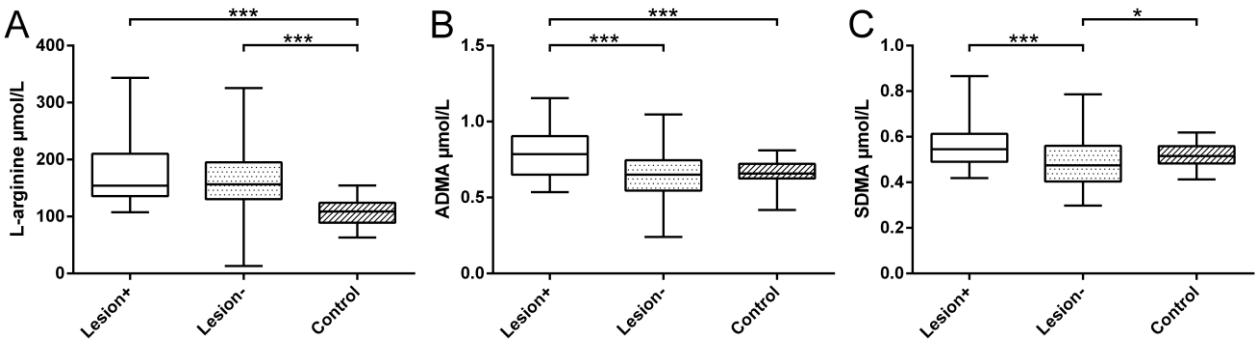

Figure 2. *L-arginine, asymmetric and symmetric dimethylarginine (ADMA and SDMA) serum concentrations in migraineurs and controls. The figure demonstrates the differences between the lesion+, and the lesion- migraine groups and the control group. Whiskers are set at minimum and maximum, the horizontal line marks the median, whereas the box indicates the interquartile range (25–75%). The statistically significant differences between the groups are indicated by * on the figures (*P < 0.05, ***P <0 .001).*

2.3.6 ADMA levels

L+ migraine patients (0.781 ± 0.155) showed higher serum concentrations than L- patients (0.650 ± 0.159) and controls (0.663 ± 0.077) ($P < 0.001$, in both comparisons) (Table 2, Figure 2*)*. Controlling for the relevant covariates using multiple linear regression analyses, there was no change in the pattern of significance. When comparing the L+ group to the control group no additional significant predictors (e.g., age, gender) were found. In the comparison of the L+ and L- groups, aura and age proved to be additional significant predictors; the presence of WML, aura and increasing age indicated an increased ADMA level ($P = 0.008$, .047 and 0.012 respectively). There were no interactions between the independent variables.

2.3.7 SDMA levels

SDMA levels of L+ migraineurs (0.559 ± 0.094) were higher than L- patients (0.488 ± 0.106) ($P < 0.001$) and controls (0.520 ± 0.047), but the latter comparison was not statistically significant ($P = 0.07$) (Table 2, Figure 2). SDMA levels of controls were higher than L- patients ($P = 0.017$). When comparing L+ and L- groups using a multiple linear regression model, age

proved to be an additional significant predictor; the presence of lesions and increasing age indicated an increased SDMA level (P = 0.017 and 0.001, respectively). Comparing the L- and control groups, age proved to be a significant predictor (increasing age associated with increased SDMA level, P = 0.011), but the group difference (i.e., L- vs. control) was no longer significant (P = 0.145). A significant interaction was found between the grouping variable and age (P = 0.037).

2.3.8 Binary logistic regression analysis

The logistic regression model explained 37.4% (Nagelkerke R^2) of the variance in WMLs and correctly classified 71.6% of WMLs. ADMA levels were the most significant independent predictors of WML presence, with more than an 85-fold higher likelihood of exhibiting WMLs in those with a 1 μmol/L elevation in ADMA (Exp(B) = 85.33, P = 0.006). Increasing age and total number of migraine attacks were also associated with an increased likelihood of exhibiting WML (Exp(B) = 1.06, P = .017 and Exp(B) = 1.001, P = 0.026, respectively). There were no interactions between the independent predictors. None of the other examined variables added significantly to the model.

2.3.9 Spearman's correlations

The ADMA level was positively correlated with both L-arginine and SDMA levels (ρ = .158, P < .05 and ρ = 0.403, P < 0.001, respectively). No significant correlation was found between the L-arginine and SDMA levels. A significant positive correlation was found between age and disease duration in the whole migraine group (ρ = 0.628, P < 0.001) and in both subgroups (L+, ρ = 0.703, P < 0.001; L-, ρ = 0.479, P < 0.001), as well.

2.4 Discussion

The main finding of the present study is the elevated serum ADMA concentration in migraine patients with WMLs outside the attack period. ADMA levels were the best predictor of WMLs, independent of other significant factors such as age and estimated life-time headache attack number. Higher SDMA serum levels distinguished lesional migraine patients from lesion-free patients, while the elevated L-arginine concentrations differentiated migraineurs from controls.

2.4.1 Age, vascular risk factors, migraine characteristics

Increasing age was associated with an increased likelihood of exhibiting WMLs, and older age proved to be a predictor of higher ADMA and SDMA in migraineurs with lesions. Since positive correlation was found between age and disease duration, and there were no significant effects of age on ADMA and SDMA concentrations in controls, long duration of migraine, rather than older age, may explain the elevation of dimethylarginines.

Increased plasma ADMA concentrations have been associated with the presence of numerous vascular risk factors and chronic diseases such as obesity, hypertension, diabetes, dyslipidemia, hyperhomocysteinemia, ischemic heart disease, transient ischemic attack, silent brain infarcts, ischemic stroke, renal and liver failure, smoking and physical inactivity (19,31). Vascular risk factors were rare in both patients and controls in our study, and all of them were treated during the study period. It is known that lipid-lowering therapy and smoking cessation reverse the endothelial dysfunction, if present (32). Therefore, a significant influence of vascular risk factors on the findings is unlikely.

In the present study, migraine patients with WMLs had a longer disease duration than non-lesional patients, and it was associated with a higher number of lifetime headache attacks. The number of lifetime headache attacks proved to be an independent predictor of WMLs, while the

presence of aura indicated higher ADMA levels in migraineurs. Beyond the investigated migraine parameters, there are other factors such as attack intensity and duration, frequency and severity of the cortical spreading depression, which may have an influence on lesion formation. In concordance with previous studies (22,27), the interictal elevation of ADMA in lesional patients indicate that oxidative stress can be present in both the ictal and interictal phases in migraine.

2.4.2 Effects of migraine on the biosynthesis and metabolism of dimethylarginines and L-arginine

NO is produced by vascular endothelial cells and plays a crucial role in the regulation of blood pressure, cerebral blood flow and neuroprotection (18,33). The amino acid L-arginine is the main precursor of NO (Figure 3) (20,34). Posttranslational methylation of arginine residues on nuclear proteins leads to the generation of ADMA, SDMA and N-monomethyl-L-arginine (L-NMMA) that are released into the cytosol upon proteolysis, then migrate into the extracellular space and thence into the blood plasma (35). Both ADMA and the less potent L-NMMA are competitive inhibitors of all three isoforms of NOS, thus lowering the NO levels (35). The concentration of ADMA and L-NMMA is regulated mainly by degradation in the endothelial cells by the dimethylarginine dimethylaminohydrolase-2 isoform (DDAH-2), and in a smaller degree by urinary excretion (35,36). SDMA is the structural counterpart of ADMA, and like ADMA, blocks cellular L-arginine uptake competitively, thus indirectly influencing NO bioavailability by reducing substrate availability (37-39).

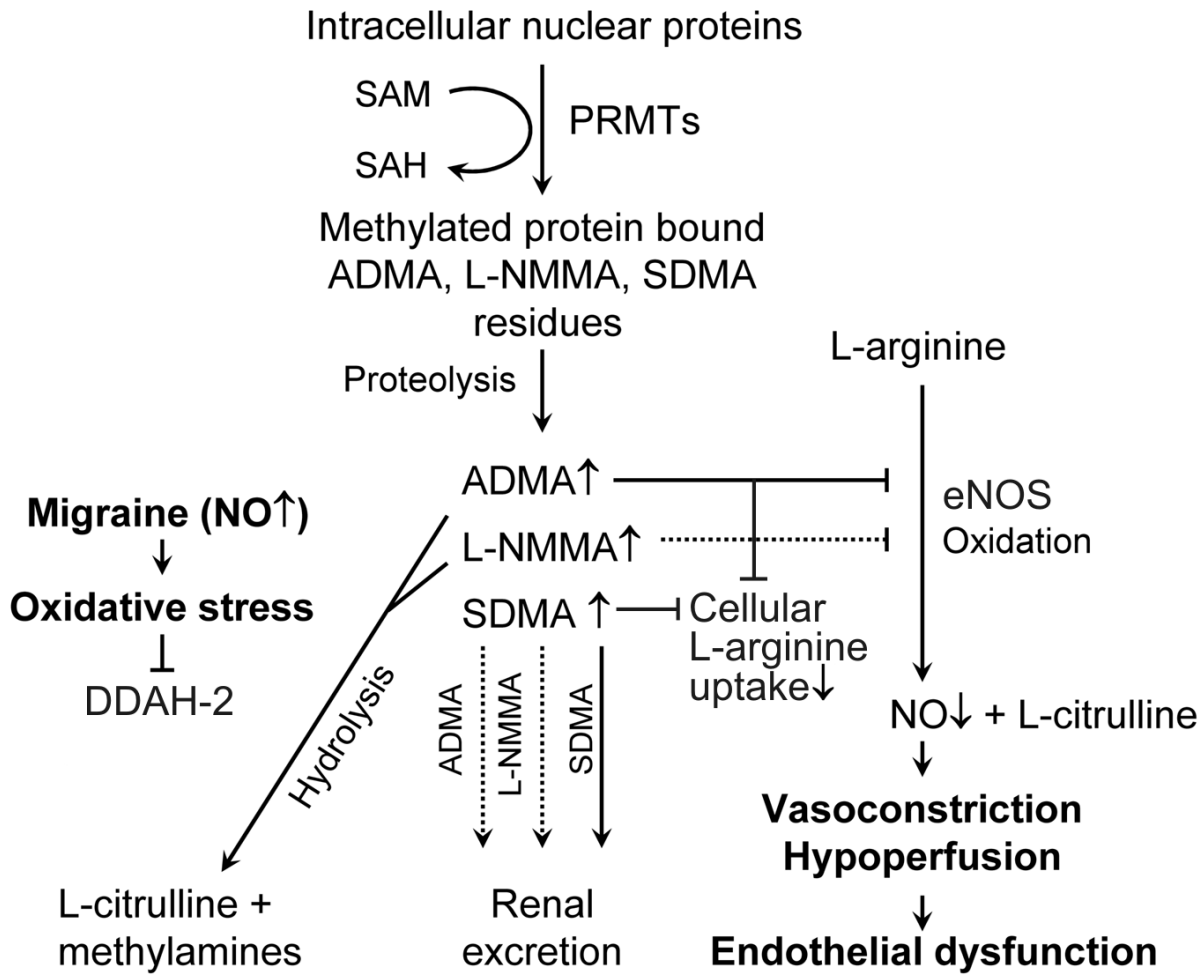

Figure 3. *Effects of migraine headache on the biosynthesis and metabolism of dimethylarginines and L-arginine. SAM = S-adenosyl-methionine, SAH = S-adenosyl-homocysteine, PRMTs = protein arginine methyltransferases, ADMA = asymmetric dimethylarginine, L-NMMA = N-monomethyl-L-arginine, SDMA = symmetric dimethylarginine, NO = nitric oxide, DDAH-2 = dimethylarginine dimethylaminohydrolase-2, eNOS = endothelial nitric oxide synthase.*

Since DDAH-2 is sensitive to oxidative stress triggered by the excessive generation of NO, under pathophysiological conditions, e.g., cardiovascular diseases and migraine, reactive O2 species (ROS) inhibit DDAH-2 activity with corresponding accumulation of ADMA (35). It is conceivable that the NO-caused vasodilatation is overbalanced by the increased amount of ADMA during migraine headache which may lead to a reduced bioavailability of NO, vasoconstriction

with cerebral hypoperfusion and endothelial dysfunction (22). Since elevation of NO and ADMA concentrations are not restricted to the ictal state (22), inhibition of eNOS can also be present in a headache-free period. Chronic endothelial dysfunction has a role in mediating impaired cerebral autoregulation, and in the small perforating cerebral blood vessels it could result in poor white matter blood supply and the accumulation of white matter injury (40,41).

It is probable that migraine is a systemic factor of vascular endothelial injury and WML development during the active headache years. Our findings support the importance of migraine headache management to avoid vascular complications of migraine. This includes not only acute and prophylactic headache treatments, but also the pharmacotherapy of comorbid cerebro- and cardiovascular diseases, as well as lifestyle changes.

2.4.3 Comparability of serum and plasma L-arginine and dimethylarginine measurements

The samples were measured with the internal standard method using L-homoarginine as the internal standard in the present study. L-homoarginine with its similar structure and chemical properties to ADMA, SDMA and L-arginine provides a chance to eliminate the matrix effects and the loss of the components during the sample preparation. Furthermore, the calibrants were prepared the same way as the samples. Thus, it can be assumed that there is no significant influence on the comparability of the results because the matrix effect can be eliminated.

2.4.4 Study limitations

The lack of age- and sex-matched study subgroups may limit the comparability of groups. Although hyperhomocysteinemia is a cardiovascular risk factor, blood homocysteine levels were not measured in the present study.

2.5 Conclusion

The elevated ADMA levels in lesional migraineurs may indicate a role of migraine-related vascular endothelial dysfunction in the development of WMLs, supporting the ischemic injury theory of WML formation. These findings indicate that elevated ADMA concentrations may be a risk factor for clinically silent brain WMLs and point out the necessity of therapeutic interventions. SDMA may also have a role in lesion formation by indirect inhibition of NO bioavailability. The higher L-arginine serum concentrations might reflect an increased demand for NO synthesis in migraine.

2.5.1 Clinical implications

- Elevated ADMA concentrations may be a risk factor for clinically silent brain WMLs.
- Detection of ADMA levels in the clinic may help to estimate the risk of vascular endothelium impairment in migraineurs.
- The elevation of ADMA in migraine patients with lesions point to the importance of the clinical management of migraine headache and the pharmacotherapy of comorbid cerebral and cardiovascular diseases.

3 Influence of Hemispheric White Matter Lesions and Migraine Characteristics on Cortical Thickness and Volume

3.1 Introduction

Cortical thickness is both a marker of neurological development and a reflection of cortical function (1,2). The cerebral cortex contains high neuronal density, and its thickness varies from 1.5 mm to 5 mm (3). Both the pyramidal neurons and the interneurons travel through the white matter within the hemisphere during prenatal brain development, and both types of cortical neuronal cells receive projection fibres from the thalamus, and association and commissural fibres from other cortical areas (3).

Migraine is a primary headache disorder (4) that may cause structural and functional alterations in the cerebral cortex (5-8). Migraine-related intracerebral WMLs are likely to be microvascular in nature and can be found in all four lobes implicating the deep white matter, the subcortical, the periventricular and the callosal commissure locations (9-11). Based on the above-mentioned data, we hypothesized that the WMLs – areas of focal axonal and glial cell (astrocyte, oligodendrocyte, microglia) injuries in association with decreased intracellular energy metabolism due to impairment of mitochondria – may cause cortical changes in migraine. For that reason, we investigated migraine patients with or without WMLs to assess the effects of these tissue damages on cortical thickness and volume. In this respect, the potential role of migraine characteristics was tested, as well. Female patients were selected, because migraine is much more prevalent in adult women than men (12) and to avoid the gender-related differences (e.g., longer headache duration, higher intensity of attacks, more frequent nausea, phonophobia and photophobia in women) existing between women and men (13).

3.2 Methods

3.2.1 Subjects

Between 2010 and 2017, a total of 161 female patients fulfilling the IHS classification criteria for migraine with or without aura (4) were prospectively screened from the Outpatient Headache Clinic of the Department of Neurology, Medical School, University of Pécs, Hungary. At the time of the study period, all migraineurs had recurrent headaches, and none of them were on chronic prophylactic therapy. For acute migraine treatment, eletriptan, sumatriptan, ibuprofen, diclofenac, acetylsalicylic acid and/or acetaminophen were utilized. The demographic and clinical data of migraineurs were the following: mean age 39.3 ± 12.5, range 18-73 years; disease duration 15.6 ± 11.9, range 1-57 years; attack frequency/month 5.6 ± 4.5, range 0.2-14.8; total number of estimated lifetime migraine attacks (average monthly attack number × 12 × number of migraine disease years to date) 966 ± 1158, range 12-6840; n = 52 with WMLs (L+ patients); n = 63 with aura (Table 1). Migraineurs had no other types of headaches. None of the included migraine patients' headache or aura was unilaterally side-locked in nature. MRI was performed in a headache-free period for each patient. Medical comorbidities that could influence migraine characteristics or lead to the formation of WMLs were excluded (hypertension, diabetes mellitus, kidney disease, hepatopathy, high low density lipoprotein (LDL)-cholesterol, hyperuricemia, elevated CRP level, thyroid gland disease, systemic autoimmune disease, smoking, cardiac source of embolism, obesity). Based on self-report, all migraineurs were right-handed. As controls, 40 age-matched healthy female subjects were included (mean age 38.3 ± 10.0, range 19-66 years, Table 1). Controls were recruited by family physicians in Baranya County, Hungary. Similar to migraine patients, all controls were right-handed. All control subjects were free of headache, and their brain MRI studies did not show any structural abnormalities.

Table 1. *Demographic and clinical data of migraine patients and healthy controls*

	Lesion+ (*n* = 52)	Lesion- (*n* = 109)	Controls (*n* = 40)	Differences (*p* value) L+ vs. L-	L+ vs. C	L- vs. C
Age (years)	44.6 ± 13.1 (20-72)	36.7 ± 11.4 (18-73)	38.3 ± 10.0 (19-66)	0.0003[a]	0.018[a]	0.422[a]
Disease duration (years)	19.8 ± 12.9 (1-57)	13.7 ± 10.9 (1-43)	-	0.003[a]	-	-
Migraine attack frequency/month	5.4 ± 4.2 (0.2-14.8)	5.7 ± 4.6 (0.5-14.5)	-	0.807[a]	-	-
Total number of migraine attacks	1213.2 ± 1249.9 (60-6840)	848.1 ± 1097.7 (12-6552)	-	0.022[a]	-	-
Patients with aura	31	32	-	0.0003[b]	-	-

Lesion+/L+: Migraine patients with lesions; Lesion-/L-: Migraine patients without lesions; C: Control subjects; Values are given as mean ± standard deviation (minimum-maximum); [a]Mann–Whitney U-test; [b]Fisher's exact test

3.2.2 MRI acquisition

All subjects were scanned on the same 3 Tesla (3T) MRI scanner (Magnetom TIM Trio, Siemens AG, Erlangen, Germany) using a 12-channel head coil. The MRI measurements of all patients were performed in a headache-free period. Whole-brain 3D magnetization-prepared rapid gradient-echo (MPRAGE) was acquired using the following parameters: TR/TI/TE=1900/900/3.4 ms; bandwidth=179 Hz/px; flip angle=9°; FOV=210×240 mm^2, matrix size=224×256, slice thickness=0.94 mm, 176 axial slices. Beyond the routine T1- and T2-weighted measurements the scanning protocol also included 2D turbo spin-echo FLAIR imaging (TR/TI/TE=13200/2600/100 ms; bandwidth=401 Hz/px; echo trains=14; FOV=186×220 mm^2, matrix size=162×192, slice thickness=1.5 mm, 100 axial slices). WML was considered if visible as hyperintensity on T2-weighted and FLAIR MRI but without hypointensity on T1-weighted MRI and larger than 3 mm, appearing in at least two consecutive slices (14).

3.2.3 MR Image analysis

Supratentorial WMLs were marked manually on the FLAIR images using 3D Slicer software (http://www.slicer.org, Version 4.6.2). An example of WML is displayed on Figure 1. Total and lobar numbers/volumes of WMLs were calculated for each subject. The borders of lobes were defined as previously described (10).

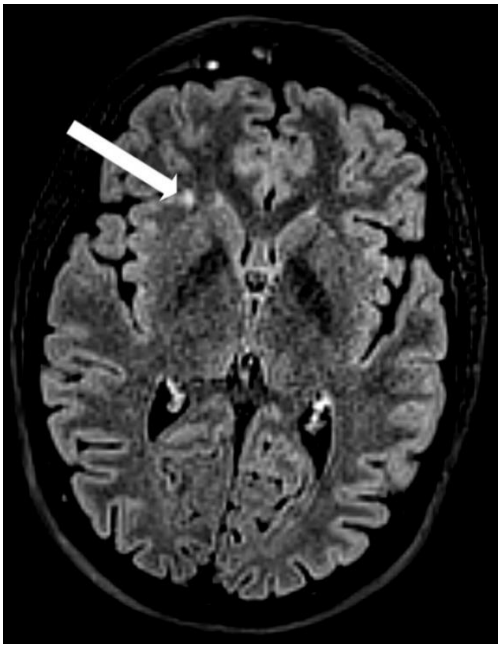

Figure 1. *Hemispheric white matter lesion of a migraine patients. The arrow shows a deep brain white matter lesion in the right frontal lobe seen on the axial fluid-attenuated inversion recovery image. The image is in radiological convention.*

Cortical reconstruction and segmentation were performed on the T1-weighted 3D MPRAGE images using Freesurfer 5.3 image analysis suite (https://surfer.nmr.mgh.harvard.edu/fswiki). The details of the image processing pipeline are described in prior publications (15,16). Quality control was performed throughout the automatic processing stream. When the reconstruction was inaccurate, error correction was performed based on the recommended workflow

(http://surfer.nmr.mgh.harvard.edu/fswiki/RecommendedReconstruction).

The automatic cortical parcellation was based on Freesurfer's "Desikan–Killiany–Tourville" (DKT) atlas (17), which has 31 cortical regions per hemisphere. The segmented regions were divided into four lobes, and surface-area weighted average cortical thickness was calculated for each lobe using the following equation:

$$\boldsymbol{LobarThickness} = \frac{\mathrm{Thickness}_1 * \mathrm{Area}_1 + \cdots + \mathrm{Thickness}_n * \mathrm{Area}_n}{\mathrm{Area}_1 + \cdots + \mathrm{Area}_n},$$

where "Area" means the surface area and 1..n is the index of left and right hemispheric cortical regions included in the given lobe. For the definition of frontal, parietal, temporal and occipital lobes please see Table 2.

Table 2. *Definition of lobes based on Freesurfer labels*

Frontal	**Parietal**	**Temporal**	**Occipital**
Superior Frontal	Superior Parietal	Superior, Middle, and Inferior Temporal	Lateral Occipital
Rostral and Caudal Middle Frontal	Inferior Parietal	Fusiform	Lingual
Pars Opercularis, Pars Triangularis, Pars Orbitalis	Supramarginal	Transverse Temporal	Cuneus
Lateral and Medial Orbitofrontal	Postcentral	Entorhinal	Pericalcarine
Precentral	Precuneus	Parahippocampal	
Paracentral	Posterior Cingulate		
Rostral Anterior Cingulate	Isthmus Cingulate		
Caudal Anterior Cingulate			

Lobar volumes were calculated as the sum of regional volumes from both hemispheres. Beyond the four lobes, insula was also investigated as the fifth hemispheric lobe of the brain (18). Insular volume was defined as the sum of left and right insular volumes and mean insular thickness was calculated by averaging the thickness of the left and right insula. Cortical thicknesses and volumes were calculated from both hemispheres because of the following reasons: (i) we had no

hypothesis on lateralized effects of migraine attacks (i.e., no migraine patient with unilateral side-locked headache or migraine aura), (ii) the left and right differences in lobar and insular volumes/thicknesses (i.e., lateralities) were not different among our three groups (see the Results section), (iii) we hypothesized a similar degree of white matter damage in the two hemispheres based on an earlier study reporting no differences between the left and right hemispheres in the number of WML, total WML volume, and average WML size in migraine (19).

3.2.4 Statistical analysis

Statistical analyses were performed using SPSS 20.0 software (IBM Corp., Armonk, NY). Differences in age between the whole migraine group and healthy controls were assessed by the Mann-Whitney U-test. Age differences among migraine subgroups (L+ and L-) and the healthy control group were assessed by Kruskal–Wallis test followed by pair-wise comparison using the Mann–Whitney U-test. Continuous migraine-related variables (i.e., disease duration, migraine attack frequency, total number of migraine attacks) were compared between migraine subgroups (L+ and L-) via the Mann–Whitney U-test, while differences in the rate of aura between the same subgroups were assessed using Fisher's exact test. The left and right differences in lobar and insular volumes/thicknesses (i.e., lateralities of volumes/thicknesses) were compared between our three groups (L+, L-, control) using analysis of variance (ANOVA). Cortical thickness and volume differences between the three groups (L+, L-, control) were assessed using analysis of covariance (ANCOVA) with age as covariate for the thickness, and age and total intracranial volume (ICV) as covariates for the volume. Concentrating on the whole migraine group, the possible effects of migraine characteristics (i.e., disease duration, migraine attack frequency, total number of migraine attacks, aura) on cortical thickness and volume were tested using stepwise multiple linear regression analyses. In these models age (for both thickness and volume) and ICV (for volume)

were also included as possible predictors. Concentrating on migraine subgroup with lesion (L+), we assessed the potential effects of lesion number/volume on lobar and insular thicknesses/volumes. Unfortunately, all of our total and lobar lesion number/volume data were definitely not normally distributed (right skewed with several extreme values). In order to perform powerful statistical analyses, L+ group was divided into patients with mild and patients with moderate-severe lesions. The division was performed based on the median split of whole brain as well as each lobar lesion number/volume, thereby creating binarized subgroup variables: e.g., subgroup with low (below or equal to the median) vs. subgroup with high number of frontal lobe lesions. Since lesions were rare in the occipital lobe with a median lesion number of 0, the division based on lesion number/volume of this lobe was not performed. The effects of these subgroup variables on the insular and lobar thicknesses/volumes were tested using stepwise multiple linear regression analyses, including subgroup variables from the whole brain (for both insular and lobar thicknesses and volumes) and from the same lobe as that of dependent variable (for the lobar thicknesses volumes), age (for both thicknesses and volumes) and ICV (for volumes) as possible predictors. The level of statistical significance was set as < 0.05.

3.3 Results

There was no significant difference in age between the whole migraine (including both L+ and L- patients) and the control groups ($P = 0.738$). The Kruskal–Wallis test revealed significant age differences among migraine subgroups (L+ and L-) and controls ($P = 0.001$). Post-hoc testing indicated that L+ subgroup was significantly older than the L- ($P = 0.0003$) and the control ($P = 0.018$) groups (Table 1). Disease duration ($P = 0.003$), the total number of migraine attacks ($P = 0.022$) and the rate of aura ($P = 0.0003$) were also significantly higher in L+ patients than in L- patients (Table 1).

The left and right differences in lobar and insular volumes/thicknesses (i.e., lateralities) were not different among our groups (L+, L-, control); P = 0.626, 0.965, 0.425, 0.859 and 0.989 for the frontal, parietal, temporal, occipital and insular thicknesses and P = 0.598, 0.252, 0.855, 0.732 and 0.136 for the frontal, parietal, temporal, occipital and insular volumes, respectively. Cortical thickness and volume measurements of the five lobes were not statistically different among our three groups (L+, L-, control), (Table 3.).

Table 3. *Group differences in cortical thickness and volume*

	Groups			ANCOVA test					
				Group effect		Age effect		ICV effect	
	Lesion+	Lesion-	Control	F	P	F	P	F	P
Frontal thickness (mm)	2.51 (0.11)	2.55 (0.09)	2.51 (0.10)	1.41	0.246	63.12	<0.001[a]	-	-
Parietal thickness (mm)	2.23 (0.11)	2.26 (0.11)	2.26 (0.11)	0.51	0.603	72.13	<0.001[a]	-	-
Temporal thickness (mm)	2.81 (0.11)	2.83 (0.11)	2.80 (0.09)	0.92	0.401	35.91	<0.001[a]	-	-
Occipital thickness (mm)	1.90 (0.10)	1.92 (0.09)	1.89 (0.08)	0.81	0.445	28.65	<0.001[a]	-	-
Insula thickness (mm)	3.05 (0.14)	3.09 (0.14)	3.06 (0.12)	0.23	0.796	29.35	<0.001[a]	-	-
Frontal volume (mm^3)	158308 (16892)	163774 (15889)	161019 (15212)	0.34	0.713	83.22	<0.001[a]	92.04	<0.001[b]
Parietal volume (mm^3)	103341 (11878)	106370 (10566)	106016 (8878)	0.11	0.897	70.13	<0.001[a]	69.83	<0.001[b]
Temporal volume (mm^3)	98752 (9514)	102308 (8639)	100105 (8294)	1.71	0.184	36.60	<0.001[a]	78.76	<0.001[b]
Occipital volume (mm^3)	40789 (5254)	42196 (4531)	40721 (4414)	1.23	0.294	33.85	<0.001[a]	42.13	<0.001[b]
Insula volume (mm^3)	10937 (1066)	11039 (994)	10938 (995)	0.28	0.758	24.25	<0.001[a]	27.53	<0.001[b]

Lesion+: Migraine patients with lesions; Lesion-: Migraine patients without lesions; ICV: total intracranial volume; All thicknesses/volumes were excluded from the analysis, where the corresponding standardized residuals from the ANCOVA model were below -3 or above 3. Maximum three subjects had to be excluded from each group. Thicknesses and volumes are presented as uncorrected mean (standard deviation); [a]negative (inverse) association with thickness/volume, [b]positive association with volume

For both thickness and volume, the mean differences between our groups and the Bonferroni corrected 95% confidence intervals of mean differences are presented in Table 4.

Table 4. *Differences in marginal means and 95% confidence intervals*

	Mean Difference[a] (95% Confidence Interval for Difference[b])		
	L+ minus L-	L+ minus C	L- minus C
Frontal thickness (mm)	-0.0002 (-0.037 to 0.037)	0.026 (-0.019 to 0.072)	0.027 (-0.013 to 0.066)
Parietal thickness (mm)	0.016 (-0.024 to 0.056)	0.006 (-0.044 to 0.055)	-0.010 (-0.053 to 0.032)
Temporal thickness (mm)	0.010 (-0.033 to 0.052)	0.029 (-0.023 to 0.082)	0.019 (-0.026 to 0.065)
Occipital thickness (mm)	0.002 (-0.033 to 0.038)	0.021 (-0.023 to 0.064)	0.018 (-0.020 to 0.056)
Insula thickness (mm)	-0.004 (-0.059 to 0.051)	0.012 (-0.055 to 0.080)	0.016 (-0.042 to 0.075)
Frontal volume (mm^3)	-1217 (-5905 to 3470)	200 (-5539 to 5938)	1417 (-3527 to 6361)
Parietal volume (mm^3)	-270 (-3560 to 3020)	-767 (-4795 to 3260)	-497 (-3967 to 2972)
Temporal volume (mm^3)	-1944 (-4803 to 915)	-336 (-3836 to 3164)	1608 (-1407 to 4623)
Occipital volume (mm^3)	-422 (-2073 to 1229)	704 (-1315 to 2724)	1126 (-616 to 2868)
Insula volume (mm^3)	84 (-290 to 459)	139 (-323 to 600)	54 (-346 to 455)

L+: Migraine patients with lesions; L-: Migraine patients without lesions; C: Control subjects
Differences in marginal means are presented as mean (95% confidence interval for the difference)
[a] Based on estimated marginal means (adjusted for age in case of thickness; adjusted for age and ICV in case of volume)
[b] Adjustment for multiple comparisons: Bonferroni

Age showed a significant negative association with both thickness and volume in each examined lobe ($P < 0.001$). ICV showed a significant positive association with the volumes of all regions ($P < 0.001$). There were no significant group*age, group*ICV or age*ICV interactions in the performed analyses.

In the whole migraine group, none of the migraine characteristics were selected by stepwise linear regression as significant predictors of cortical thickness or volume. Only age (for both thickness and volume) and ICV (for volume) were identified as significant predictors ($P < 0.001$). Focusing on the L+ patients, none of the binarized total or lobar lesion number/volume variables

were selected by stepwise linear regression as significant predictors of the insular or lobar thicknesses/volumes. The main features of WMLs in the L+ group are presented in Table 5.

Table 5. Location, number and size of WMLs in the migraine group with lesions

Location	Number of WMLs	Total WML volume (mm^3)
Frontal lobe	6.5 (2-11)	168.4 (66.0-447.1)
Parietal lobe	2 (0-7)	54.2 (0-318.5)
Temporal lobe	0.5 (0-2)	8.9 (0-42.3)
Occipital lobe	0 (0-1)	0 (0-22.2)
Whole brain	10.5 (3-19)	338.0 (119.1-963.2)

WML: white matter lesion; The number and volume of lesions failed the Shapiro-Wilk normality test, thus values are presented as median (25^{th} -75^{th} percentile); Total WML volume was calculated by the sum of individual WML volumes in the given lobe.

3.4 Discussion

In this study, we investigated a homogeneous (female migraineurs without medical comorbidities) migraine group to explore the potential effects of WMLs and migraine characteristics on cortical lobar thickness and volume. The WMLs and clinical characteristics failed to show any effects on the lobar cortical measures. When the lesion + group was divided into two subgroups by median split of total and lobar lesion number and volume, the cortical measurements (thickness and volume) did not show any significant difference between the groups with low vs. high lesion number/volume by stepwise linear regression. Only age and ICV proved to be significant predictors; the former for both cortical thickness and volume, while the latter for cortical volume.

The lack of the impact of WMLs on cortical thickness and volume may be the consequence of the not reaching the critical size of injured white matter territory, the intralobar separations of lesions with differences in distributions, or less severe intralesional tissue damage. Although the

clinically silent brain WMLs are predominantly progressive in nature, smaller lesions may improve in size or even disappear (10). In addition, the normal-appearing white matter (NAWM) did not show MRI signs of tissue injury in migraine patients with or without WML (9).

The negative association of age with cortical thickness and volume raises the possibility that WMLs are age-related. WMLs can develop at any age during the active migraine years, and their presence does not correlate with age (20-23). Disease duration and attack frequency are the main indicators for brain damage in migraine (22,24). Usually, ageing associates with longer disease duration and higher lifetime attack number, and these factors may increase the risk of oxidative stress-related endothelial injury and atherosclerosis (11). Furthermore, a wide range of vascular risk factors contribute to lesion formation (22,25). In the present study, both migraineurs and controls lacked any medical comorbidity, thus the role of ageing in lesion development is less likely.

Previous studies presented several different cortical regions with morphological abnormalities, sometimes with contradictory findings. Due to differences in study aims, morphometric MRI studies, analytical approaches and number of study participants, our results are not directly comparable to earlier studies (6-8, 26-36).

Some of these studies used a voxel-based morphometry (VBM) approach (7,8,26,27,29,32,36), which analyses the brain on a voxel by voxel basis, while our approach examines brain changes at a larger territory level (i.e., lobar level). In addition, several other methodological differences exist between VBM and our surface-based analysis. VBM does not distinguish between different cortical morphological properties and various methodological factors, including cortical thickness, surface area, cortical volume, gyrification pattern, T1 signal alterations within a physiologic range, registration artefacts and smoothing (37-39). Moreover, the significant group differences reported by a VBM analysis are not necessarily homogenous in terms

of the underlying factors (37). Based on these differences, VBM and our method should be considered as complementary techniques (38).

Others used surface-based analysis to examine local cortical thickness changes in migraine (6,28,30,31,33-35). Most of these studies were not interested in the direct thickness differences between migraineurs and controls, but rather examined between-hemisphere cortical differences related to the headache side (6), interregional cortical thickness correlations to differentiate groups of migraine patients from healthy controls (34), and differences in cortical thickness-to-pain threshold correlations between migraineurs and controls (33). Another study reported significantly decreased left anterior midcingulate cortical thickness in the migraine group, but the number of subjects was low (17 migraine patients vs. 18 controls) (31). From the largest studies, one reported no significant cortical thickness differences at all (28), while the other found significant differences between migraineurs and controls in several small brain regions (30). The latter should be interpreted in the context of statistically controlling for the whole-hemisphere average cortical thickness during vertex-wise statistical analysis of thickness. Since the overall average cortical thickness was significantly increased in the migraineurs, such correction may add noise and provide inaccurate data. A vertex-based study conducted on females found higher cortical thickness in the superior frontal gyrus, paracentral gyrus, temporal pole, precuneus and lower cortical thickness in the anterior cingulate of migraineurs (25). Interestingly, the authors were unable to detect the well-established cortical atrophy with advancing age (40) for the insula in the migraine group, while we could demonstrate it by measuring both cortical thickness and cortical volume without any group*age interaction. Two recent studies investigated only migraine patients with aura (41, 42). One of them found slightly thicker cortical visual areas in female migraineurs with aura (41), while the other found no solid evidence for cortical thickness differences between patients with aura and

controls (42). The second study found reduced volume of the left fusiform gyrus in migraineurs with aura compared to controls. We conducted a region-of-interest (ROI) surface-based analysis, which has unique strengths and limitations compared to the vertex-wise surface-based method (e.g., ROI analysis does not need smoothing or inter-subject registration, and the problem of multiple comparisons is substantially reduced compared to a vertex-wise comparison). The measurement at a single vertex is often quite noisy, which may reduce the statistical power (43). However, if the structural differences cross the boundaries of the predefined ROIs, then only the vertex-wise approach may find them (44). Moreover, the vertex-wise approach doesn't need an a priori hypothesis. From the above cited studies, only Datta et al. (28) and Gaist at al. (41) used a similar approach to our one, and although the results of Datta et al. (28) were also negative, the investigated ROIs were quite different in both of these studies compared to the present study.

In one study the cortical thickness measures by Freesurfer agreed to those obtained using traditional neuropathologic techniques within 0.2 mm (with a mean difference of 0.077 mm) (45); in another study the accuracy was better than 0.5 mm compared to manual measures on MRI data (46), while test-retest within-scanner error in local cortical thickness measurement was found to be about 0.12 mm in average (47). Based on the order of these numbers, in the present study, only cortical thickness differences of at least 0.1 mm was considered scientifically relevant. Since our Bonferroni corrected 95% confidence intervals of the cortical thickness differences were within the range of -0.059 to +0.082 mm (Table 4) for each lobe and each subgroup comparison, equivalence testing suggests that mean lobar cortical thicknesses of all three groups are equivalent within the pre-defined practically relevant limits (i.e., ± 0.1 mm).

3.4.1 Strengths and limitations

The main strengths of our study include the relatively large single centre sample size,

assessing both cortical thickness and volume, and performing equivalence testing. Our study was cross-sectional, where the detection of subtle cortical changes is hindered by inter-individual differences. Longitudinal studies eliminating much of these differences are also needed to further support that no cortical changes occur in migraine. Instead of searching for very subtle structural differences, our main goal was to examine whether migraine lesions cause thickness/volume changes at the level of larger cortical territories (i.e., lobar level). This approach is useful to detect more robust lobar changes even if there are individual variations of morphometric changes within the lobes. However, we acknowledge that very subtle structural changes with consistent anatomical locations can exist, which may be detected by vertex-wise analysis or more detailed ROI analysis, while such abnormalities may be averaged out and overlooked when examining at the lobar level.

3.5 Conclusions

In summary, we investigated a female migraine group, and found that neither the lesions nor other clinical characteristics have a detectable effect on cortical thickness and volume of bilateral intracerebral lobes. Cortical thicknesses were equivalent within the range of ± 0.1 mm. Only age and ICV proved to be significant predictors; the former for both cortical thickness and volume, while the latter for cortical volume.

3.6 New Findings

This is the first published study with oxidative stress in migraine-free period in lesional migraine patients. The elevated ADMA levels in lesional migraineurs may indicate a role of migraine-related vascular endothelial dysfunction in the development of WMLs, supporting the ischemic injury theory of WML formation. These findings indicate that elevated ADMA concentrations may be a risk factor for clinically silent brain WMLs and point out the necessity of therapeutic interventions. SDMA may also have a role in lesion formation by indirect inhibition of NO bioavailability. The higher L-arginine serum concentrations might reflect an increased demand for NO synthesis in migraine. Migraine may be a systemic factor of vascular endothelial injury during the active headache years.

We investigated a female migraine group and found that neither the lesions nor other clinical characteristics have a detectable effect on cortical thickness and volume of bilateral intracerebral lobes. Only age and ICV proved to be significant predictors; the former for both cortical thickness and volume, while the latter for cortical volume. These are good news for migraineurs.

4 Acknowledgement

„There are two things parents should give their children roots and wings. Roots to give them bearing and a sense of belonging, but also wings to help free them from constraints and prejudices and give them other ways to travel (or rather, to fly).”

(Johann Wolfgang von Goethe)

The value and quality which were given to me during my personal and professional development as basic values are the grounding of my everyday life. The effects of the impulses of the countless personal relationships and contacts form me day by day.

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Zoltán Pfund M.D., Ph.D. besides our working relationship directed my thinking towards scientific activities.

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5 Publications

5.1 Related to the Thesis

1. Influence of hemispheric white matter lesions and migraine characteristics on cortical thickness and volume. **Komáromy H**, He M, Perlaki G, Orsi G, Nagy SA, Bosnyák E, Kamson Olayinka D, John F, Trauninger A, Pfund Z. J Headache Pain. 2019 Jan 10;20(1):4. doi: 10.1186/s10194-019-0959-2. Impact Factor (2018): 3.403

2. Serum L-arginine and dimethylarginine levels in migraine patients with brain white matter lesions. Erdélyi-Bótor S, **Komáromy H**, Kamson DO, Kovács N, Perlaki G, Orsi G, Molnár T, Illes Z, Nagy L, Kéki S, Deli G, Bosnyák E, Trauninger A, Pfund Z. Cephalalgia. 2017 May; 37(6):571-580. doi: 10.1177/0333102416651454. Impact factor: 3.882

5.2 Other Publications Related to the Topic of Headache

3. Risk factors of migraine-related brain white matter hyperintensities: an investigation of 186 patients. Trauninger A, Leél-Ossy E, Kamson DO, Pótó L, Aradi M, Kövér F, Imre M, **Komáromy H**, Erdélyi-Botor S, Patzkó A, Pfund Z. *J Headache Pain.* 2011 Feb;12(1):97-103. doi: 10.1007/s10194-011-0299-3. **Impact Factor: 2.427**

5.3 Other Publications

4. Intrauterine diagnosis and pathology of fetal choroid plexus carcinoma--a case study. Joó GJ, Reiniger L, Papp C, Csaba A, **Komáromy H**, Rigó J *Jr. Pathol Res Pract.* 2014 Dec; 210(12):1156-9. doi: 10.1016/j.prp.2014.01.009. **Impact Factor: 1,397**

5. A statistical model for intervertebral disc degeneration: determination of the optimal T2 cut-off values. Nagy SA, Juhasz I, **Komaromy H**, Pozsar K, Zsigmond I, Perlaki G, Orsi G, Schwarcz A, Walter N, Doczi T, Bogner P. *Clin Neuroradiol.* 2014 Dec; 24(4):355-63. doi: 10.1007/s00062-013-0266-2. **Impact Factor: 2,25**

6. Unfavorable outcome of aggressive lowering of high blood pressure. Case report. Kuperczkó D, Csécsei P, **Komáromy H**, Szapáry L, Fehér G. *Orv Hetil.* 2014 Oct 19;155(42):1685-9. doi: 10.1556/OH.2014.30011. **Impact Factor:** NA

7. Multi-modal magnetic resonance imaging in the acute and sub-acute phase of mild traumatic brain injury: can we see the difference? Toth A, Kovacs N, Perlaki G, Orsi G, Aradi M, **Komaromy H**, Ezer E, Bukovics P, Farkas O, Janszky J, Doczi T, Buki A, Schwarcz A. *J Neurotrauma.* 2013 Jan 1; 30(1):2-10. doi: 10.1089/neu.2012.2486 **Impact Factor: 3,968**

8. Bi-exponential diffusion signal decay in normal appearing white matter of multiple sclerosis. Nagy SA, Aradi M, Orsi G, Perlaki G, Kamson DO, Mike A, **Komaromy H**, Schwarcz A, Kovacs A, Janszky J, Pfund Z, Illes Z, Bogner P. *Magn Reson Imaging.* 2013 Feb; 31(2):286-95. doi: 10.1016/j.mri.2012.07.007. **Impact Factor: 2,022**

9. Aspirin resistance in cerebrovascular patients. Feher A, Pusch G, Harang G, Gasztonyi B, Papp E, Werling D, Menyhart M, **Komaromy H**, Szapary L, Feher G. *Int J Cardiol.* 2011 Oct 6;152(1):111-2. doi: 10.1016/j.ijcard.2011.07.028. **Impact Factor:** N/A (letter)

10. Anti-NMDA-receptor encephalitis: description of the syndrome in connection with the first Hungarian patient. Hollódy K, Csábi G, Láng A, Rózsai B, **Komáromy H**, Bors L, Illés Z. *Ideggyogy Sz.* 2011 Mar 30;64(3-4):119-25. **Impact Factor: 0,488**

11. Transverse myelitis as a rare, serious complication of Mycoplasma pneumoniae infection. Csábi G, **Komáromy H**, Hollódy K. *Pediatr Neurol.* 2009 Oct; 41(4):312-3. doi: 10.1016/j.pediatrneurol.2009.04.029. **Impact Factor: 1,497**

12. Perifériás motoros tünettannal társuló kórképek. Deli G, **Komáromy H**, Pál E, Pfund Z. *Ideggyógyászati Szemle/Clinical Neuroscience* 2019. **Impact Factor: 0.252**

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6.1 Introduction

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6.2 Serum L-arginine and Dimethylarginine Levels in Migraine Patients with Brain White Matter Lesions

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6.3 Influence of Hemispheric White Matter Lesions and Migraine Characteristics on Cortical Thickness and Volume

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