Cerebrospinal fluid and plasma amine profiles in interictal migraine

Onderwater, G.L.J.; Dongen, R.M. van; Harms, A.C.; Zielman, R.; Oosterhout, W.P.J. van; Klinken, J.B. van; ... ; Ferrari, M.D.

Cerebrospinal Fluid and Plasma Amine Profiles in Interictal Migraine

Gerrit L. J. Onderwater, MD, PhD,[1#] Robin M. van Dongen, MD, PhD,[1#] Amy C. Harms, PhD,[2] Ronald Zielman, MD, PhD,[1] Willebrordus P. J. van Oosterhout, MD, PhD,[1] Jan B. van Klinken, PhD,[3,4] Jelle J. Goeman, PhD,[5] Gisela M. Terwindt, MD, PhD,[1] Arn M. J. M. van den Maagdenberg, PhD,[1,3] Thomas Hankemeier, PhD,[2] and Michel D. Ferrari, MD, PhD, FANA, FRCP[1]

Objective: Impaired amine metabolism has been associated with the etiology of migraine, that is, why patients continue to get migraine attacks. However, evidence from cerebrospinal fluid (CSF) is lacking. Here, we evaluated individual amine levels, global amine profiles, and amine pathways in CSF and plasma of interictal migraine patients and healthy controls.
Methods: CSF and plasma were sampled between 8:30 AM and 1:00 PM, randomly and interchangeably over the time span to avoid any diurnal and seasonal influences, from healthy volunteers and interictal migraine patients, matched for age, sex, and sampling time. The study was approved by the local medical ethics committee. Individual amines (n = 31), global amine profiles, and specific amine pathways were analyzed using a validated ultraperformance liquid chromatography mass spectrometry platform.
Results: We analyzed n = 99 participants with migraine with aura, n = 98 with migraine without aura, and n = 96 healthy volunteers. Univariate analysis with Bonferroni correction indicated that CSF L-arginine was reduced in migraine with aura (10.4%, $p < 0.001$) and without aura (5.0%, $p = 0.03$). False discovery rate-corrected CSF L-phenylalanine was also lower in migraine with aura (6.9%, $p = 0.011$) and without aura (8.1%, $p = 0.001$), $p = 0.088$ after Bonferroni correction. Multivariate analysis revealed that CSF global amine profiles were similar for both types of migraine ($p = 0.64$), but distinct from controls ($p = 0.009$). Global profile analyses were similar in plasma. The strongest associated pathways with migraine were related to L-arginine metabolism.
Interpretation: L-Arginine was decreased in the CSF (but not in plasma) of interictal patients with migraine with or without aura, and associated pathways were altered. This suggests that dysfunction of nitric oxide signaling is involved in susceptibility to getting migraine attacks.

ANN NEUROL 2023;93:715–728

Migraine is a common brain disorder, characterized by disabling attacks of headache and associated symptoms for up to 3 days.[1,2] In one third of patients, attacks may include aura.[3] Women are affected 3 times more often.[1–3] Objective diagnostic biomarkers are lacking.[2] Median attack frequency is 1.5 per month, and one quarter

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26576

Received Mar 1, 2022, and in revised form Nov 18, 2022. Accepted for publication Dec 7, 2022.

Address correspondence to Dr Ferrari, Department of Neurology, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands. E-mail: m.d.ferrari@lumc.nl

[#]G.L.J.O. and R.M.v.D. contributed equally.

From the [1]Department of Neurology, Leiden University Medical Center, Leiden, the Netherlands; [2]Division of Analytical Biosciences, Leiden Academic Center for Drug Research, Leiden, the Netherlands; [3]Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands; [4]Department of Clinical Chemistry, Laboratory Genetic Metabolic Diseases, Amsterdam University Medical Center, Amsterdam, the Netherlands; and [5]Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, the Netherlands

Additional supporting information can be found in the online version of this article.

of patients experience weekly attacks.[3] Genetic and non-genetic risk and susceptibility-modifying factors are involved.[4] Although the mechanisms of migraine *attacks* are reasonably well understood,[5,6] little is known about the pathogenesis of migraine *disease*, that is, the tendency to have recurrent migraine attacks.[1,4] This has hindered the development of a causal prophylactic therapy that prevents the initiation and occurrence of migraine attacks. Even the recently emerging calcitonin gene-related peptide (CGRP) inhibitors have limited efficacy, probably because they most likely mainly act at the end of the initiation cascade of the migraine attack rather than at the beginning.[6,7]

Unraveling the neurochemistry of migraine could help elucidate its etiology and the reason people get recurrent attacks.[4,8] Most biochemical studies in migraine have focused on blood and urine.[5,9] Although these fluids are easier to collect, they reflect systemic rather than cerebral changes.[10] Cerebrospinal fluid (CSF) could better reflect brain neurochemistry,[11,12] but its sampling is hampered by logistical and ethical issues.

More than 70 metabolites from different molecular classes such as neurotransmitters, neuropeptides, cytokines, hormones, and ions have been measured in the CSF of migraine patients.[9,13] Several amines such as glutamate, glutamine, γ-aminobutyric acid (GABA), and serotonin have been implicated in the pathogenesis of migraine.[4,9,14] However, glutamate was the only amine measured in the CSF of migraineurs in 3 or more different studies and was found to be elevated during the ictal phase and in the chronic form of migraine.[9]

Studies in the CSF of migraineurs were small and had significant chemical detection and clinical methodological limitations. Details of the measurement methods used and their validation were often reported only to a limited extent.[9] Moreover, only a single molecule was usually measured rather than several simultaneously, and in only a limited number of cases were multiple molecules measured simultaneously in both CSF and plasma.[9] This prevented a more detailed analysis of the cerebral and systemic profiles and pathways. In addition, controls were rarely matched for important confounding factors such as sex, age, and diurnal and seasonal timing of sampling.[9] They were also rarely truly healthy, as control CSF was usually obtained from lumbar punctures in people with neurological symptoms to exclude neurological disease.[9] Finally, many studies compared CSF collected between attacks only with CSF collected during attacks, but not with CSF obtained from healthy controls.[9] Thus, these studies focused on changes occurring during and mechanisms of the *attack* rather than on the pathogenetic mechanisms of migraine *disease*, to identify mechanisms for why people get recurrent migraine attacks.[9]

In our search for mechanisms of susceptibility to getting migraine attacks, we investigated whether amine metabolism is impaired in people with migraine outside of attacks. To this end, we determined and compared the levels of 31 amines and their global profiles and pathways in CSF and plasma of n = 99 interictal patients with migraine with aura, n = 98 with migraine without aura, and n = 96 healthy volunteers. Healthy volunteers had no personal or family history of migraine. The 3 groups were matched for age, sex, and diurnal and seasonal timing of sampling.

Subjects and Methods

Study Design and Participants

We enrolled patients with migraine with or without aura[2] and healthy controls who were group-matched for sex and age by adhering to 5-year age strata; age ranged from 18 to 69 years. Patients were diagnosed according to the International Classification of Headache Disorders criteria (ICHD3) by experienced physicians (supervised by G.M.T. and M.D.F.).[2] For the first few years, we used the ICHD2 version without essential differences between patients.[15] Participants were recruited from the LUMINA (Leiden University Medical Center Migraine Neuro Analysis) project, which includes migraine patients and healthy controls from the general Dutch population who agreed to participate in migraine-related scientific research. Some controls and patients were recruited through a public advertisement or through the Leiden University Medical Center Headache Clinic. Migraineurs did not use acute migraine drugs on more than 8 days per month. CSF and plasma were collected interictally when patients had been attack-free for ≥3 days. Sampling occurred between April 2008 and May 2016. Healthy volunteers had no obvious signs or symptoms of a disease and had no history of headache (except for infrequent tension-type headaches) or other pain syndromes. To exclude any underlying genetic risk, they also did not have first-degree relatives with migraine or trigeminal autonomic cephalalgia. Participants did not have a severe psychiatric disorder, nor a history of oncological disease, or a contraindication for lumbar puncture (signs and symptoms of increased intracranial pressure, local skin infection, or a coagulopathy including use of anticoagulant drugs or platelet inhibitors). The study was conducted according to the criteria of the Declaration of Helsinki and approved by the Leiden University Medical Center institutional ethics committee. All participants provided written informed consent prior to participation and received financial compensation according to standard fees for participation in similar studies.

Sample Collection

Patients and controls were sampled, after standard neurological examination, between 8:30 AM and 1:00 PM and in random order and interchangeably over the time span to avoid diurnal and seasonal variation. They abstained from eating or drinking, other than water, for at least 8 hours prior to sampling to minimize the influence of food or drinks. CSF was sampled by lumbar

puncture in the L3/L4, L4/L5, or L5/S1 interspace. Intracranial pressure was measured, and 3.0ml CSF was sampled for routine diagnostics (cell count, glucose and total protein levels). Next, 3.8ml of CSF was sampled directly in a 15ml polypropylene Falcon tube prechilled on ice and centrifuged at 4°C for 5 minutes (2,000rpm, 747 × *g*). The supernatant was transferred into a new chilled 15ml polypropylene Falcon tube, inverted several times, and divided into 0.5ml aliquots (1.8ml cryotubes) that already contained 1.0ml of cold ethanol. Cryotubes were inverted several times to mix CSF and ethanol. Samples were placed on dry ice within 30 minutes of sampling and stored at −80°C within 60 minutes. Blood was collected from the median cubital vein in ethylenediaminetetraacetic acid plasma tubes, immediately after lumbar puncture, and centrifuged at 21°C for 20 minutes (2,000rpm, 622 × *g*). The supernatant was transferred to a new 15ml polypropylene Falcon tube, inverted several times, and divided into 0.5ml aliquots (1.0ml Nunc [Rochester, NY] cryotubes). Plasma samples were stored at −80°C within 60 minutes from sampling. All CSF and plasma samples remained at −80°C until sample preparation; no extra freeze–thaw cycles were allowed. See Figure S1 for detailed information on sample processing. To monitor possible complications, participants were followed for 3 days, or longer if necessary.

Amine Measurements

Amines were measured with an ultraperformance liquid chromatography mass spectrometry (UPLC-MS) method specifically developed and validated for amine profiling.[16] Samples were randomized across 5 CSF batches and 5 plasma batches. All batches included calibration lines, blanks, and quality control samples. Blanks were used to subtract background levels from study samples. Quality control samples were analyzed every 10 samples and were used to monitor data quality and to correct for instrument response.[17]

For the analysis of CSF, 30µl of CSF/ethanol sample was spiked with an internal standard solution. For the analysis of plasma, 5µl of starting material was used. For the analysis of amino acids, $^{13}C^{15}N$-labeled analogues were used.[16] For other amines, the closest-eluting internal standard was added. The target list is included as Table S1. After spiking, proteins were precipitated by the addition of methanol and the mixture was vortexed for 10 seconds and centrifuged (9,400 × *g* for 10 minutes at 10°C). The supernatant was transferred to a new Eppendorf tube and taken to dryness in a Speedvac. The residue was reconstituted in borate buffer (pH 8.5) with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate reagent. Next, vials were transferred to an autosampler tray and cooled to 4°C until injection, when 1.0µl of the reaction mixture was injected into the UPLC-MS system. Chromatographic separation was achieved by an ACQUITY UPLC system (Waters, Milford, MA) on an AccQ-Tag Ultra column (Waters) with a flow of 0.7ml/min over a 11-minute gradient. The UPLC was coupled to electrospray ionization on a triple quadrupole mass spectrometer (Qtrap 6,500; AB SCIEX, Nieuwekerk aan den Ijssel, the Netherlands). Analytes were detected in the positive ion mode and monitored by multiple reaction monitoring (MRM) using nominal mass resolution. Acquired data were evaluated using MultiQuant Software for Quantitative Analysis (v3.0.2, AB SCIEX), by integration of assigned MRM peaks and normalization using internal standards. Unit-free concentrations are reported in this paper (ie, relative response ratios, calculated as "amine target area"/"internal standard area").

Statistical Analysis

Amine concentrations were first corrected based on quality control samples.[17] Only metabolites with relative standard deviations of quality controls below 15% were included in further analyses. Outlier detection was performed using principal component analysis with a 99% confidence interval (Fig S2). Log-transformed concentrations were used for further statistical analyses.

Univariate. To detect univariate metabolite differences, analysis of covariance was performed with age and sex as covariates. Bonferroni was used for multiple testing correction, separately for CSF (30 tests) and plasma (31 tests). Additionally, we analyzed the data using the less conservative false discovery rate (FDR). For significant metabolites ($p < 0.05$ after multiple testing correction) post hoc comparisons were tested according to Shaffer's method.[18]

Multivariate, Global Amine Profiles and Specific Pathways. To detect multivariate differences in global metabolite profiles or amine pathways, the global test approach[19] was used (separately for CSF and plasma). First, global amine profiles of the 3 groups were compared with age and sex as covariates. Second, pathways were tested. We used MetaboAnalyst to obtain Kyoto Encyclopedia of Genes and Genomes (KEGG) identifiers[20] from the 31 amines and subsequently downloaded KEGG metabolite set pathway definitions from ConsensusPathDB.[21] Because most KEGG pathways were not covered in a coherent fashion by the amines measured on our platform, we filtered the metabolite sets by eliminating those amines that were disconnected from the rest in the pathway diagram. Specifically, we computed a metabolic interaction network of the 31 amines from a curated version of the human Genome-Scale Metabolic Model (GSMM) Human Metabolic Reaction 2.0,[22] using a generic path-finding algorithm that was developed in house. Only biochemical paths of one or two reaction steps were considered, and each path was checked for stoichiometric and thermodynamic consistency. In addition, only substrate–product mappings were considered that involved the transfer of carbon-based moieties, except for S-adenosylmethionine, which was considered a hub metabolite. As a consequence, half-reactions involving the transfer of electrons, amino or phosphate groups were decoupled from the main reaction in the path-finding

procedure. For example, in the reaction nicotinamide adenine dinucleotide (NAD)H + pyruvate <=> NAD+ + lactate, only NADH and NAD+ are linked in the network, and pyruvate and lactate are linked. Likewise, in the reaction glutamate + pyruvate <=> α-ketoglutarate + alanine, only glutamate and α-ketoglutarate are linked, and pyruvate and alanine are linked. In this way, we prevented the creation of a crowded, highly connected network in which all amines are interconnected via a few hub metabolites such as H+, H2O, adenosine triphosphate (ATP), and NADH. Finally, transporter reactions that transferred compounds over the cellular membranes and (half) reactions that involved uniquely produced metabolites were given a weight of zero in the path-finding procedure. Computations were performed in MATLAB 2019b (MathWorks, Natick, MA).

Network analysis of the GSMM resulted in 2 larger connected networks of 9 and 10 amines, respectively, and 2 smaller networks of only ethanolamine and o-phosphoethanolamine and of phenylalanine, tyrosine, and 3-methoxytyrosine. Overlapping the network with the KEGG metabolite sets and eliminating disconnected amines resulted in 10 independent pathways that contained a subnetwork of 3 amines or more:

1. Cysteine and methionine metabolism
2. Glycine, serine, and threonine metabolism
3. Lysine degradation
4. GABAergic synapse (same set definition as alanine, aspartate, and glutamate metabolism)
5. Arginine and proline metabolism (glutathione metabolism is a subset of this pathway)
6. Arginine biosynthesis
7. Aminoacyl-tRNA biosynthesis (same set definition as protein digestion and absorption)
8. Central carbon metabolism in cancer
9. Mineral absorption
10. ATP-binding cassette transporters

After inspection of the new list of metabolite sets, we decided to exclude the last 4 pathways, because they involved unspecific biological processes that were not relevant for this study. To test whether the defined pathways differed between the 3 groups, we used the global test function with the pathways as subsets and age and sex as covariates. Bonferroni was used to correct for the final number of pathways (n = 6).

Finally, to determine whether amine profiles could predict diagnosis, a logistic regression model was developed. Migraine diagnosis was set as dependent variable and amines, age, and sex were used as predictors. Overfitting of the model was prevented using L2 ridge penalization.[23] Predictions resulted from cross-validation. Statistical analyses were done with R (v3.4.1), R packages global test (v5.30.0) and penalized (v0.9-50), and Statistical Package for the Social Sciences (v23). Two-sided hypothesis testing was used.

Results

Study Population

We included n = 96 healthy volunteers and n = 197 patients with migraine, n = 99 with aura and n = 98 without aura. After inclusion, n = 5 healthy controls were found to have a first-degree relative with migraine. However, their results were kept in the study because CSF from healthy volunteers is difficult to obtain and a separate analysis of these 5 controls did not yield any abnormal results. In 1 patient with migraine with aura, we were unable to collect plasma. Additionally, 2 outliers were excluded from further data analysis (Fig S2). There were no differences in clinical characteristics between the final study groups (Table 1), except for a higher monthly attack frequency (2.8 ± 2.6) in migraine without aura versus migraine with aura (2.1 ± 1.9, $p = 0.026$). In total, 92 (31.4%) participants developed post-dural puncture headache (fewer in patients with migraine than in controls, 31.0% vs 32.3%; see Table 1[24]), of whom 18 required a blood patch.

Univariate Results

In total, 30 amines were reliably detected in CSF, and 31 in plasma. In CSF, L-arginine levels were different between the 3 groups (Table 2; $p = 0.042$ after Bonferroni correction). Post hoc analysis showed 10.4% lower levels in migraine with aura ($p < 0.001$) and 5.0% lower levels in migraine without aura ($p = 0.027$) versus controls, without difference between migraine subtypes ($p = 0.153$; Fig 1). Because L-arginine is potentially related to cardiovascular status,[25] we repeated the analysis after exclusion of participants with possible cardiovascular comorbidities (n = 12) or antihypertensive medication for migraine prevention (n = 20), but differences remained significant (Table S2). Participants who developed a migraine attack ≤3 days after sampling (and thus potentially were in a preictal phase), did not show different results (Fig S3). The ability of L-arginine to predict migraine was modest, with an area under the curve of 0.657 for migraine with aura and 0.560 for migraine without aura (Fig S4).

None of the other metabolites in CSF differed significantly after Bonferroni correction (see Table 2). Using FDR correction, CSF L-phenylalanine also differed; concentrations were reduced in migraine with aura (6.9%, $p = 0.011$) and without aura (8.1%, $p = 0.001$; Fig S5).

TABLE 1. Clinical Characteristics of Participants

Characteristic	Healthy Controls	Migraine with Aura	Migraine without Aura	*p*
Number of participants[a]	95	98	98	
Subject characteristics				
Females	56 (58.9)	65 (66.3)	60 (61.2)	0.553[b]
Age, yr	38.8 (14.5)	41.7 (13.6)	42.0 (12.9)	0.170[c]
BMI	23.7 (2.8)	24.0 (2.7)	23.6 (2.5)	0.602[d]
Smoking	20 (21.1)	13 (13.3)	13 (13.3)	0.245[b]
Overnight fasting				
Fasting time, h	11.6 (2.4)	11.7 (1.7)	11.9 (1.6)	0.105[c]
Migraine characteristics				
Migraine frequency, attacks/mo	—	2.1 (1.9)	2.8 (2.6)	0.026[e,f]
Headache days, days/mo	0.3 (0.7)	5.2 (4.2)	6.0 (4.9)	<0.001[e,c]
Migraine <3 days after LP				0.168[a]
No	—	85 (86.7)	79 (80.6)	
Yes	—	11 (11.2)	18 (18.4)	
Unknown	—	2 (2.0)	1 (1.0)	
Medication use				
Triptan	—	58 (59.6)	71 (72.4)	0.050[b]
Prophylactic medication	—	17 (17.3)	17 (17.3)	1.000[b]
B-blocker	—	11 (11.2)	9 (9.2)	0.637[b]
Antiepileptic drug	—	4 (4.1)	4 (4.1)	1.000[g]
ACE inhibitors	—	1 (1.0)	1 (1.0)	1.000[g]
Angiotensin II receptor antagonist	—	2 (2.0)	1 (1.0)	1.000[g]
Sampling characteristics				
Opening pressure in mmH_2O	19.1 (4.4)	18.8 (4.0)	18.0 (4.7)	0.250[d]
CSF characteristics				
Erythrocytes, count/3μl	154 (934)	2,299 (20,682)	130 (505)	0.509[c]
Leukocytes, count/3μl	6.0 (6.0)	22.0 (89.0)	5.0 (7.0)	0.587[c]
Protein concentration, g/l	0.35 (0.13)	0.36 (0.25)	0.35 (0.10)	0.702[c]
Glucose, mmol/l	3.2 (0.3)	3.2 (0.3)	3.1 (0.2)	0.738[d]
Post-dural puncture headache[h]				
Cases	31 (32.3)	24 (24.2)	37 (37.8)	0.121
Duration, days	5.35 (3.34)	4.52 (2.8)	4.15 (2.33)	0.206
Blood patch	5 (5.2)	7 (7.0)	6 (6.1)	0.953

Data are presented as n, mean (standard deviation [SD]), or n (%), unless otherwise stated.
[a]Shown for CSF after exclusion of 2 outliers (see Subjects and Methods and Fig S2).
[b]Chi-squared test.
[c]Kruskal–Wallis test.
[d]One-way analysis of variance.
[e]Statistically significant at $p < 0.05$.
[f]Mann–Whitney test.
[g]Fisher exact test.
[h]For these variables, all participants who received an LP were included.
ACE = angiotensin-converting enzyme; BMI = body mass index; LP = lumbar puncture; CSF = cerebrospinal fluid.

TABLE 2. Univariate Analysis Results in Cerebrospinal Fluid

Metabolite	Sex, *p*	Age, *p*	Diagnosis, *p*	Bonferroni	FDR
L-Arginine	0.0002	0.0000	0.0014	0.0422[a]	0.0422[a]
L-Phenylalanine	0.0078	0.0000	0.0029	0.0881	0.0441[a]
L-Asparagine	0.2080	0.0000	0.0101	0.3022	0.0767
Ethanolamine	0.1481	0.5817	0.0102	0.3069	0.0767
L-Methionine	0.0001	0.0000	0.0149	0.4482	0.0896
L-Glutamine	0.0000	0.0000	0.0200	0.5987	0.0998
Taurine	0.0000	0.0000	0.0510	1.0000	0.2519
Ornithine	0.0010	0.0000	0.0598	1.0000	0.2503
L-Homoserine	0.7510	0.0204	0.0860	1.0000	0.5000
L-Tyrosine	0.0169	0.0000	0.0871	1.0000	0.2335
L-Tryptophan	0.8322	0.2757	0.0909	1.0000	0.2585
L-2-Aminoadipic-acid	0.8585	0.0004	0.0997	1.0000	0.4966
L-Isoleucine	0.0000	0.0000	0.1012	1.0000	0.7089
γ-Aminobutyric acid	0.0120	0.0000	0.1172	1.0000	0.8132
L-Leucine	0.0000	0.0000	0.1251	1.0000	0.5119
Citrulline	0.0000	0.0000	0.1343	1.0000	0.2335
L-4-Hydroxyproline	0.0047	0.0224	0.1465	1.0000	0.2335
N6-N6-N6-Trimethyl-lysine	0.0416	0.0026	0.2104	1.0000	0.2503
L-Lysine	0.0357	0.0000	0.2514	1.0000	0.3970
Putrescine	0.0000	0.0001	0.2720	1.0000	0.6620
L-Valine	0.0000	0.0000	0.3495	1.0000	0.8864
L-Alanine	0.0506	0.0000	0.3642	1.0000	0.6935
Glycine	0.1652	0.0000	0.3834	1.0000	0.2335
L-Histidine	0.4174	0.2717	0.4095	1.0000	0.2335
L-Proline	0.0082	0.0000	0.5517	1.0000	0.4966
3-Methoxytyrosine	0.0126	0.0006	0.5957	1.0000	0.3507
L-Threonine	0.5953	0.0102	0.6242	1.0000	0.2242
L-α-Aminobutyric acid	0.1929	0.0000	0.6616	1.0000	0.4081
L-Glutamic acid	0.1449	0.7921	0.7861	1.0000	0.2186
L-Serine	0.0053	0.0328	0.8864	1.0000	0.6874

Probability values depicted are from an analysis of covariance between the study groups (diagnosis) with age and sex as covariates. Metabolites are ranked by *p* value of diagnosis.

[a]$p < 0.05$ after multiple testing correction.

FDR = false discovery rate correction.

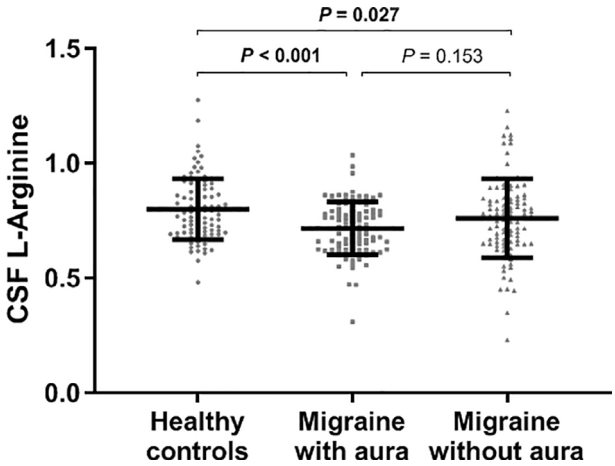

FIGURE 1: Cerebrospinal fluid (CSF) L-arginine levels in participants with migraine and healthy controls. Individual CSF L-arginine levels are plotted (*dots*) with group means ± standard deviation (*bars*). Data are adjusted for age. Probability values are from post hoc analysis after analysis of covariance.

In plasma, there were no metabolite differences after correction for multiple comparisons (Table 3 and Fig S6).

Multivariate Results

In CSF, global metabolite profiles differed between groups ($p = 0.035$). Whereas there were no differences between migraine subtypes ($p = 0.640$), profiles for migraine with aura ($p = 0.029$), migraine without aura ($p = 0.020$), and all migraine participants combined ($p = 0.009$) did differ from those in controls (Fig 2). Again, the ability to predict migraine was modest, with an area under the curve of 0.67 for migraine with aura and 0.63 for migraine without aura (Fig S7).

In plasma, results were similar to those in CSF. Profiles differed between groups ($p = 0.021$). Whereas there were no differences between migraine subtypes ($p = 0.275$), profiles for migraine with aura ($p = 0.028$), migraine without aura ($p = 0.018$), and all migraine ($p = 0.011$) did differ from those in controls. The area under the curve of the prediction model was 0.61 for migraine with aura and 0.57 for migraine without aura (Fig S8).

Pathways present in our metabolite set are visualized in Figure 3. In CSF, the pathway "arginine biosynthesis" was significantly associated with migraine with aura (Fig 4; $p = 0.004$). The "arginine/proline metabolism" pathway showed the strongest association with migraine without aura, but this was not multiple testing resistant. In plasma, the strongest associated pathways were "arginine biosynthesis" with migraine with aura and "arginine/proline metabolism" with migraine without aura (see Fig 4), but these were only significant before multiple testing correction. Exclusion of L-arginine, to study the effect of other metabolites in these pathways, showed similar results (Table S3).

Discussion

We compared the individual levels, global profiles, and pathways of 31 amines in the CSF and plasma of n = 197 interictal patients with migraine with or without aura with those of n = 96 truly healthy volunteers. The 3 groups were matched for age, sex, and diurnal and seasonal timing of sampling. L-Arginine (and possibly also L-phenylalanine) levels were reduced in the CSF, but not in the plasma of interictal individuals with migraine with or without aura, and global amines profiles were abnormal. Pathway analysis pointed toward a disrupted L-arginine metabolism in migraine with aura. Contrary to previous findings in the literature, no differences were found in the levels of L-glutamic acid (glutamate), L-glutamine, and GABA.

L-Arginine has never been measured in the CSF of migraineurs.[9,13] Studies in serum, plasma, platelets, saliva, and urine have yielded inconsistent findings for and within different biofluids.[26–33] In migraine with and without aura, plasma or serum L-arginine levels were found to be higher,[29,30] whereas in chronic migraine lower levels were found compared to healthy controls.[31] However, 3 other studies failed to detect a difference in L-arginine level between migraine patients and controls.[28,32,33] These inconsistent results can perhaps be explained by different inclusion criteria for patients and controls, different confounding factors, and the lack of adjustment for sex and age. In our study, sex was found to be a highly significant factor determining L-arginine levels.

Because we found no differences in plasma levels but solely in CSF, this could indicate only central metabolism (that is, in the central nervous system [CNS]) of L-arginine is affected and not systemic metabolism. In the CNS, L-arginine is unevenly distributed, with the greatest pool in astrocytes, less so in neuronal tissue, and absent in oligodendrocytes.[34] L-Arginine is involved in endocrine activity, immune system modulation, regulation of vascular tone, and peptide and protein production in the CNS.[34] It is also suggested to be neuroprotective; in ischemic stroke models, L-arginine suppresses the elicited proinflammatory response of microglia.[35] In the CNS, the metabolism of L-arginine is closely linked to the metabolism of L-citrulline and L-ornithine (see Fig 3 and Fig 5), which can be synthesized and degraded into each other (see Oja et al[34] and Wu and Morris[36] for extensive review on arginine metabolism). L-Arginine is oxidized by nitric oxide synthase (NOS), resulting in citrulline and nitric oxide. Additionally,

TABLE 3. Univariate Analysis Results in Plasma

Metabolite	Sex, *p*	Age, *p*	Diagnosis, *p*	Bonferroni	FDR
Citrulline	0.0000	0.0000	0.0063	0.1965	0.0857
L-Arginine	0.0001	0.0570	0.0079	0.2439	0.0857
L-4-Hydroxyproline	0.0000	0.0008	0.0083	0.2572	0.0857
O-Phosphoethanolamine	0.5608	0.1047	0.0435	1.0000	0.3370
Taurine	0.0830	0.8983	0.0592	1.0000	0.3673
Ethanolamine	0.0000	0.0571	0.0820	1.0000	0.4234
N6-N6-N6-Trimethyl-lysine	0.0000	0.1537	0.1298	1.0000	0.4982
L-Tryptophan	0.0000	0.0021	0.1580	1.0000	0.4982
L-Lysine	0.0086	0.0262	0.1631	1.0000	0.4982
L-Isoleucine	0.0000	0.1417	0.1679	1.0000	0.4982
L-Glutamic acid	0.0000	0.6273	0.2162	1.0000	0.4982
L-Leucine	0.0000	0.9974	0.2188	1.0000	0.4982
L-Glutamine	0.0000	0.0003	0.2240	1.0000	0.4982
3-Methoxytyrosine	0.1295	0.0971	0.2348	1.0000	0.4982
L-Histidine	0.0625	0.0018	0.2547	1.0000	0.4982
L-2-Aminoadipic acid	0.0000	0.0001	0.2592	1.0000	0.4982
L-Methionine	0.0000	0.0439	0.3018	1.0000	0.4982
L-Phenylalanine	0.0000	0.0868	0.3228	1.0000	0.4982
L-Proline	0.0000	0.8231	0.3337	1.0000	0.4982
L-Homoserine	0.0151	0.2065	0.3469	1.0000	0.4982
L-Valine	0.0000	0.0774	0.3510	1.0000	0.4982
L-Asparagine	0.3512	0.1308	0.3536	1.0000	0.4982
L-Alanine	0.0001	0.2295	0.4013	1.0000	0.5408
Putrescine	0.0115	0.1437	0.4306	1.0000	0.5562
L-Tyrosine	0.0000	0.0000	0.5789	1.0000	0.6961
L-Threonine	0.3757	0.2104	0.5839	1.0000	0.6961
L-Serine	0.6010	0.7000	0.7065	1.0000	0.8112
γ-Aminobutyric acid	0.9494	0.4597	0.8354	1.0000	0.9249
Glycine	0.0983	0.0000	0.8879	1.0000	0.9492
L-α-Aminobutyric acid	0.1532	0.0008	0.9282	1.0000	0.9591
Ornithine	0.0000	0.0000	0.9817	1.0000	0.9817

Probability values depicted are from an analysis of covariance between the study groups (diagnosis) with age and sex as covariates. Metabolites are ranked by *p* value of diagnosis.
FDR = false discovery rate correction.

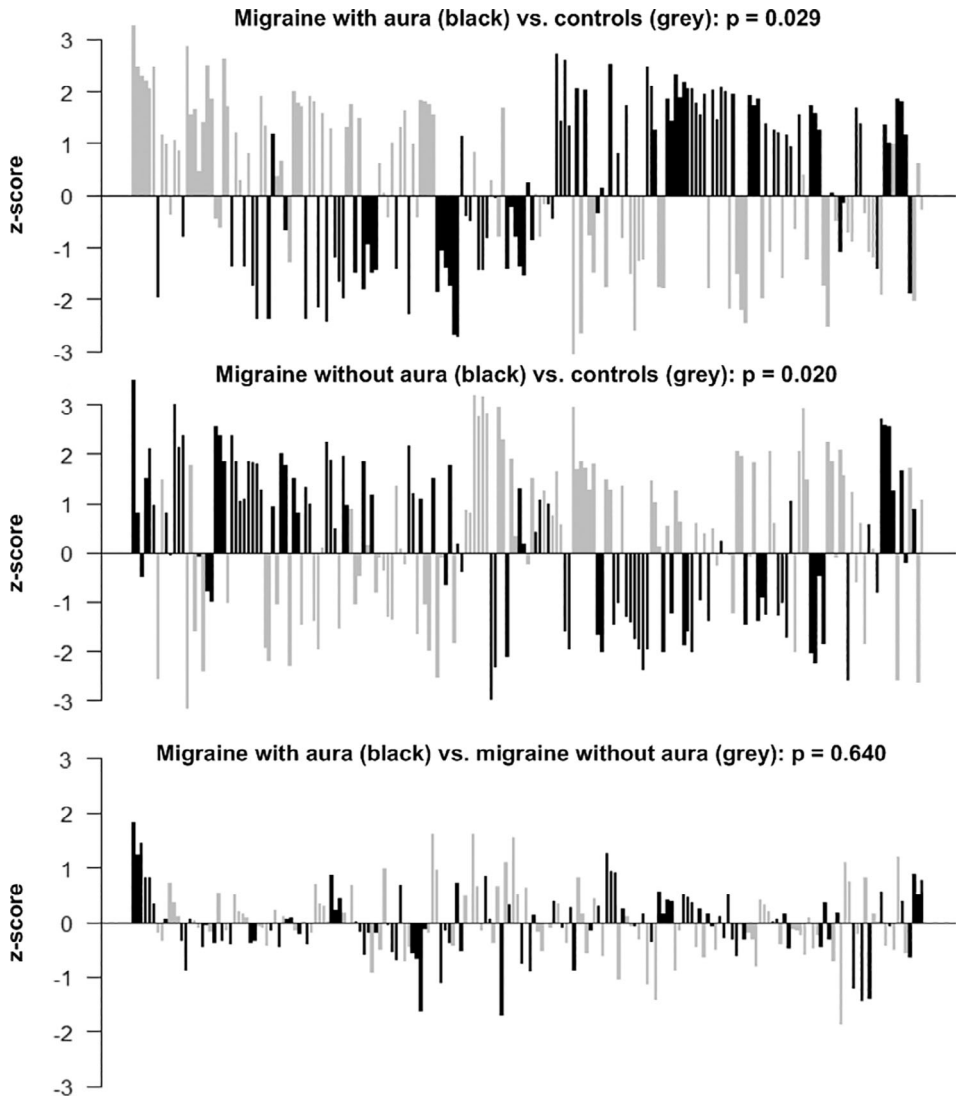

FIGURE 2: Subjects plot of global test with all amines in cerebrospinal fluid. For each participant (*bars*) the *z*-score is plotted. A positive *z*-score indicates the metabolite profile of the participant matches with the profile of his or her group. A negative *z*-score indicates the profile of the participant matches better with the profile of the other group. The number of the *z*-score reflects the strength of the match. For the comparisons migraine with aura versus controls (upper panel) and migraine without aura versus controls (middle panel), there are relatively more participants with high positive *z*-scores than for the comparison migraine with aura versus migraine without aura (lower panel). Probability values are from the global test.

L-arginine can be metabolized in L-ornithine (and urea) by arginase. Finally, L-arginine can be metabolized by arginine decarboxylase, resulting in formation of agmatine (and afterward putrescine), or by arginine:glycine amidinotransferase, resulting in guanidinoacetic acid (and eventually creatine). Of the above metabolites, we measured L-citrulline, L-ornithine, and putrescine, and we observed no differences in their concentrations. We studied several ratios (L-citrulline/L-arginine, L-ornithine/L-arginine, and putrescine/L-arginine) to investigate whether one of those was particularly altered in migraine patients compared to controls, but this was not the case (data not shown). Because L-citrulline, L-ornithine, and putrescine levels are also dependent on amines other than L-arginine, this could explain why altered L-arginine levels are not reflected in their concentrations.

The link between L-arginine and nitric oxide formation is of special interest in migraine. Glyceryl trinitrate, a nitric oxide donor, can provoke migraine-like attacks in migraineurs but not in nonmigraineurs.[37] Nitric oxide increases cyclic guanosine monophosphate. This second messenger is believed to play a central role in migraine pathophysiology, because it is the common pathway of not only glyceryl trinitrate but also other migraine provocation models.[37] The nitric oxide pathway is probably more upstream in the migraine cascade than the CGRP pathway, because CGRP levels were found to be elevated in migraine attacks provoked by nitric oxide[38] and

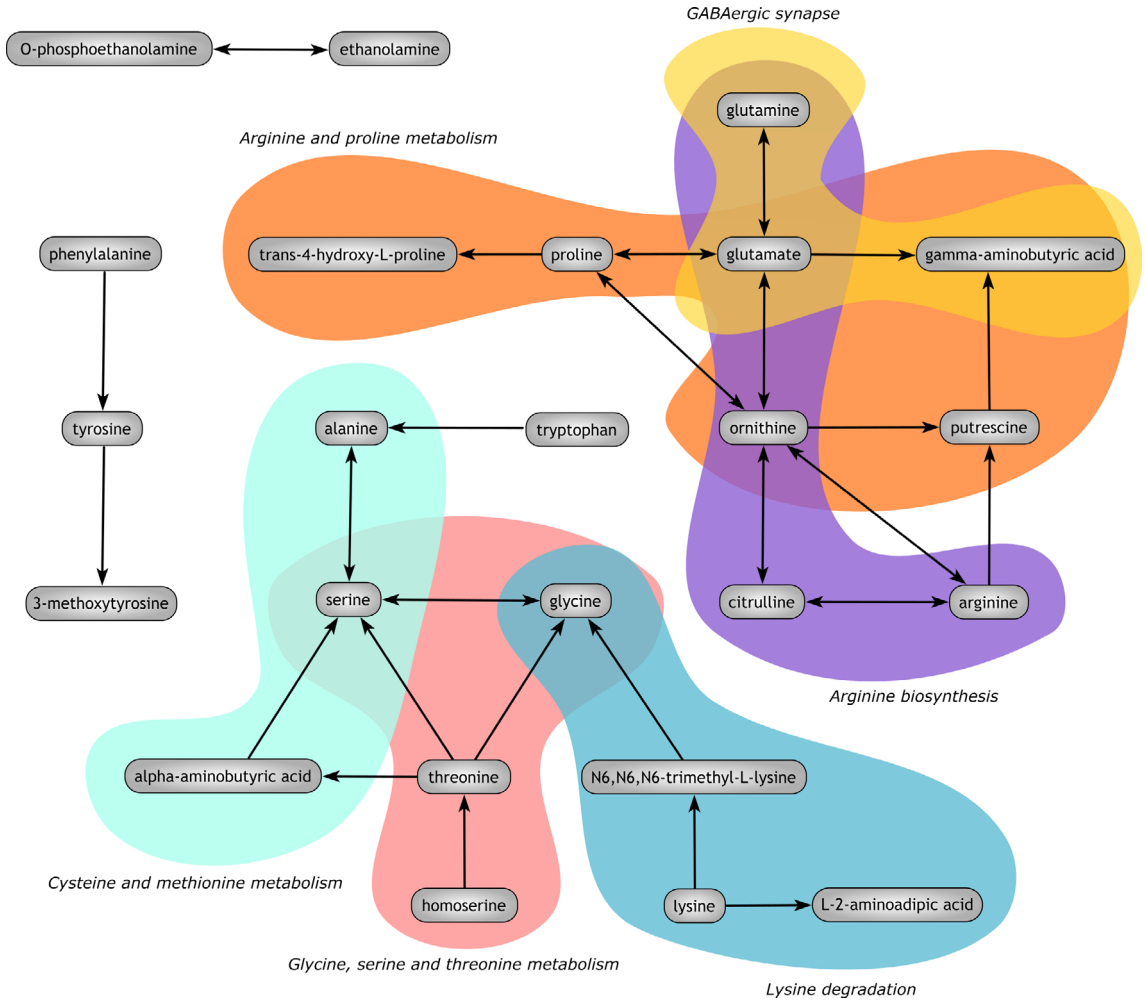

FIGURE 3: Metabolic network. Visualization of the generated Genome-Scale Metabolic Model (GSMM) network and the corresponding Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolite sets after filtering. Arrows represent biochemical paths consisting of one or two reaction steps. All amines could be mapped to the GSMM, except L-asparagine, L-methionine, taurine, L-isoleucine, L-leucine, L-valine, and L-histidine. These were not connected with the rest of the network and are therefore excluded from the visualization. Colors indicate the KEGG pathways that were tested using the global test approach, results of which are presented in Figure 4. GABAergic = γ-aminobutyric acidergic. [Color figure can be viewed at www.annalsofneurology.org]

premonitory symptoms are frequently reported in glyceryl trinitrate-provoked attacks[39,40] while being rare in CGRP-provoked attacks.[41] L-Arginine serves as the only available nitrogen-containing substrate for NOS.[34] The observed lower L-arginine concentrations might therefore reflect overactivity of NOS, elevating nitric oxide levels and making migraine patients susceptible to a next attack, although this hypothesis could not be studied in our study design. There are 3 isoforms of NOS: endothelial, neuronal (n), and inducible (i) NOS.[34] A nonselective NOS inhibitor was reported to be effective in aborting spontaneous migraine attacks,[42,43] whereas specific iNOS inhibitors failed, and trials of combinations of an nNOS blocker and a triptan produced conflicting results.[37,43,44] Future research should aim to study the various NOS activities, especially that of neuronal NOS, and L-arginine concentrations in the CSF of different types of migraine.

At first glance, the biological significance of only a 10% decrease seems small. However, we must remember that this was found in the CSF of *interictal* migraineurs, that is, outside of migraine attacks, when they are symptom-free. Patients with paroxysmal brain disorders such as episodic migraine only experience neurological symptoms during attacks. Outside of these, they are clinically completely normal. A biochemical dysregulation that might underlie susceptibility to having recurrent migraine attacks probably fluctuates in severity and is only minor and subtle outside of attacks. Moreover, migraine is probably caused by a local (not generalized) brain abnormality. Focal biochemical abnormalities are expected to be diluted in the CSF. The regional change in L-arginine is likely to be much larger than 10%. Therefore, finding any biochemical abnormality in the CSF of interictal migraineurs that also survives strict statistical Bonferroni correction

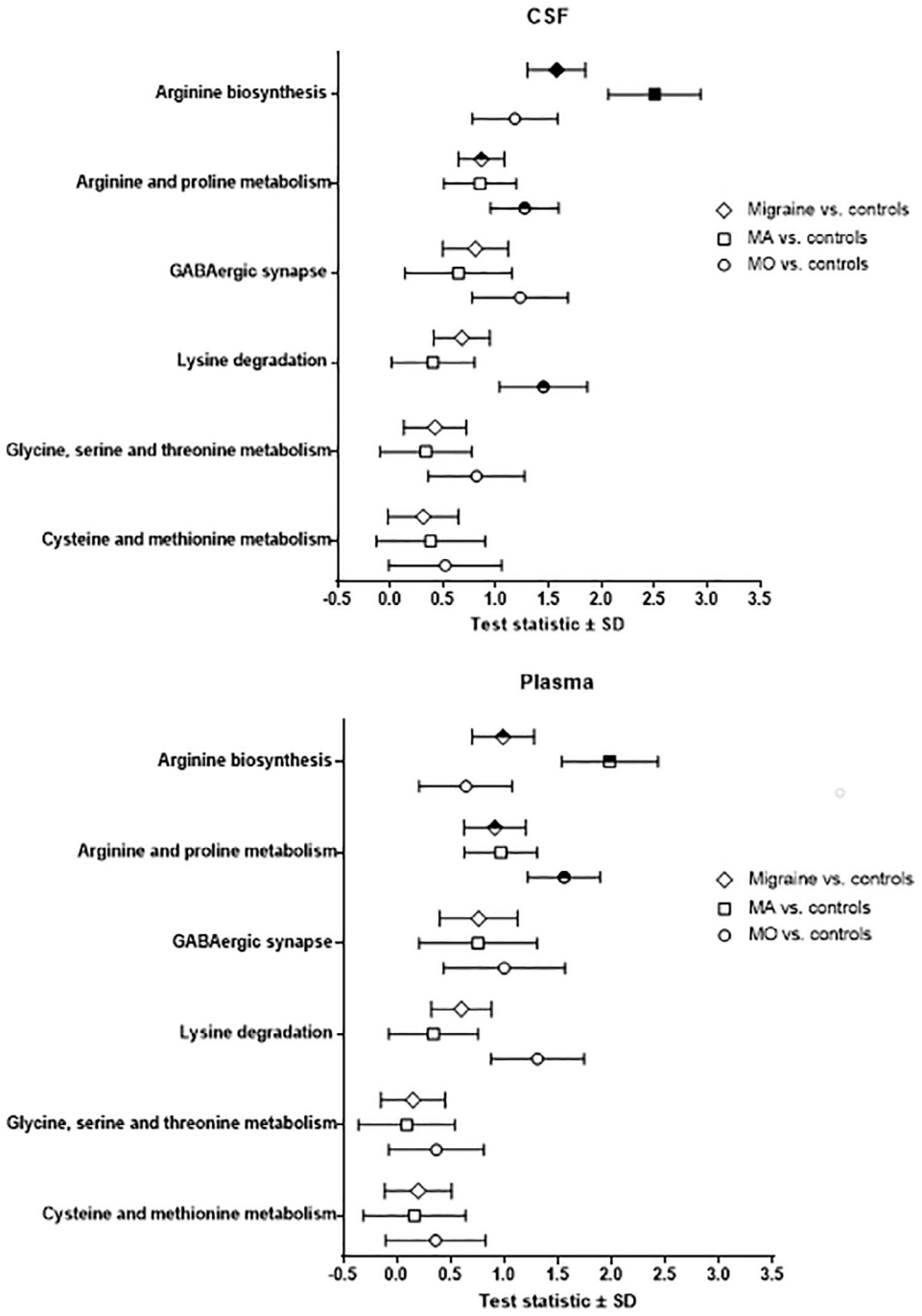

FIGURE 4: Results from the global test approach on pathways. The global test is aimed at associations between sets of connected metabolites and disease status and does not test the direction of the association, that is, whether sets of metabolites are up- or downregulated. Therefore, there is no mean difference to plot, and one can only visualize the test statistic and corresponding standard deviation (SD). The full black symbols indicate $p < 0.05$ after multiple testing correction with Bonferroni, and the half-black symbols indicate $p < 0.05$ before multiple testing correction. CSF = cerebrospinal fluid; GABAergic = γ-aminobutyric acidergic; MO = migraine without aura; MA = migraine with aura; Migraine = both subtypes combined.

seems all the more remarkable and should warrant further focused investigation.

Using the less conservative FDR correction, L-phenylalanine levels were also lower in the CSF of both migraine subtypes. Humans cannot synthesize L-phenylalanine de novo.[34] Therefore, the concentrations are fully dependent on dietary intake. Although participants were fasting, long-term dietary differences cannot be excluded, and therefore findings must be interpreted with caution. However, that we found no difference in plasma levels argues against a dietary cause. We observed no difference in L-tyrosine, the hydrolyzation product of L-phenylalanine.[34]

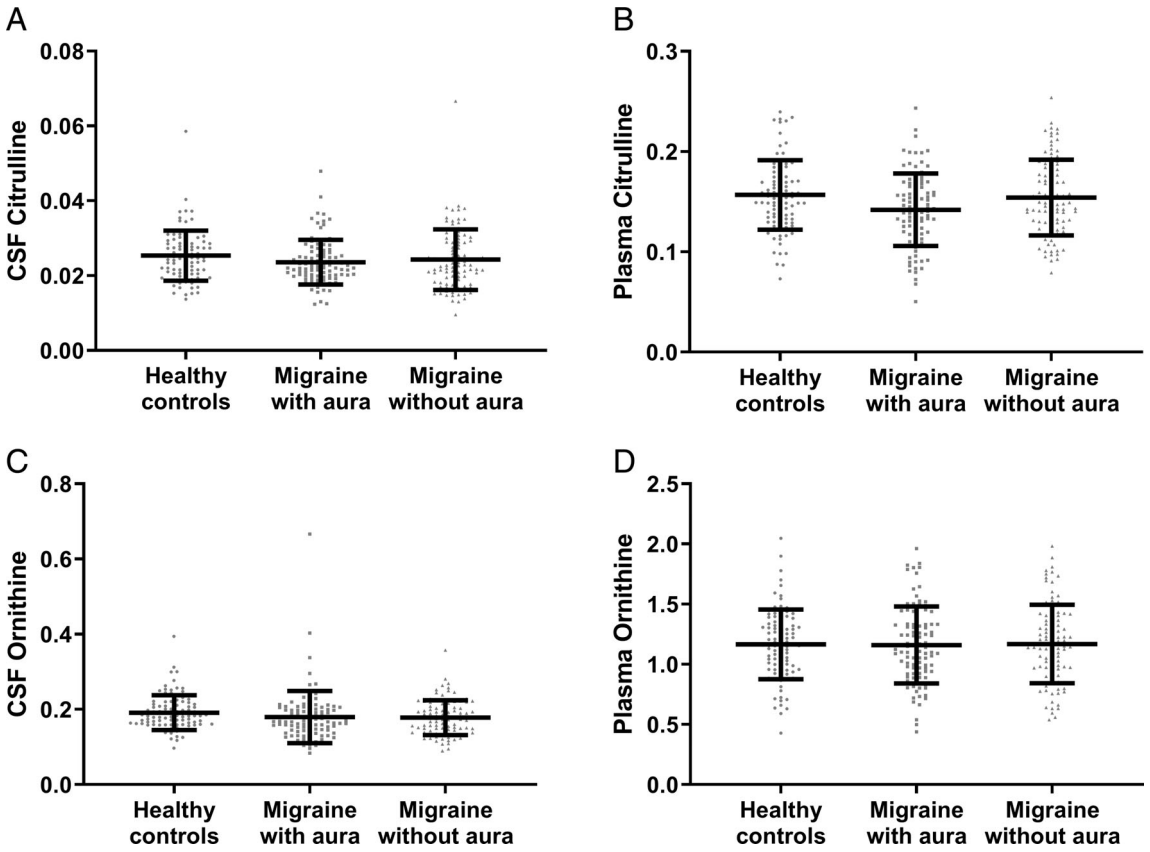

FIGURE 5: Cerebrospinal fluid (CSF) and plasma L-citrulline and L-ornithine levels in participants with migraine and healthy controls. (A) Individual CSF L-citrulline levels are plotted (*dots*) with group means ± standard deviation (SD; *bars*). (B) Individual plasma L-citrulline levels are plotted (*dots*) with group means ± SD (*bars*). (C) Individual CSF L-ornithine levels are plotted (*dots*) with group means ± SD (*bars*). (D) Individual plasma L-ornithine levels are plotted (*dots*) with group means ± SD (*bars*). Data are adjusted for age.

With our strict methodology and inclusion criteria, we could not confirm the previously reported changes in L-glutamic acid (glutamate), L-glutamine, or GABA.[9] However, most of these findings have been made in chronic migraine, and these compounds have never previously been studied in the CSF of interictal episodic migraineurs. A meta-analysis of several small studies with heterogeneity in study quality did show a modest increase in glutamate in the blood.[9] Altogether, the data suggest that glutamate may be elevated during or immediately before or after attacks, but not outside of migraine attacks.

Our study has several strengths. The study groups were significantly larger than those in other studies,[9] and they were matched for important potential confounding factors such as sex, age, and diurnal and seasonal timing of sampling. Control CSF was as normal as possible. Unlike in other studies,[9] the controls were truly healthy. For example, control CSF had not been collected as part of a diagnostic process due to neurological symptoms. In addition to having no personal history of migraine, controls also had no first-degree relatives with migraine. This minimized the genetic risk of later developing migraine.[4,45] We also made an extra effort to obtain strictly interictal values without any potential effect of attacks. Patients were examined at least 3 days after and at least 3 days before an attack. For this reason, patients with high-frequency or chronic migraine were excluded from participation. Finally, whereas other studies measured only one or a few molecules, and only in CSF or in plasma,[9] our study measured multiple amines simultaneously in both CSF and plasma using strict, very high-quality control standards. Only amines for which the relative standard deviation of the quality control samples was <15% were included. Because the concentrations of amines are lower in CSF than in plasma or serum, measuring amines in CSF is also more difficult. Therefore, we used a specifically validated UPLC-MS platform for measuring amines in CSF. This also allowed us to perform additional profile and pathway analyses.

We used a network-based pathway analysis approach to investigate which pathways underlie the altered CSF and plasma amine profiles of migraine patients. We found this approach especially useful for metabolomics data, where coverage of the pathway definitions is low

compared to, for example, transcriptomics data. Consequently, it is only possible to make assertions about part of the pathway, that is, not the full KEGG pathway. If studied metabolites only reside on extreme ends of the pathway diagram or are only linked through enzymatic steps that do not occur in humans, it would be incorrect to draw statistical conclusions regarding the full KEGG pathway. Therefore, we used the described filtering step. Combining classic pathway definitions with knowledge about how the measured amines are connected through human metabolism, we were able to define metabolite sets for pathway analysis that are more accurate and relevant from a biochemical point of view.

Our study also has limitations. Ideally, we should have replicated our findings in an independent study population, but collecting a second, sufficiently large, matched sample of CSF and plasma from migraineurs and healthy volunteers is logistically challenging, expensive, and time-consuming. Alternatively, we could have divided the total study population into two portions and used a second portion for replication. However, statistical analysis showed that this would have resulted in a lower power to detect and replicate statistically significant findings. Moreover, to assess the specificity of our findings, we should also have investigated other non-migraine headaches, for example, tension-type headache. Furthermore, extended pathway coverage by measuring additional amines and other pathways is needed to improve our understanding of migraine biochemistry. However, because we considered reliable quantification more important than a broader detection range, and because we had not previously focused on L-arginine but rather on a broad screening of amines in interictal migraine, we did not measure all amines and metabolites involved in nitric oxide metabolism. A detailed, hypothesis-driven, follow-up study on this should definitely be conducted. Another limitation is the cross-sectional nature of our study. Repeated sampling, ideally across the entire migraine attack cycle, would have afforded more detailed insight into migraine biochemistry, but seems ethically impossible. Finally, the effects in our study were modest and overlap; hence, the observed differences are unlikely to be useful as diagnostic tests or offer direct clinical implications. Notwithstanding the aforementioned limitations, we believe that our study is a good illustration of the many methodological aspects that must be considered when studying biochemical changes in the CSF of people with a paroxysmal brain disorder such as migraine. Our study shows how metabolomics could be studied in the CSF of patients with paroxysmal brain disease and the specific methodological problems and interpretation issues that may arise.

In conclusion, extensive analyses of amine profiles and pathways in the CSF and plasma of interictal migraineurs with or without aura show that L-arginine is reduced in the CSF of migraineurs outside of attacks. This suggests a permanent dysfunction of nitric oxide signaling underlying susceptibility to having recurrent migraine attacks. After confirmation and additional analysis of other amines and metabolites involved in nitric oxide metabolism during the different phases of the migraine attack cycle, this may serve as a potential more etiological new target for preventive therapy of migraine attacks.

Acknowledgments

This work was supported by the Netherlands Organization for Scientific Research (VICI grant 918.56.601 and Spinoza 2009 to M.D.F.), the Netherlands Organization for Health Research and Development (Clinical Fellowship grant 90700217 and Vidi grant 917-11-31 to G.M.T.), and European Community-funded FP7-EUROHEADPAIN (grant 602633 to M.D.F. and A.M.J.M.v.d.M.).

We greatly acknowledge all study participants; the medical students who assisted with recruitment of participants; Drs F. Bakels, N. Pelzer, L. Wilbrink, and A. van der Plas for sample collection; and E. van Zwet and S. Koval for their advice on the applied statistics.

Author Contributions

G.L.J.O., R.M.v.D., R.Z., W.P.J.v.O., G.M.T., A.M.J.M.v.d.M., T.H., and M.D.F. contributed to the conception and design of the study. All authors contributed to the acquisition and analysis of data. G.L.J.O., R.M.v.D., J.B.v.K., and M.D.F. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390:1211–1259.

2. Headache classification Committee of the International Headache Society (IHS). The international classification of headache disorders, 3rd edition. Cephalalgia 2018;1:1–211.

3. Launer LJ, Terwindt GM, Ferrari MD. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology 1999;53:537–542.
4. Ferrari MD, Klever RR, Terwindt GM, et al. Migraine pathophysiology: lessons from mouse models and human genetics. Lancet Neurol 2015;14:65–80.
5. Goadsby PJ, Holland PR, Martins-oliveira M, et al. Pathophysiology of migraine—a disorder of sensory processing. Physiol Rev 2017;97:553–622.
6. Edvinsson L, Haanes KA, Warfvinge K, Krause DN. CGRP as the target of new migraine therapies—successful translation from bench to clinic. Nat Rev Neurol 2018;14:338–350.
7. Charles A, Pozo-Rosich P. Targeting calcitonin gene-related peptide: a new era in migraine therapy. Lancet 2019;394:1765–1774.
8. Stankewitz A, May A. The phenomenon of changes in cortical excitability in migraine is not migraine-specific – a unifying thesis. Pain 2009;145:14–19.
9. van Dongen RM, Zielman R, Noga M, et al. Migraine biomarkers in cerebrospinal fluid: a systematic review and meta-analysis. Cephalalgia 2017;37:49–63.
10. Wishart DS, Lewis MJ, Morrissey JA, et al. The human cerebrospinal fluid metabolome. J Chromatogr B 2008;871:164–173.
11. Brown PD, Davies SL, Speake T, Millar ID. Molecular mechanisms of cerebrospinal fluid production. Neuroscience 2004;129:957–970.
12. Deisenhammer F, Teunissen CE, Tumani H. *Cerebrospinal Fluid in Neurologic Disorders*. Vol 146. 1st ed., Amsterdam, Netherlands: Elsevier, 2017.
13. Zielman R, Postma R, Verhoeven A, et al. Metabolomic changes in CSF of migraine patients measured with 1 H-NMR spectroscopy. Mol Biosyst 2016;12:3674–3682.
14. Humphrey PPA, Feniuk W, Perren MJ, et al. Serotonin and migraine. Ann N Y Acad Sci 1990;600:587–598.
15. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders: 2nd edition. Cephalalgia 2004;24:9–160.
16. Noga MJ, Zielman R, van Dongen RM, et al. Strategies to assess and optimize stability of endogenous amines during cerebrospinal fluid sampling. Metabolomics 2018;14:44.
17. Van Der Kloet FM, Bobeldijk I, Verheij ER, Jellema RH. Analytical error reduction using single point calibration for accurate and precise metabolomic phenotyping. J Proteome Res 2009;8:5132–5141.
18. Shaffer JP. Modified sequentially Rejective multiple test procedures. J Am Stat Assoc 1986;81:826–831.
19. Hendrickx DM, Hoefsloot HCJ, Hendriks MMWB, et al. Global test for metabolic pathway differences between conditions. Anal Chim Acta 2012;719:8–15.
20. Kanehisa M, Sato Y, Kawashima M, et al. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 2016;44:D457–D462.
21. Kamburov A, Stelzl U, Lehrach H, Herwig R. The ConsensusPathDB interaction database: 2013 update. Nucleic Acids Res 2013;41:D793–D800.
22. Mardinoglu A, Agren R, Kampf C, et al. Genome-scale metabolic modelling of hepatocytes reveals serine deficiency in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:3083.
23. Goeman JJ. L1 penalized estimation in the cox proportional hazards model. Biom J 2010;52:70–84.
24. Van Oosterhout WPJ, Van Der Plas AA, Van Zwet EW, et al. Postdural puncture headache in migraineurs and nonheadache subjects: a prospective study. Neurology 2013;80:941–948.
25. Moss MB, Siqueira MA, Mann GE, et al. Platelet aggregation in arterial hypertension: is there a nitric oxide-urea connection? Clin Exp Pharmacol Physiol 2010;37:167–172.
26. Sjaastad O, Gjesdahl P, Gjessing LR. Amino acids in urine in spontaneous migriane attacks. Eur J Neurol 1972;7:137–145.
27. Rajda C, Tajti J, Komoróczy R, et al. Amino acids in the saliva of patients with migraine. Headache 1999;39:644–649.
28. D'andrea G, Welch K, Perini F, et al. Decreased collagen-induced platelet aggregation and increased platelet arginine levels in migraine: a possible link with the NO pathway. Cephalalgia 1994;14:352–356.
29. Reyhani A, Celik Y, Karadag H, et al. High asymmetric dimethylarginine, symmetric dimethylarginine and L-arginine levels in migraine patients. Neurol Sci 2017;38:1287–1291.
30. Erdélyi-Bótor S, Komáromy H, Kamson DO, et al. Serum L-arginine and dimethylarginine levels in migraine patients with brain white matter lesions. Cephalalgia 2017;37:571–580.
31. D'Andrea G, Gucciardi A, Giordano G, et al. Study of arginine metabolism in medication overuse chronic migraine: possible defect in NO synthesis. Neurol Sci 2022;43:2745–2749. https://doi.org/10.1007/s10072-021-05672-7.
32. Van Der Schueren BJ, Verbrugge FH, Verbesselt R, et al. No arguments for increased endothelial nitric oxide synthase activity in migraine based on peripheral biomarkers. Cephalalgia 2010;30:1354–1365.
33. Gruber HJ, Bernecker C, Lechner A, et al. Increased nitric oxide stress is associated with migraine. Cephalalgia 2010;30:486–492.
34. Oja SS, Schousboe A, Saransaari P. Amino acids and peptides of the nervous system. Ch 5 arginine, citrulline and ornithine. *Handbook of Neurochemistry and Molecular Neurobiology*, New York, NY: Springer Science+Business Media, 2007:59-115.
35. Chen SF, Pan MX, Tang JC, et al. Arginine is neuroprotective through suppressing HIF-1α/LDHA-mediated inflammatory response after cerebral ischemia/reperfusion injury. Mol Brain 2020;13:1–13.
36. Wu G, Morris SM. Ch 10. Arginine metabolism in mammals. *Metabolic and therapeutic aspects of amino acids in clinical nutrition*, Florida, United States: CRC Press, 2004:153-167.
37. Ashina M, Hansen JM, á Dunga BO, Olesen J. Human models of migraine — short-term pain for long-term gain. Nat Rev Neurol 2017;13:713–724.
38. Juhasz G, Zsombok T, Modos EA, et al. NO-induced migraine attack: strong increase in plasma calcitonin gene-related peptide (CGRP) concentration and negative correlation with platelet serotonin release. Pain 2003;106:461–470.
39. Afridi SK, Kaube H, Goadsby PJ. Glyceryl trinitrate triggers premonitory symptoms in migraineurs. Pain 2004;110:675–680.
40. Onderwater GLJ, Dool J, Ferrari MD, Terwindt GM. Premonitory symptoms in glyceryl trinitrate triggered migraine attacks: a case-control study. Pain 2020;161:2058–2067.
41. Guo S, Vollesen ALH, Olesen J, Ashina M. Premonitory and nonheadache symptoms induced by CGRP and PACAP38 in patients with migraine. Pain 2016;157:2773–2781.
42. Lassen LH, Ashina M, Christiansen I, et al. Nitric oxide synthase inhibition in migraine. Lancet 1997;349:401–402.
43. Goadsby PJ. Bench to bedside advances in the 21st century for primary headache disorders: migraine treatments for migraine patients. Brain 2016;139:2571–2577.
44. Macone AE, Perloff MD. Triptans and migraine: advances in use, administration, formulation, and development. Expert Opin Pharmacother 2017;18:387–397.
45. Russell MB, Olesen J. Increased familial risk and evidence of genetic factor in migraine. BMJ 1995;311:541–544.