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Cerebrospinal fluid and plasma amine profiles in migraine

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Abstract

Importance

Impaired cerebral and systemic amine metabolism have been implicated in migraine etiology. Direct evidence from cerebrospinal fluid (CSF) is, however, lacking.

Objective

To assess amine levels (primary outcome), global amine profiles and amine pathways (secondary outcomes) in CSF and plasma of interictal patients with migraine compared to healthy controls.

Design, Setting, and Participants

In this case-control study CSF and plasma were sampled between 08:30 am and 1:00 pm, at random from healthy volunteers (n=96) and interictal patients with migraine (n=99 with aura; n=98 without aura). Groups were matched for age and sex. The study was approved by the Local Medical Ethics Committee.

Main outcomes and measures

Individual amines (n=30 in CSF; n=31 in plasma), global amine profiles, and specific amine pathways were analyzed using an ultra-performance liquid chromatography mass spectrometry (UPLC-MS) platform that was validated for amine measurements.

Results

We analyzed n=197 participants with migraine (n=99 with aura; n=98 without aura) and n=96 healthy volunteers. Univariate analysis with Bonferroni correction indicated that CSF L-Arginine levels were reduced in migraine with aura (-10.4%; $P < 0.001$) and without aura (-5.0%; $P = 0.03$). FDR-corrected CSF L-Phenylalanine levels were also lower in both migraine with aura (6.9%; $P = 0.011$) and without aura (-8.1%; $P = 0.001$). The effect, however, failed to reach significance after Bonferroni correction ($P = 0.088$). Other amines did not differ. 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. Strongest associated pathways with migraine were related to L-Arginine metabolism.

Conclusions and relevance

Cerebral and systemic amine profiles are disrupted in migraine with and without aura. Reduced CSF L-Arginine and altered associated pathway analyses suggest dysfunction of nitric oxide signaling in migraine, which may serve as a potential preventive treatment target.

Introduction

Migraine is a common neurovascular brain disorder, characterized by disabling attacks of headache and associated symptoms for up to three days.^{1,2} In one third of patients, attacks may include aura.³ Women are affected three times more often.^{1,2,3} Objective diagnostic biomarkers are lacking.² Median attack frequency is 1.5 per month and one quarter of patients experience weekly attacks.³ Genetic and non-genetic risk and susceptibility-modifying factors are involved.⁴ Although mechanisms for aura and headache are reasonably well understood,^{5,6} little is known about how attacks are initiated and why they continue recurring.^{1,4} This has hampered the development of causal therapy. Even the recently emerging CGRP inhibitors have limited effectiveness.^{6,7}

Unravelling migraine neurochemistry might help to elucidate its etiology.^{4,8} Most biochemical studies in migraine have focused on blood and urine.^{5,9} While easier to collect, these biofluids mainly reflect systemic rather than cerebral changes.¹⁰ CSF might better reflect brain neurochemistry.^{11,12} but its sampling has been hampered by logistic and ethical issues.

Various amines such as glutamate, glutamine, GABA and serotonin have been implicated in migraine,^{4,9,13} but CSF studies were small and had methodological limitations. Controls were rarely matched for confounding factors such as sex, age, and diurnal and seasonal timing of sampling. They were also rarely truly healthy, which could potentially affect CSF composition. Lumbar punctures were usually done to exclude neurological disease in patients with neurological complaints. In some studies, ictal CSF was only compared with interictal CSF, without healthy controls. These reports focused on the pathophysiology of the attack, rather than on pathogenetic mechanisms of “the disease migraine” (i.e. why does someone experience recurring migraines?). Details of the measurement methods and their validation were often limited.⁹ Finally, studies only reported on single or just a few molecules, rather than on multiple substances, simultaneously measured in both CSF and plasma and allowing for unbiased analysis of global profiles and pathways.

To identify abnormalities in amine levels, global profiles, and pathways, we assessed 31 amines in CSF and plasma of 96 healthy volunteers and 197 interictal migraineurs with (n=99) or without (n=98) aura. Groups were matched for age, sex, and timing of sampling.

Methods

Study design and participants

We enrolled patients with migraine with or without aura² and healthy controls who were group-matched for sex and age (by adhering to 5-year age strata). 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. 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 contra-indication for lumbar puncture. The study was conducted according to the criteria of the Declaration of Helsinki and approved by the Leiden University Medical Centre 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

Sampling occurred between 08:30 a.m. and 1:00 p.m., randomly for patients and controls, to mitigate diurnal and seasonal variation differences. Participants refrained from eating or drinking, apart from water, for at least 8 hours prior to sampling. After standard neurological examination, CSF was sampled by lumbar puncture between the L3/L4, L4/L5, or L5/S1 interspace. Intracranial pressure was measured, and 3.0 mL CSF was sampled for routine diagnostics (cell count, glucose and total protein levels). Next, 3.8 mL of CSF was sampled directly in a 15-mL polypropylene falcon tube pre-chilled on ice and centrifuged at 4°C for 5 minutes (2,000 rpm, 747 g). The supernatant was transferred into a new chilled 15-mL polypropylene falcon tube, inverted several times, and divided in 0.5 mL aliquots (1.8-mL cryotubes) that already contained 1.0 mL of cold EtOH. Cryotubes were inverted several times to mix CSF and EtOH. 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 EDTA plasma tubes, immediately after lumbar puncture, and centrifuged at 21°C for 20 minutes (2,000 rpm, 622 g). The supernatant was transferred to a new 15-mL polypropylene falcon tube, inverted several times, and divided in 0.5 mL aliquots (1.0-mL Nunc™ 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 eFigure 1 for detailed information on sample processing. To monitor complications, participants were followed for three days, or longer if necessary.

Amine measurements

Amines were measured with an ultra-performance liquid chromatography mass spectrometry (UPLC-MS) method specifically developed for amine profiling.¹⁴ Samples were randomized across five CSF batches and five 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.¹⁵ See eMethods and eTable 1 for details on UPLC-MS methodology and target list.

Statistical analysis

Amine concentrations were first corrected based on quality control samples.¹⁵ Only metabolites with relative standard deviations of quality controls below 15% were included in further analyses. Outlier detection was performed using principal component analysis (PCA) with a 99% confidence interval (eFigure 2). Log-transformed concentrations were used for further statistical analyses.

To detect univariate metabolite differences, ANCOVA analysis 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.¹⁶

To detect multivariate differences in global metabolite profile or amine pathways, the Global Test approach¹⁷ was used (separately for CSF and plasma). First, global amine profiles of the three groups were compared with age and sex as covariates. Second, pathways were tested. We used MetaboAnalyst to obtain KEGG (Kyoto Encyclopedia of Genes and Genomes) identifiers from the 31 amines and subsequently downloaded KEGG metabolite set pathway definitions from ConsensusPathDB (07-11-2018).

To ensure that metabolite sets represented coherent pathways, we computed a metabolic interaction network of the 31 amines from the human Genome-Scale Metabolic Model (GSMM) HMR 2.0,¹⁸ where metabolites are connected when they lie within two reaction steps of each other. Amines that were not connected to others in a pathway set were excluded, and only pathways consisting of at least three amines were considered (eMethods). To test whether these pathways differed between the three 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 ($n = 6$). To visualize the generated amine network and examine the reaction paths between connected metabolites, we developed an interactive HTML/JavaScript document (eMethods).

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.¹⁹ Predictions resulted from cross validation. Statistical analyses were done with R (version 3.4.1), R packages global test (version 5.30.0) and penalized (version 0.9-50) and SPSS (version 23). Two-sided hypothesis testing was used.

Results

Study population

We included 96 healthy volunteers and 197 patients with migraine, 99 with aura and 98 without aura. Five healthy controls turned out to have a first-degree relative with migraine but were kept in the study because CSF of healthy volunteers is so precious and separate analysis of these five controls did not yield aberrant results. In one patient with migraine with aura we were unable to collect plasma. Additionally, two outliers were excluded from further data analysis (eFigure 2). 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 (eTable 2) of which eighteen 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 three 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 ($P = 0.153$) (Figure 1). Since L-Arginine is potentially related to cardiovascular status,²⁰ 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 (eTable 3). Participants who developed a migraine attack ≤ 3 days after sampling (and thus potentially were in a preictal phase), did not show different results (eFigure 3). 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 (eFigure 4).

None of the other metabolites in CSF differed significantly after Bonferroni correction (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%; $P = 0.001$; eFigure 5).

In plasma there were no metabolite differences after correction for multiple comparisons (eTable 4 and eFigure 6).

Table 1. Clinical characteristics of participants

	Healthy controls	Migraine with aura	Migraine without aura	P-value
Number of participants^a	95	98	98	
Subject characteristics				
Females	56 (58.9)	65 (66.3)	60 (61.2)	0.553 ^c
Age, years	38.8 (14.5)	41.7 (13.6)	42.0 (12.9)	0.170 ^d
BMI	23.7 (2.8)	24.0 (2.7)	23.6 (2.5)	0.602 ^e
Smoking	20 (21.1)	13 (13.3)	13 (13.3)	0.245 ^c
Overnight fasting				
Fasting time, hours	11.6 (2.4)	11.7 (1.7)	11.9 (1.6)	0.105 ^d
Migraine characteristics				
Migraine frequency, attacks/month	-	2.1 (1.9)	2.8 (2.6)	0.026^f
Headache days, days/month	0.3 (0.7)	5.2 (4.2)	6.0 (4.9)	<0.001^d
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 ^c
Prophylactic medication	-	17 (17.3)	17 (17.3)	1.000 ^c
B-blocker	-	11 (11.2)	9 (9.2)	0.637 ^c
Antiepileptic drug	-	4 (4.1)	4 (4.1)	1.000 ^g
Ace-inhibitors	-	1 (1.0)	1 (1.0)	1.000 ^g
Angiotensine II receptor antagonist	-	2 (2.0)	1 (1.0)	1.000 ^g
Sampling characteristics				
Opening pressure in mmH ₂ O	19.1 (4.4)	18.8 (4.0)	18.0 (4.7)	0.250 ^c
CSF characteristics				
Erythrocytes, count/3μL	154 (934)	2,299 (20,682)	130 (505)	0.509 ^d
Leukocytes, count/3μL	6.0 (6.0)	22.0 (89.0)	5.0 (7.0)	0.587 ^d
Protein concentration, g/L	0.35 (0.13)	0.36 (0.25)	0.35 (0.10)	0.702 ^d
Glucose, mmol/L	3.2 (0.3)	3.2 (0.3)	3.1 (0.2)	0.738 ^e
Post-dural puncture headache^b				
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

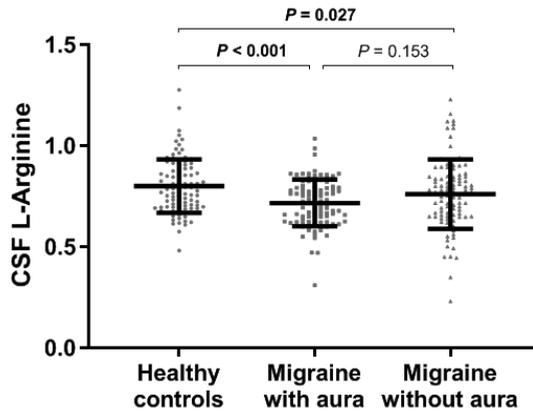
Legend: Data are n, mean (SD), or n (%), unless otherwise stated. LP=lumbar puncture; CSF=cerebrospinal fluid a Shown for CSF after exclusion of two outliers (see Methods), bfor these variables all participants who received an LP were included. cChi-square test. dKruskal-Wallis Test. eOne-way ANOVA. fMann-Whitney test. gFisher's Exact Test. P < 0.05 are depicted in bold.

Table 2. Univariate analysis results in CSF

Metabolite	Sex	Age	Diagnosis		
	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	Bonferroni	FDR
L-Arginine	0.0002	0.0000	0.0014	0.0422	0.0422
L-Phenylalanine	0.0078	0.0000	0.0029	0.0881	0.0441
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
Gamma-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-hydroxy-proline	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-Alpha-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

Legend: *P*-values depicted are from an analysis of covariance (ANCOVA) between the study groups (diagnosis) with age and sex as covariates. Metabolites are ranked by *P*-value of diagnosis. In bold: $P < 0.05$ after multiple testing correction. FDR=false discovery rate correction.

Figure 1. CSF L-Arginine levels in participants with migraine and healthy controls



Legend: Individual CSF L-Arginine levels are plotted (dots) with group means \pm SD (bars). Data are adjusted for age. P-values are from post-hoc analysis after ANCOVA.

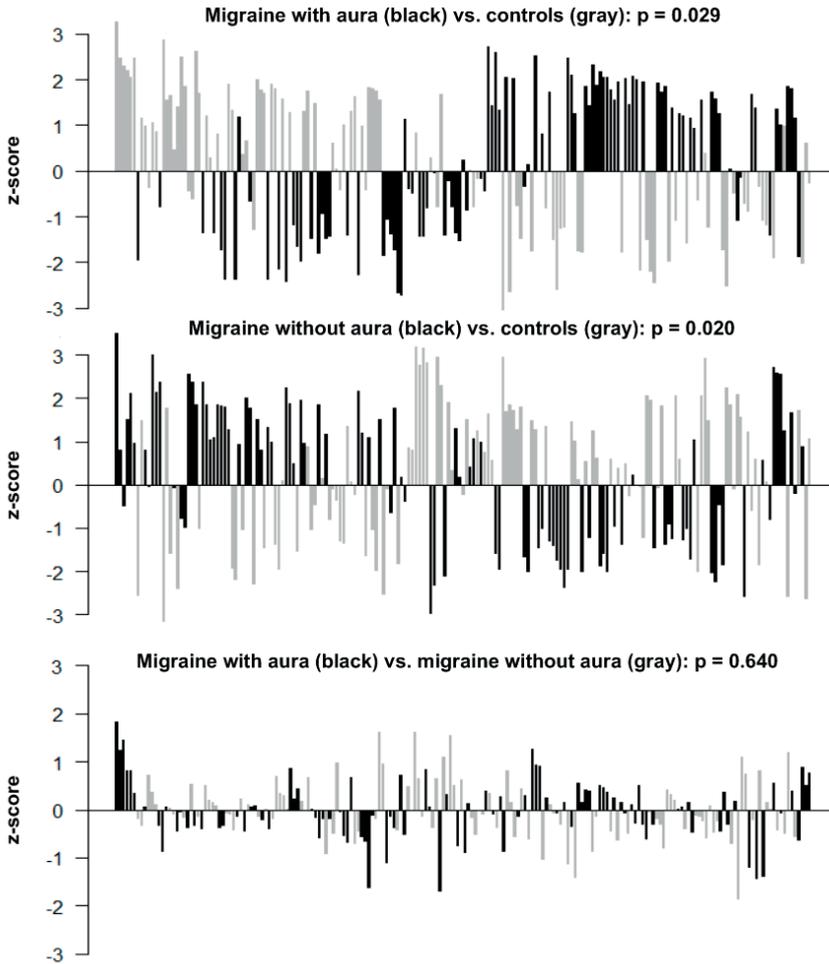
Multivariate results

In CSF, global metabolite profiles differed between groups ($P = 0.035$). While 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 ($P = 0.009$) differed from those in controls (Figure 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 (eFigure 7).

Plasma, results were similar to those in CSF. Profiles differed between groups ($P = 0.021$). While 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 ($P = 0.011$) differed 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 (eFigure 8).

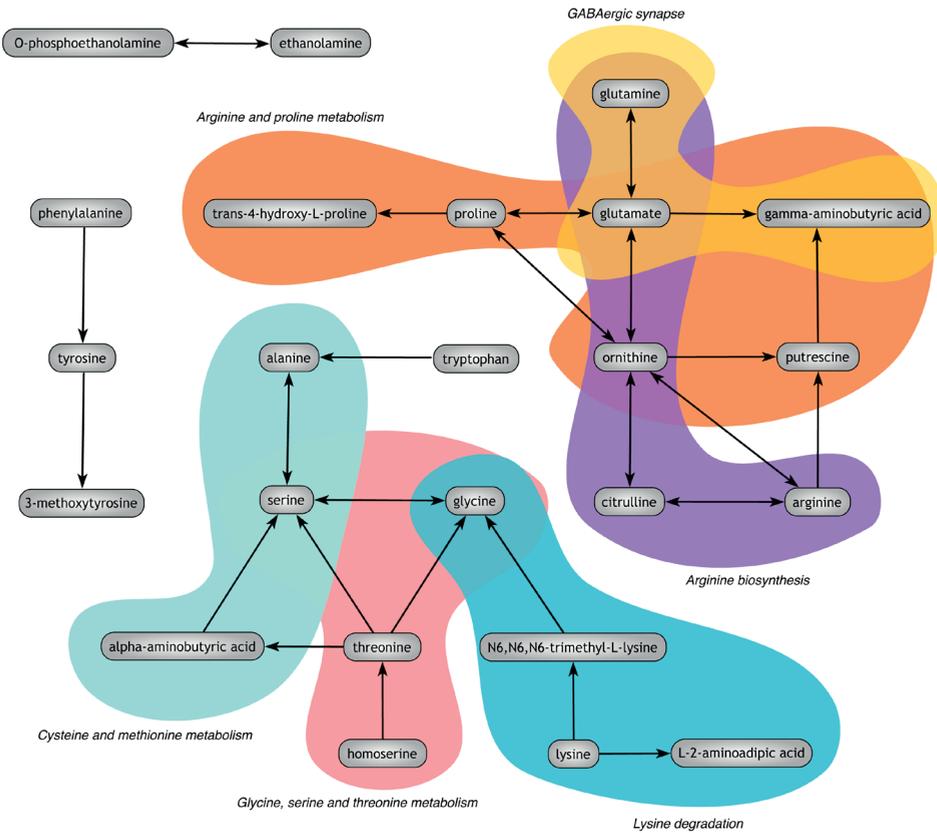
Pathways present in our metabolite set are visualized in Figure 3. In CSF, the pathway “arginine biosynthesis” was significantly associated with migraine with aura (eFigure 9). The “arginine/proline metabolism” pathway showed the strongest association with migraine without aura, but this was not multiple testing resistant. In plasma, strongest associated pathways were “arginine biosynthesis” with migraine with aura and “arginine/proline metabolism” with migraine without aura (eFigure 9), but only significant before multiple testing correction. Exclusion of L-Arginine, to study the effect of other metabolites in these pathways, showed similar results (eTable 5).

Figure 2. Subjects plot of global test with all amines in CSF



Legend: P -values are from the global test. 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).

Figure 3. Metabolic network



Legend: Visualization of the generated GSMM network. Only metabolites that lie within two reaction steps of each other are visualized (thereby excluding L-asparagine, L-methionine, taurine, L-isoleucine, L-leucine, L-valine and L-histidine). Colors indicate KEGG pathways that were tested using the global test approach.

Discussion

We analyzed individual amines, global amine profiles and amine pathways in the CSF and plasma of a uniquely large study population of interictal patients with migraine with or without aura and healthy volunteers. CSF L-Arginine levels were 10.4% reduced in migraine with aura and 5.0% in migraine without aura. Global CSF and plasma amine profiles were similar in migraineurs with and without aura but differed from those of healthy volunteers. Pathway analysis results also point toward L-Arginine metabolism in migraine with aura.

L-Arginine has never been measured in CSF of migraineurs.^{9,21} Previous studies in serum, plasma, platelets, saliva, and urine have produced inconsistent findings across and within biofluids.²²⁻²⁵ The compound is unevenly distributed in the CNS, with the greatest pool in astrocytes, less so in neuronal tissue, and absent in oligodendrocytes.²⁶ L-Arginine is involved in endocrine activity, immune system modulation, regulation of vascular tone, and CNS peptide and protein production.²⁶ In the CNS, metabolism of L-Arginine is closely connected with the metabolism of citrulline and ornithine, that can be synthesized and degraded into each other. Citrulline and ornithine, however, were not abnormal in our study (eFigures 10 and 11). Nonetheless, pathway analysis revealed that although the results seem primarily driven by L-Arginine, there was still an association between migraine and 'arginine biosynthesis' when excluding L-Arginine (although not multiple testing resistant). This suggests that other metabolites from these pathways, including citrulline and ornithine, could still be involved. Catabolism of L-Arginine leads to formation of agmatine, guanidonoacetic acid (ultimately forming creatine) or urea²⁶; all metabolites that could not be measured on our platform.

L-Arginine is also linked to the formation of nitric oxide, which has been implicated in migraine pathophysiology.²⁶ Glycerine trinitrate, a nitric oxide donor, can provoke migraine-like attacks in migraineurs but not in non-migraineurs.²⁷ Nitric oxide elevates the levels of cyclic guanosine monophosphate (cGMP), an important second messenger which is believed to play a central role in migraine pathophysiology, since it is the common pathway of not only glycerin trinitrate but also other migraine provocation models.²⁷ Nitric oxide production is catalyzed by nitric oxide synthase (NOS) for which L-Arginine serves as the only available nitrogen-containing substrate.²⁶ There are three isoforms of NOS: endothelial (e), neuronal (n), and inducible (i) NOS.²⁶ The observed lower CSF L-Arginine concentrations might therefore reflect overactivity of NOS. A non-selective NOS inhibitor was suggested to be effective in spontaneous migraine attacks,^{28,29} while specific iNOS inhibitors failed and trials with combinations of a nNOS blocker and a triptan showed conflicting results.^{27,29,30} Still, our findings offer support that nitric oxide signaling is involved in migraine pathophysiology, also outside attacks. The nitric oxide pathway could be more upstream in the migraine cascade than the CGRP pathway, since CGRP levels were found to be elevated in migraine attacks provoked by nitric oxide.³¹ Furthermore, premonitory symptoms (symptoms which precede migraine headache) are frequently reported in glycerine trinitrate provoked attacks,³² while being rare in CGRP-provoked attacks.³³ Therefore, L-Arginine might be a more upstream target than CGRP.

Using the less conservative FDR correction, L-Phenylalanine CSF levels were lower in both migraine subtypes. Humans cannot synthesize L-Phenylalanine *de novo*.²⁶ 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. We observed no difference in tyrosine, the hydrolyzation product of L-Phenylalanine but tyrosine also has a dietary component.²⁶

Our study has several strengths. Study groups were considerably larger than in other studies⁹ and matched for major confounding factors such as sex, age, and timing of sampling. Control CSF was as normal as possibly could. Unlike in other studies,⁹ controls were truly healthy. CSF was not collected because of neurological symptoms. Controls were also not to have first degree relatives with migraine, to minimize the genetic risk of still developing migraine later on.^{4,34} Finally, where other studies only measured single or a just few molecules, and either in CSF or plasma,⁹ we on the contrary, simultaneously assessed multiple amines in both CSF and plasma, using a dedicated and validated UPLC-MS platform, allowing for additional profile and pathway analyses.

We used a network-based pathway analysis approach to investigate which pathways could 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 e.g. 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 connected through enzymatic steps that do not occur in humans, it would be incorrect to draw statistical conclusions of 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.

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. Moreover, to assess the specificity of our findings, we should also have investigated other non-migraine headaches, e.g. tension-type headache. Furthermore, extended pathway coverage by measuring additional amines and other pathways is needed to improve our understanding of migraine biochemistry. However, we prioritized reliable quantification above a broader range of detection. 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.

Conclusion

Extensive amine profiling in CSF and plasma shows that brain and systemic amine profiles are altered in interictal patients with migraine, similarly in migraineurs with or without aura. Reduced CSF L-Arginine and altered associated pathway analyses suggest dysfunction of nitric oxide signaling in migraine, which may serve as a potential preventive treatment target.

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