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Large-scale plasma metabolome analysis reveals alterations in HDL metabolism in migraine

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Abstract

Objective
To identify a plasma metabolomic biomarker signature for migraine.

Methods
Plasma samples from 8 Dutch cohorts (n = 10,153: 2,800 migraine patients and 7,353 controls) were profiled on a ^{1}H-NMR-based metabolomics platform, to quantify 146 individual metabolites (e.g., lipids, fatty acids, and lipoproteins) and 79 metabolite ratios. Metabolite measures associated with migraine were obtained after single-metabolite logistic regression combined with a random-effects meta-analysis performed in a nonstratified and sex-stratified manner. Next, a global test analysis was performed to identify sets of related metabolites associated with migraine. The Holm procedure was applied to control the family-wise error rate at 5% in single-metabolite and global test analyses.

Results
Decreases in the level of apolipoprotein A1 (β −0.10; 95% confidence interval [CI] −0.16, −0.05; adjusted $p = 0.029$) and free cholesterol to total lipid ratio present in small high-density lipoprotein subspecies (HDL) (β −0.10; 95% CI −0.15, −0.05; adjusted $p = 0.029$) were associated with migraine status. In addition, only in male participants, a decreased level of omega-3 fatty acids (β −0.24; 95% CI −0.36, −0.12; adjusted $p = 0.033$) was associated with migraine. Global test analysis further supported that HDL traits (but not other lipoproteins) were associated with migraine status.

Conclusions
Metabolic profiling of plasma yielded alterations in HDL metabolism in migraine patients and decreased omega-3 fatty acids only in male migraineurs.

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Glossary

apoA1 = apolipoprotein A1; **BBMRI** = Biobanking and BioMolecular resources Research Infrastructure; **BMI** = body mass index; **CI** = confidence interval; **ERF** = Erasmus Rucphen Family study; **^{1}H-NMR** = proton nuclear magnetic resonance; **HDL** = high-density lipoprotein; **HDL-C** = high-density lipoprotein cholesterol; **ICHD** = International Classification of Headache Disorders; **LDL** = low-density lipoprotein; **LDL-C** = low-density lipoprotein cholesterol; **LUMINA** = Leiden University Migraine Neuro-Analysis; **NESDA** = Netherlands Study of Depression and Anxiety; **NTR** = Netherlands Twin Registry; **RS** = Rotterdam Study; **S-HDL-FC** = free cholesterol to total lipid ratio in small high-density lipoprotein ratio; **TMS** = The Maastricht Study; **VLDL** = very low-density lipoprotein.

Migraine is an episodic brain disorder affecting about 15% of the general population, occurs 3 times more frequently in women than men, and is ranked as the second most disabling disease worldwide.[1–4] In one-third of patients, transient focal neurologic symptoms precede the headache (migraine with aura).[1] Migraine, especially in women, has been linked to an increased risk for cerebrovascular and cardiovascular diseases.[5–8] Systemic (micro) vascular dysfunction, but not atherosclerosis,[9,10] has been suggested to be the underlying cause for this association.[11,12]

Previous studies showed elevations of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglyceride levels, and decreases of high-density lipoprotein cholesterol (HDL-C) levels, to be associated with migraine.[13] However, results were not consistently replicated due to methodologic variability,[13] emphasizing the need for a systematic approach. High-throughput proton nuclear magnetic resonance (^{1}H-NMR) allows for the rapid simultaneous identification and quantification of hundreds of metabolite measures in body fluids, providing metabolic profiles in large patient cohorts[14] that hopefully provide more detailed pathophysiologic insight, beyond the traditional blood-based measurements. Identifying circulating biomarkers might provide insights into molecular signature of migraine, and perhaps its relation with cerebrovascular and cardiovascular disease.[12]

We performed large-scale metabolic profiling of plasma on a ^{1}H-NMR platform measuring >220 metabolite measures in 8 large Dutch cohorts.[14] The platform was designed for a detailed assessment of cholesterol measures, triglycerides, creatine, lipids, fatty acids, apolipoproteins, amino acids, glycolysis-related metabolites, and ketone bodies.[14] We aimed to find circulating biomarkers and functionally related metabolite sets in plasma associated with migraine. Furthermore, we investigated these separately for female or male participants.

Methods

Study population

Eight Dutch cohorts, which collaborate in the Dutch Biobanking and BioMolecular resources Research Infrastructure (BBMRI; bbmri.nl/), provided samples: The Leiden University Migraine Neuro-Analysis (LUMINA),[15] The Netherlands Study of Depression and Anxiety (NESDA-1, NESDA-2),[16] The Netherlands Twin Registry (NTR),[17] The Erasmus Rucphen Family study (ERF),[18,19] The Rotterdam Study (RS),[20] The Maastricht Study (TMS),[21] and LifeLines.[22,23] These cohorts include population-based cohorts (NTR, ERF, RS, and LifeLines), web-based (clinic-based) (LUMINA) cohorts, and mixed clinic- and population-based cohorts (NESDA-1, NESDA-2, and TMS). Participants were unrelated, except for NTR and ERF participants. NTR participants included twins, their parents, siblings, and spouses. ERF participants originated from a genetically isolated population in the southwest of the Netherlands. Cases were patients diagnosed with migraine. Probable migraine cases were not included. The control group consisted of participants negative for (probable) migraine. Apart from probable migraine patients, no participants were excluded. Information on migraine symptomatology, used for migraine assessment, was collected by means of surveys based on the International Classification of Headache Disorders (ICHD) criteria (NESDA, NTR, and TMS),[24] self-reported only (LifeLines), or a combination of questionnaires based on the ICHD criteria and a follow-up (telephone) interview (LUMINA, ERF, and RS).[24,25] For details regarding the cohorts, migraine assessments, other relevant disorders, and sampling procedures, see e-Methods (doi.org/10.5061/dryad.p698mn7). All blood samples were measured essentially in one batch in 2014, with the exception of part of the samples from NESDA (the NESDA-2 samples), which were analyzed a few months later.

Standard protocol approvals, registrations, and patient consents

All participants of the respective cohorts provided written informed consent. The study was approved by the local ethics committees of each study.

Metabolite quantification

Metabolites were quantified from EDTA plasma samples of 10,174 individuals (after quality control, 10,153 samples remained), analyzed using the same high-throughput ^{1}H-NMR metabolomics platform (Nightingale Health Ltd., Helsinki, Finland; nightingalehealth.com/).[14] This platform provides simultaneous quantification of 147 individual metabolites and 79 metabolite ratios; for example, routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, esterified fatty acid composition, and various low-molecular metabolites including amino acids, ketone bodies, and gluconeogenesis-related metabolites in molar concentration units. Details of the experimentation and applications of the NMR metabolomics platform have been described previously.[14]

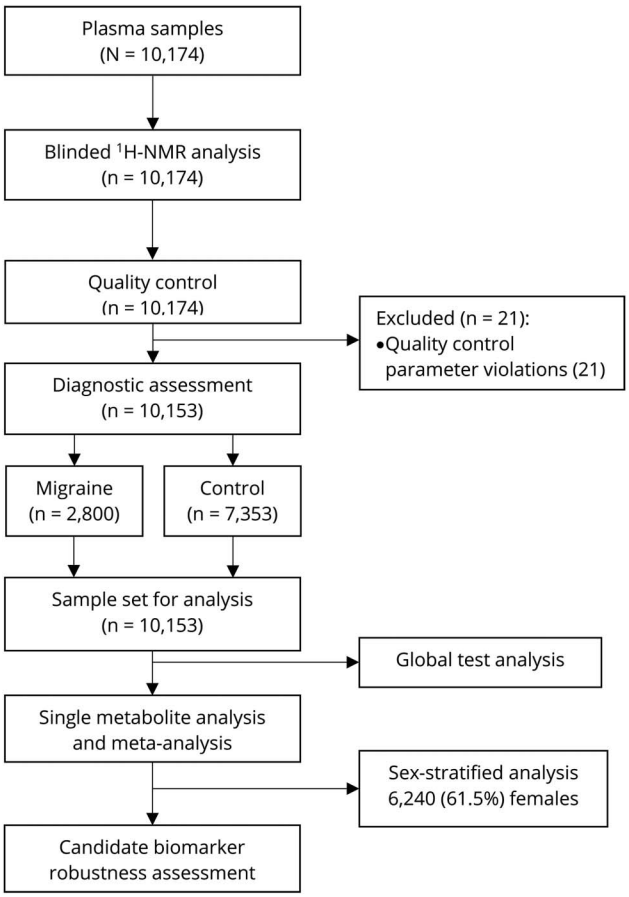
Data preprocessing

The study flowchart is presented in figure 1. Metabolite measures that failed quality control (in particular glutamine, pyruvate, glycerol, β-hydroxybutyrate, and acetate) were excluded from the analysis. Metabolite measures with >10% missing values were excluded entirely. The final set of metabolite measures comprised 146 metabolites and 79 ratios, totaling 225 metabolite measures. Second, outliers (>5 SD) were removed in concordance with previous research in this field.[26] Third, metabolite measurements were raised by 1 to allow log-transformation. Thereafter all metabolite values were log-transformed and scaled to approximate normality using a *z*-transformation prior to the analyses of each cohort. This process was conducted using R 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Single-metabolite logistic regression

For each metabolite measure separately, logistic regression was performed with the metabolite measure, age at blood draw, and sex as independent variables, and migraine status as dependent variable. The obtained estimates and standard errors for the metabolite measures were used in the subsequent random-effects meta-analysis. A random-effects model was chosen to account for possible heterogeneity due to differences in migraine assessment, sample processing, and sample collection between cohorts. Heterogeneity was assessed using the I^2 statistic and by visual inspection of forest plots. The family-wise error rate (the probability of making at least one type I error [false-positive] in a set of measures) was controlled at 5% with the Holm procedure (Holm-Bonferroni).[27] This multiple testing correction procedure was used because it is appropriate in case of strongly correlated measures, as is the case for our 225 metabolite measures. We investigated the influence of familial relatedness on metabolite levels in NTR and ERF using Pearson correlation analysis, but the effect of heritability on metabolite measure estimates (NTR $r = 0.984$ and ERF $r = 0.838$) and p values (NTR $r = 0.972$ and ERF $r = 0.702$) was negligible (figure e-1; doi.org/10.5061/dryad.p698mn7). Therefore, we did not include relatedness in the model. Meta-analyses were conducted with the meta software package for R 3.3.2.

Figure 1 Study flowchart



Determination of the sample set used for data analysis and the different data analysis approaches performed in the current study. ¹H-NMR = proton nuclear magnetic resonance.

Influence of other covariates

First, we independently assessed, within LUMINA and NESDA, the influence of depression, smoking, fasting status, body mass index (BMI), and lipid-lowering medication usage on the metabolite levels of the candidate biomarkers identified in the single-metabolite logistic regression, using stratification plots. LUMINA and NESDA cohorts were selected, because the catalogue of covariates was most complete for these cohorts and because the current migraine assessment (LUMINA) and depression assessment (NESDA) were most accurate and detailed. Furthermore, NESDA was the only cohort that was measured in 2 separate batches. BMI and lipid-lowering medication usage showed to be of influence on the candidate biomarkers and were added to the single-metabolite logistic regression model in all 8 cohorts. Subsequently, meta-analyses were repeated.

Stability measure

We studied the stability of metabolite measures in LUMINA participants (n = 41) that were sampled twice and measured in the same batch on the ¹H-NMR platform.[14] For these participants, time between blood draws ranged from 15 days to almost 4 years (average 833 ± 434 days). To investigate correlation between measurements and assess the effect of time on metabolite levels, the absolute values on the first and second measurement and the value difference between the paired measurements vs the days between the measurements was computed and analyzed with Pearson correlation analysis. p Values <0.05 were regarded as statistically significant. Analyses were performed using SPSS 23.0 (SPSS Inc., IBM, Armonk, NY) and GraphPad Prism version 7.02 for Windows (GraphPad Software, La Jolla, CA).

Sex-stratified analysis

In order to ascertain if the association of metabolites with migraine status may be different between male and female participants, we performed analyses stratified for sex in

accordance with the aforementioned single-metabolite logistic regression and random-effects meta-analysis model with family-wise error rate at 5% controlled using the Holm procedure (Holm-Bonferroni).[27]

Global test analysis

Associations of predefined sets of related metabolites with migraine status were tested with the global test framework,[28,29] adjusted for sex, age at blood draw, BMI, and lipid-lowering medication usage. The global test is aimed at associations between particular sets of (functionally) related metabolites and migraine status and does not test the direction of the association, that is, whether sets of metabolites are upregulated or downregulated. Metabolites were assigned to 23 different groups (tables e-1 and e-2; doi.org/10.5061/dryad.p698mn7) in agreement with the Kyoto Encyclopedia of Genes and Genomes pathways and in accordance with a previous pathway analysis conducted with the same NMR platform.[30] The test statistics for the separate cohorts (p values) from the global test were meta-analyzed using the Fisher combination method.[31] p Values < 0.05 after Holm-Bonferroni correction were considered statistically significant. Statistical analyses were conducted using the global test 5.30.0 software package for R 3.3.2.

Data availability

The data that support the findings of this study will be available in the BBMRI-omics atlas (bbmri.researchlumc.nl/atlas) and in the depository (datadryad.org/review?doi=doi:10.5061/dryad.p698mn7).

Results

Study population

Reliable quantification of 146 blood plasma metabolites and 79 metabolite ratios were available for 10,153 participants from 8 different cohorts: 2,800 migraine patients (80.6% female) and 7,353 controls (54.1% female) (see study flowchart [figure 1]). Clinical characteristics from all cohorts are shown in table 1.

Single-metabolite logistic regression

To identify potential metabolite biomarkers associated with migraine status, we performed a separate logistic regression for each metabolite measure in each cohort (table e-3; doi.org/10.5061/dryad.p698mn7). Corresponding results were used in a random-effects meta-analysis. Migraine was associated with decreased apolipoprotein A1 levels (apoA1, an apoprotein with specific association with high-density lipoprotein [HDL]) (β −0.10, 95% confidence interval [CI] −0.16, −0.05, adjusted $p = 0.029$) and decreased free cholesterol to total lipid ratio in small HDL (S-HDL-FC ratio; β −0.10, 95% CI −0.15, −0.05, adjusted $p = 0.029$) (figure 2). Heterogeneity between cohorts was minimal with $I^2 = 0\%$ for both metabolite measures. A β of −0.10 translates to an odds ratio for having migraine of 1.22 when comparing an individual with a typical low metabolite score ($z = -1$ or 1 SD below average) and an individual with a typical high metabolite score (z = 1 or 1 SD above average). Other HDL particle measures (XL-HDL–[C, CE, FC, L, P, and PL], L-HDL–[C, CE, FC, L, P, PL, and TG], total cholesterol in HDL and HDL2, the mean diameter for HDL particles, and the total cholesterol to total lipids ratio in very large HDL) were also reduced in migraine, but failed to reach significance after correction for multiple comparisons (table e-4; doi.org/10.5061/dryad.p698mn7). Despite the high negative correlation between HDL and very low-density lipoprotein (VLDL) or low-density lipoprotein (LDL) measures, only a few associations with LDL or VLDL measures were found nominally significant and none remained significant after correction for multiple comparisons.

Candidate biomarker robustness assessment

Next, we assessed the influence of smoking, fasting status, depression, lipid-lowering medication usage, and BMI (figures e-2–e-6; doi.org/10.5061/dryad.p698mn7) on apoA1 levels and the S-HDL-FC ratio in the LUMINA and NESDA cohorts. Small effects of lipid-lowering medication usage and BMI on apoA1 and S-HDL-FC ratio plasma levels were identified. Other covariates did not influence these levels. For all cohorts, BMI and lipid-lowering medication usage were subsequently added to our model. The expanded model revealed that a decreased apoA1 level (β −0.092, 95% CI −0.15, −0.04) and S-HDL-FC ratio (β −0.068, 95% CI −0.12, −0.02) were still associated (uncorrected p values 0.0010 and 0.0095) with migraine. To further support the robustness of the candidate biomarkers, correlation analyses using 82 samples from 41 participants, acquired on 2 occasions (833 ± 434 days apart), revealed particularly stable results between measurements in the same individual patient for apoA1 ($r = 0.859$) and to a lesser extent for S-HDL-FC ratio ($r = 0.497$) (figure e-7; doi.org/10.5061/dryad.p698mn7).

Sex-stratified analysis

Given the preponderance of females among migraine patients, we searched for possible differences in the metabolite profile associated with migraine between male and female participants (figure 3). ApoA1 levels were significantly associated with migraine in male participants, with smaller effects, but in similar direction, in female participants. Furthermore, the apoB/apoA1 ratio was significantly higher in female migraineurs compared to female controls. The S-HDL-FC ratio (table e-5; doi.org/10.5061/dryad.p698mn7) was negatively associated with migraine in female participants, but failed to reach significance after correction for multiple testing. In male participants, no apparent relation was identified for the S-HDL-FC ratio. Associations with lower medium and large HDL measures (L-HDL–[C, CE, FC, L, P, and PL]) were significant in female participants, with a similar finding in male participants, although not significant. Interestingly, in male participants, lower omega-3 fatty acids ($p = 0.033$) were associated with migraine, an association not seen in female participants. Clinical characteristics from all cohorts stratified for sex and the sex-stratified meta-analysis are shown in table 1.

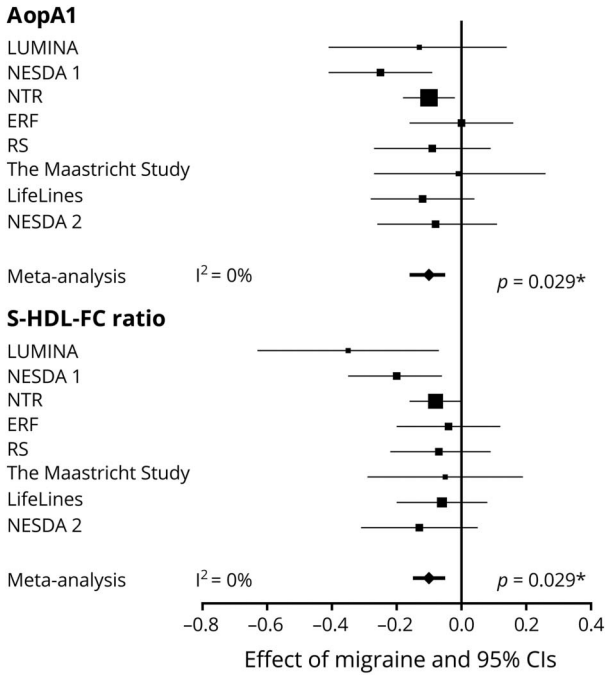
Table 1 Baseline characteristics of the study populations

	LUMINA (n = 408)				NESDA-1 (n = 1,082)				NTR (n = 2,873)				ERF (n = 1,413)			
	Cases (n = 317)		Controls (n = 91)		Cases (n = 276)		Controls (n = 806)		Cases (n = 1,360)		Controls (n = 1,513)		Cases (n = 178)		Controls (n = 1,235)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Total, n	105	212	47	44	48	228	353	453	217	1143	571	942	39	139	598	637
Age, y, mean ± SD	44.6 ± 13.0	42.4 ± 12.1	42.1 ± 13.9	36.2 ± 14.0	41.7 ± 11.6	39.7 ± 11.3	44.1 ± 12.8	41.9 ± 13.8	44.5 ± 14.0	41.4 ± 12.7	40.4 ± 14.4	39.3 ± 13.6	46.6 ± 11.9	45.8 ± 12.3	48.8 ± 14.0	48.3 ± 14.5
BMI, kg/m^2, mean ± SD	24.5 ± 2.6	23.9 ± 3.8	24.2 ± 2.7	23.4 ± 3.4	26.5 ± 5.1	25.5 ± 5.2	26.1 ± 4.5	25.1 ± 5.0	25.2 ± 3.9	24.8 ± 4.5	24.9 ± 3.4	23.9 ± 3.9	28.0 ± 5.5	27.2 ± 5.6	27.3 ± 4.3	26.4 ± 4.9
LLMU, n	1	5	2	0	8	12	40	20	22	37	37	41	3	16	73	64

	RS (n = 1,425)				TMS (n = 687)				LifeLines (n = 1,319)				NESDA-2 (n = 946)			
	Cases (n = 173)		Controls (n = 1,252)		Cases (n = 79)		Controls (n = 608)		Cases (n = 249)		Controls (n = 1,070)		Cases (n = 168)		Controls (n = 778)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Total, n	29	144	556	696	27	52	458	150	49	200	504	566	27	141	285	493
Age, y, mean ± SD	77.4 ± 4.2	79.3 ± 5.1	79.3 ± 4.6	79.5 ± 4.9	59.2 ± 8.5	61.5 ± 7.1	63.2 ± 7.3	61.8 ± 8.1	44.1 ± 11.1	43.3 ± 12.3	44.8 ± 14.1	43.9 ± 13.8	39.8 ± 9.8	41.8 ± 12.4	44.7 ± 13.5	42.7 ± 13.6
BMI, kg/m^2, mean ± SD	26.9 ± 3.0	27.6 ± 4.6	27.0 ± 3.3	27.8 ± 4.3	28.0 ± 3.4	30.0 ± 5.8	29.6 ± 4.7	30.3 ± 5.5	26.1 ± 3.3	25.6 ± 5.2	25.3 ± 3.4	24.8 ± 4.2	26.7 ± 5.2	24.8 ± 4.7	25.9 ± 4.2	24.9 ± 4.7
LLMU, n	8	31	142	153	21	31	335	113	0	8	29	20	0	4	32	36

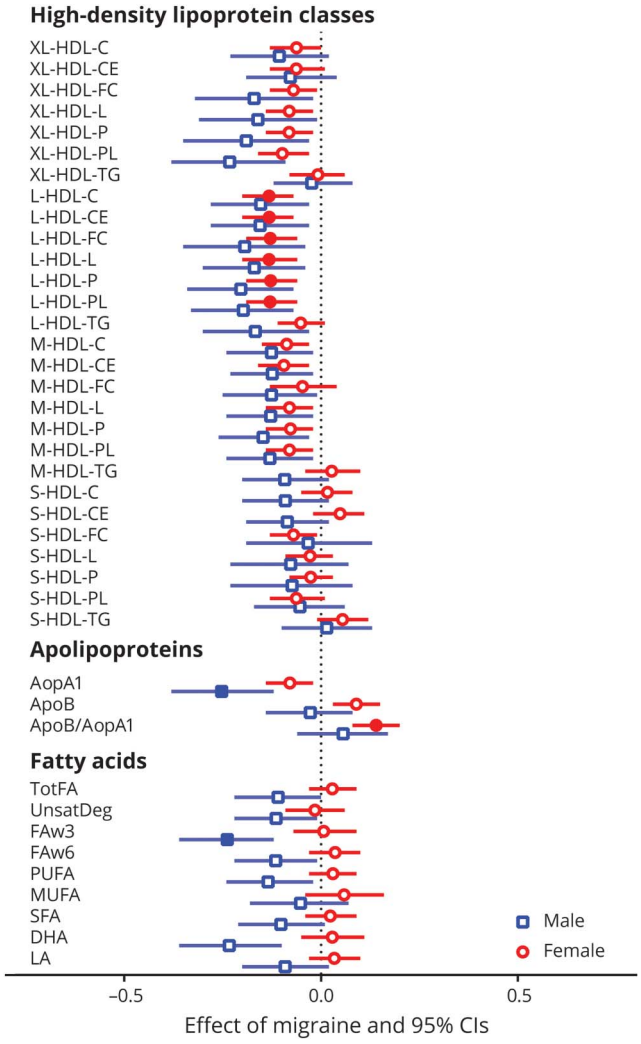
Abbreviations: BMI = body mass index; ERF = Erasmus Rucphen Family study; LLMU = lipid-lowering medication usage; LUMINA = Leiden University Migraine Neuro-Analysis; NESDA = Netherlands Study of Depression and Anxiety; NTR = Netherlands Twin Registry; RS = Rotterdam Study; TMS = The Maastricht Study.

Figure 2 Forest plots of candidate migraine biomarkers apolipoprotein A1 (apoA1) and the free cholesterol to total lipid ratio in small high-density lipoprotein ratio (S-HDL-FC)



Associations with migraine in random-effects meta-analyses. The effect sizes and 95% confidence intervals (CIs) for apoA1 and S-HDL-FC are presented per cohort and in a random-effects meta-analysis. Values from logistic regression with metabolite levels, sex, and age as independent variables and migraine status as dependent variable. Error bars denote 95% CIs. To facilitate the interpretation of the effect sizes (β coefficients), we calculated the odds ratio (OR) for having migraine for a typical low metabolite score (*z* score = −1, 1 SD below average) and a typical high metabolite score: β −0.10, OR 1.22; β −0.20, OR 1.49; β −0.30, OR 1.82; β −0.40, OR 2.22; β −0.50, OR 2.72. **p* Values after Holm-Bonferroni ($p < 0.0002$) multiple testing correction. ERF = Erasmus Rucphen Family study; I^2 = measure of heterogeneity; LUMINA = Leiden University Migraine Neuro-Analysis; NESDA = Netherlands Study of Depression and Anxiety 1 and 2; NTR = Netherlands Twin Registry; RS = Rotterdam Study.

Figure 3 Sex-stratified metabolite associations with migraine

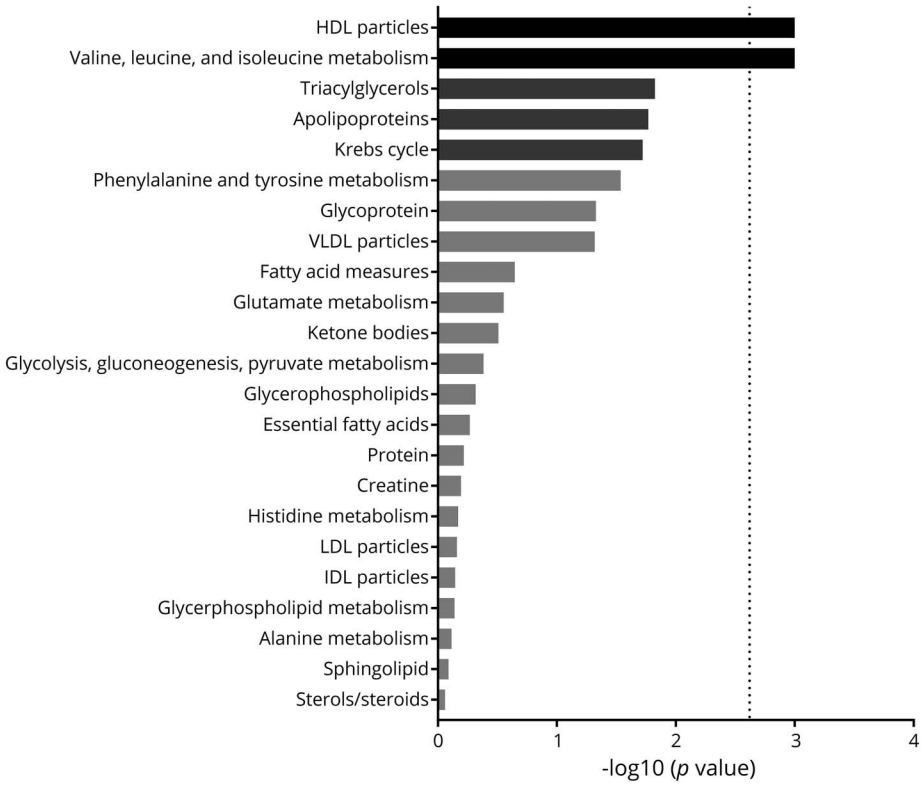


Metabolite associations with migraine in male (blue squares) and female (red circles) participants in a random-effects meta-analysis comprised of 8 cohorts. The effect sizes and 95% confidence intervals (CIs) are shown. Values are from logistic regression with metabolite levels, sex, age, body mass index, and lipid-lowering medication usage as independent variables and migraine status as dependent variable. Error bars denote 95% CIs, filled squares (male participants) or circles (female participants) indicate significance after Holm-Bonferroni ($p < 0.0002$) multiple testing correction. All other metabolite classes without significant metabolites after Holm-Bonferroni correction as well as I^2 values can be found in table e-5 (doi.org/10.5061/dryad.p698mn7). To facilitate the interpretation of the effect sizes (β coefficients), we calculated the odds ratio (OR) for having migraine for a typical low metabolite score (*z* score = −1, 1 SD below average) and a typical high metabolite score: β −0.10, OR 1.22; β −0.20, OR 1.49; β −0.30, OR 1.82; β −0.40, OR 2.22; β −0.50, OR 2.72. All metabolite abbreviations can be found in tables e-1 and e-2 (doi.org/10.5061/dryad.p698mn7).

Global test analysis

To detect if migraine status was associated with particular sets of (functionally) related metabolites, we tested the association of 23 different predefined sets of metabolites with migraine status using the global test. The global test does not evaluate each metabolite measure individually, but tests whether the levels of a group of metabolites are associated with an outcome (in this case, migraine status). We controlled for the same covariates as in the logistic regression per metabolite. The global test was first applied per cohort, after which the *p* values were combined in a meta-analysis using the Fisher method (figure 4 and table 2). The global test analysis confirmed the association of HDL-associated metabolites with migraine, already apparent from the single metabolite analysis, with large clusters of medium (M−) to very large (XL−) HDL subclasses generally associated with migraine status across the majority of cohorts (figure e-8; doi.org/10.5061/dryad.p698mn7). Interestingly, no other lipoprotein classes were associated with migraine. Somewhat surprisingly, the metabolism of valine, leucine, and isoleucine was significantly associated with migraine, and not in line with the findings from the single-metabolite analyses. This is a false-positive result, obtained because this meta-analysis method is based on nondirectional *p* values, and may provide a significant *p* value even when the direction of change is not consistent between cohorts, as is the case for these branched chain amino acids.

Figure 4 Global test analysis



Meta-analysis of the results of the 23 sets of (functionally) related metabolites tested in 8 cohorts for their association with migraine. The analysis using the global test framework has been adjusted for a sex, age, body mass index, and lipid-lowering medication usage. Glutamate metabolism does not include results from the Netherlands Twin Registry as glutamine levels could not be determined in this cohort. Bars denote $-\log^{10}$ of the *p* value (Fisher combination of global test *p* values for the individual cohorts) per pathway, where only the black bars remain significant after multiple testing correction using Holm-Bonferroni. The threshold for withstanding multiple testing correction is indicated by a dotted line. IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

Discussion

We performed high-throughput ^{1}H-NMR metabolite profiling of 225 metabolite measures in plasma samples from 8 Dutch cohorts and identified a consistent association between migraine and decreased HDL levels. We identified 2 circulating candidate migraine biomarkers, which are both related to HDL status: a decreased level of apoA1 (an apoprotein with a specific association with HDL) and a decreased S-HDL-FC ratio (the free cholesterol to total lipid ratio in small HDL). In addition, fatty acids of the omega-3 class were shown to be associated with migraine, but only in male participants.

Dyslipidemia and migraine have been extensively studied because of the comorbidity of cerebrovascular and cardiovascular disease and migraine, with the strongest associations in young women without elevated conventional cardiovascular risk profiles.[5–8,13] Large population-based studies suggested elevated total cholesterol, LDL-C, or triglycerides, and decreased levels of HDL-C, in migraine.[5–8,13] However, earlier results were conflicting[13] due to cohort variability and measurement of crude lipoprotein levels,[5–8,13] or failed to detect differences in apoA1 levels, possibly due to lack of power.[32,33] Notably, the sufficiently powered Women's Health Study observed a nonsignificant effect with decreased apoA1 levels in 5,087 female participants with a history of migraine (total population 27,626, mean age 54.7 years).[34] A more prominent association between migraine and apoA1 in men compared to women, and lower mean age in the current study, might explain the difference between the studies. To the best of our knowledge, lower omega-3 fatty acid levels have not been reported in migraine. Of note, omega-3 fatty acid supplements, due to their anti-inflammatory action, have been investigated in migraine attack prevention.[35] A recent meta-analysis found no apparent reduction in headache frequency after omega-3 fatty acid supplementation; however, a significant reduction in headache duration was found across studies.[35]

HDL subclasses are composed of proteins and lipids, each roughly representing 50% of the total mass of HDL. Major proteins are apoA1 (70%) and apoA2 (20%) together with proteins such as apoA4, apoE, apoJ, haptoglobin, paraoxanase, α2-macroglobulin, and lecithin cholesterol acyltransferase.[36] These proteins contribute to various functions of HDL, including mediating the reverse cholesterol transport pathway and antioxidative, anti-inflammatory, and antithrombotic effects.[37] Combining the different analyses conducted, we identified an association between deceased apoA1 level and S-HDL-FC ratio and migraine together with decreased levels of medium to very large HDL measures, in the absence of clear LDL, intermediate-density lipoprotein, or VLDL involvement. Thus, the observed profiles suggest that migraine is associated with alterations in specific HDL functions but not with a general dyslipidemia profile characteristic for cardiovascular conditions.

Although this biomarker discovery study was not aimed to unravel pathophysiologic mechanisms, several hypotheses

Table 2 Cohort results of global test analysis and Fisher combination method

	LUMINA, *p* value	NESDA-1, *p* value	NTR, *p* value	ERF, *p* value	RS, *p* value	TMS, *p* value	LifeLines, *p* value	NESDA-2, *p* value	Fisher method	H-B corrected *p* value
HDL particles	0.059	0.0091	0.047	0.052	0.228	0.372	0.059	0.448	0.00098	0.022
Valine, leucine, isoleucine metabolism	0.018	0.068	0.246	0.108	0.964	0.00014	0.743	0.766	0.00088	0.020
Triacylglycerols	0.099	0.507	0.567	0.225	0.236	0.473	0.00088	0.374	0.015	0.325
Apolipoproteins	0.059	0.023	0.133	0.056	0.570	0.609	0.123	0.631	0.017	0.336
Krebs cycle	0.118	0.254	0.066	0.0041	0.399	0.445	0.382	0.591	0.019	0.356
Phenylalanine and tyrosine metabolism	0.0036	0.795	0.429	0.039	0.315	0.271	0.400	0.436	0.029	0.524
Glycoprotein	0.015	0.115	0.415	0.484	0.849	0.104	0.966	0.059	0.047	0.804
VLDL particles	0.062	0.406	0.396	0.481	0.461	0.538	0.0050	0.295	0.048	0.804
Fatty acid measures	0.283	0.271	0.153	0.294	0.607	0.735	0.057	0.544	0.226	1.000
Glutamate metabolism[a]	0.190	0.069	NA	0.369	0.478	0.706	0.259	0.601	0.280	1.000
Ketone bodies	0.279	0.035	0.806	0.568	0.213	0.768	0.290	0.523	0.310	1.000
Glycolysis, gluconeogenesis, pyruvate metabolism	0.826	0.820	0.182	0.045	0.899	0.093	0.586	0.927	0.413	1.000
Glycerophospholipids	0.660	0.166	0.068	0.645	0.827	0.521	0.404	0.490	0.482	1.000
Essential fatty acids	0.608	0.045	0.663	0.416	0.483	0.775	0.645	0.334	0.539	1.000
Protein	0.639	0.777	0.086	0.591	0.179	0.451	0.588	0.800	0.606	1.000
Creatine	0.617	0.994	0.352	0.068	0.194	0.528	0.966	0.822	0.639	1.000
Histidine metabolism	0.957	0.873	0.549	0.520	0.987	0.330	0.605	0.033	0.676	1.000
LDL particles	0.205	0.378	0.775	0.122	0.972	0.866	0.443	0.626	0.691	1.000
IDL particles	0.163	0.376	0.673	0.292	0.900	0.925	0.352	0.576	0.716	1.000
Glycerolipid metabolism	0.536	0.244	0.237	0.426	0.658	0.622	0.705	0.563	0.724	1.000
Alanine metabolism	0.408	0.588	0.812	0.353	0.906	0.130	0.590	0.633	0.770	1.000
Sphingolipids	0.164	0.383	0.306	0.909	0.550	0.776	0.781	0.736	0.816	1.000
Sterols/steroids	0.232	0.428	0.965	0.268	0.922	0.880	0.343	0.981	0.871	1.000

Abbreviations: ERF = Erasmus Rucphen Family study; H-B = Holm-Bonferroni; HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; LUMINA = Leiden University Migraine Neuro-Analysis; NA = not applicable; NESDA = Netherlands Study of Depression and Anxiety; NTR = Netherlands Twin Registry; RS = Rotterdam Study; TMS = The Maastricht Study; VLDL = very low-density lipoprotein.
Corrected for age, sex, body mass index, and use of lipid-lowering medication.
[a] Determined without NTR due to nonquantified glutamine measurements.

regarding pathophysiological mechanisms emerge from the study. First, our findings provide some biochemical evidence for a link with endothelium dysfunction in migraine as HDL with its antioxidative, anti-inflammatory, and antithrombotic effects plays a role in endothelial function.[36–38] Interestingly, omega-3 fatty acids, which showed decreased levels associated with migraine in male participants, have also been shown to be vasoprotective and have been deemed to generate anti-inflammatory resolvins.[39] It is at this point, however, only speculation whether reduced protective actions of HDL and omega-3 through endothelial dysfunction may explain the association with migraine. Second, it has been suggested that omega-3 fatty acids and certain HDL subclasses can travel across the blood–brain barrier, which may have effects on a neuronal level.[40–42] Third, that omega-3 fatty acids are associated with migraine exclusively in male participants may suggest distinct sex-specific mechanisms. However, this might also be due to differences in omega-3-rich food consumption[43] and requires further specific investigation.

The strengths of this study are the large sample size (>10,000 participants) and extensive metabolic profiling (225 metabolite measures) to identify candidate biochemical biomarkers for migraine. Furthermore, similar methods (EDTA samples, ^{1}H-NMR platform, and facility) were used across cohorts. A possible limitation of the study is that migraine status was assessed with varying degrees of detail in the various cohorts, which also made us unable to look into possible differences between migraine with and without aura. Still, many cohorts used validated questionnaires based on ICHD criteria and have shown their effectiveness and precision in diagnosing migraine,[1,15,18] which is why metabolite measure associations with migraine were chosen as main study outcome. To make a clear distinction between definite migraineurs and non-migraine participants, we excluded probable migraine cases whenever possible. Additional variability due to sampling protocols used, foremost time-to-freezer and centrifuge settings, we aimed to control for by using meta-analysis approaches with a random-effects model. The low heterogeneity seen in the random-effects meta-analysis, in particular for the candidate biomarkers, seems to indicate that the aforementioned variability only had a limited influence on the study outcome. Our top metabolites related to HDL concentrations are known to be affected by BMI. Although we corrected for BMI in our analysis, we cannot exclude a residual confounding effect of this variable nor of any other variable that we have not tested. However, the robustness of our finding across different cohorts that differed in BMI distributions makes it likely that the HDL-related traits are truly associated with migraine. Genetic variability was limited because all cohorts were comprised predominantly of participants from the Netherlands, with Western European ancestry, but as a direct consequence the generalizability of our findings to other populations may be limited. The current study design does not allow the study of causality. Intervention or animal studies are needed to further explore the interplay between BMI, HDL-related traits, and migraine. Another limitation of our multicohort design using distributed data analysis algorithms is that we cannot make definitive estimates of the sensitivity and specificity of the candidate biomarkers.

The current study illustrates the power of detailed metabolite profiling for biomarker discovery in a large meta-analytic design, pointing towards consistent associations of mainly medium to very large HDL measures with migraine. Furthermore, we identified a male-specific association between migraine status and omega-3 fatty acids. Our study suggests that alterations in HDL metabolism may be involved in the association between migraine and cerebrovascular and cardiovascular disease.

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Appendix *(continued)*

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Appendix *(continued)*

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Continued

Appendix *(continued)*

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Gisela M. Terwindt, MD, PhD	Leiden University Medical Center, the Netherlands	Author	Contributed to design and conceptualization of the study, contributed to data collection and interpretation, interpreted the data, and revised the manuscript

Appendix *(continued)*

Name	Location	Role	Contributions
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