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Metabolic Dysfunction in New-Onset Idiopathic Intracranial Hypertension: Identification of Novel Biomarkers

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Objective: Idiopathic intracranial hypertension (IIH) is a neurometabolic disease with an increasing incidence. The pathophysiology is unknown, but improvement of diagnosis and management requires discovery of novel biomarkers. Our objective was to identify such candidate biomarkers in IIH, and secondarily, test for associations between identified metabolites and disease severity.

Methods: This is a prospective case–control study with collection of cerebrospinal fluid (CSF), serum, and clinical data from new-onset, treatment-naïve patients with IIH (n = 60). Patients were included consecutively from 2 tertiary headache centers in Denmark, and age, sex, and body mass index (BMI) -matched healthy controls (n = 35) were recruited. Clinical data were retrieved at ocular remission (n = 55). Samples were analyzed using non-targeted mass spectrometry.

Results: Serum sphingosine 1-phosphate (S1P), adenosine, and glutamate were 0.46-fold ($q < 0.0001$), 0.25-fold ($q = 0.0048$), and 0.44-fold ($q < 0.0001$) lower, respectively, in IIH. CSF stearoyl-lysophosphatidylcholine (LysoPC-18) and 2-palmitoyl-lysophosphatidylcholine (LysoPC-16) were 0.42 ($q = 0.0025$) and 0.37 ($q < 0.001$) -fold lower. LysoPC-18 was higher in patients with moderate–severe versus mild papilledema ($p = 0.022$). LysoPC-18 correlated positively with retinal nerve fiber layer thickness ($p = 0.0012$, $r = 0.42$) and inversely with mean deviation on automated perimetry ($p = 0.01$, $r = -0.35$). Higher baseline serum S1P ($p = 0.018$) and lower CSF LysoPC-16 ($p = 0.003$) were associated with optic nerve atrophy at ocular remission. Pathway analysis suggests dysregulated lipid metabolism and redox disturbances in new-onset IIH.

Interpretation: We identify perturbed metabolism in new-onset IIH. S1P and LysoPC-16 demonstrate potential prognostic value due to association with subsequent optic nerve atrophy. This association between specific, differential metabolites and outcome provides substantial evidence for novel biomarkers of clinical significance that should be the focus of further targeted studies.

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Idiopathic intracranial hypertension (IIH) is a neuro-ophthalmological condition intricately linked with obesity, female sex, and reproductive age.[1,2] Chronic elevation of intracranial pressure (ICP) causes papilledema, headache, and cognitive disturbances with a risk of permanent visual loss; however, the pathophysiological mechanisms remain unknown.[1–4] Scientific interest in IIH is increasing, as the burden of disease grows with pandemic obesity, but also because the study of IIH can provide novel insights in ICP regulation.[5] The simplistic understanding of IIH as merely a neuro-ophthalmological disease has been challenged by studies associating metabolic disturbances with IIH, thus suggesting IIH as a systemic, neurometabolic disorder.[6,7] IIH is associated with an elevated risk of cardiovascular disease and features of insulin resistance, which exceeds that associated with simple obesity.[7–9] Adipocytes from patients with IIH show a distinct phenotype of transcriptional priming towards storage of calories as adipose mass, compared to other obese individuals, and specifically the truncal fat mass—often associated with metabolic dysfunction—correlates with ICP in IIH.[7,10] Excess levels of specific androgens have been found in cerebrospinal fluid (CSF) from women with IIH.[9] In a cohort of IIH patients with mean disease duration of 1 year, metabolic dysfunction related to ketones, urea, and acetate is found and linked to headache and ICP dynamics.[6] Even with these advances, no diagnostic or prognostic biochemical markers exist, and diagnosis is based on exclusion of differential diagnoses. Novel, targeted therapies and improved management necessitates discovery of candidate biomarkers with pathophysiological, diagnostic, or prognostic significance.

We conducted a prospective study using untargeted metabolomics on new-onset, treatment-naïve patients with IIH, with the aim of identifying differential metabolites in serum or CSF at diagnosis. Important secondary outcomes included correlation of metabolites to disease severity and clinical phenotype.

Materials and Methods

Study Population

This was a prospective case–control study. Patients with suspected, new-onset IIH were consecutively recruited from 2 tertiary centers in Denmark, The Danish Headache Center, Rigshospitalet-Glostrup and Department of Neurology, Odense University Hospital (April 2018–April 2021). Controls were recruited via social media platforms (July 2020–May 2021). Inclusion criteria were ≥ 18 years of age, clinical suspicion of new-onset IIH with papilledema[11] (patients) and written, informed consent. After the baseline diagnostic work-up, IIH was confirmed or excluded based on the diagnostic criteria.[2] Exclusion criteria were: non-IIH (patients only), pregnancy, breastfeeding, previous IIH, IIH without papilledema, secondary causes of elevated ICP (eg, cerebral sinus venous thrombosis). Healthy controls, with matching age, sex, and BMI had ≤1 headache day/week and no symptoms associated with ICP-elevation. The healthy controls participated exclusively for research purposes. The controls were compensated for time spent, the invasive nature of the procedure and expenses related to transportation (equal to 365 US dollars excluding transportation). This compensation was pre-approved by the local ethical committee according to national regulation.

Diagnostic Work-Up and Follow-Up

Patients underwent a standardized diagnostic work-up (visit 1, Fig 1) to confirm or exclude IIH with papilledema according to the diagnostic criteria[1,2] consisting of a structured medical interview, physical exam, lumbar puncture, neuro-ophthalmological exam by a consultant neuro-ophthalmologist (fundus imaging, slit lamp exam, optic coherence tomography (OCT), Ishihara plates, visual acuity and fields), routine blood samples and neuroimaging (cerebral venography, cerebral MRI). A systematic phone interview by a medical professional assessed eligibility of controls and was followed by an in-person structured, medical interview, physical exam, lumbar puncture, and scanning laser ophthalmoscopy to exclude papilledema (Compass®). Neuroimaging was not done in healthy controls. Included patients received standard clinical care and were evaluated by a neuro-ophthalmologist at ocular remission (see supplemental methods), defined as complete resolution of papilledema (visit 2a, Fig 1). In a subset of patients CSF and serum samples were repeated after significant clinical improvement (resolution/improvement of papilledema) (visit 2b, Fig 1). Visit 2b was independent of visit 2a, and included CSF and serum samples from patients who were having a follow-up lumbar puncture on a clinical indication and had significant clinical improvement compared to baseline. The protocol did not allow sampling solely for research purposes at visit 2a; therefore, follow-up samples were only included in a minority of the cohort.

Collection of Research Samples

Blood samples to screen for coagulability disorders (INR, hemoglobin, thrombocytes) were taken before lumbar puncture in patients and controls. Lumbar puncture with CSF analysis (cells, glucose, protein) and opening pressure (OP) for patients and controls was standardized and done by an experienced physician with the participant placed in left, lateral decubitus position, legs and neck extended. OP was measured with a standard issue manometer in the relaxed participant after an observational period of at least 1 minute. CSF (4 ml) was collected in polystyrene tubes, centrifuged at 400G (20°C, 10 minutes). Serum (6 ml) was collected in BD Vacutainer® Serum tubes with silica clot activator and centrifuged at 2000G (20°C, 10 minutes). The supernatant from both was aliquoted in Sarstedt® tubes and stored at −80°C. Time to freeze was maximum 3 hours.

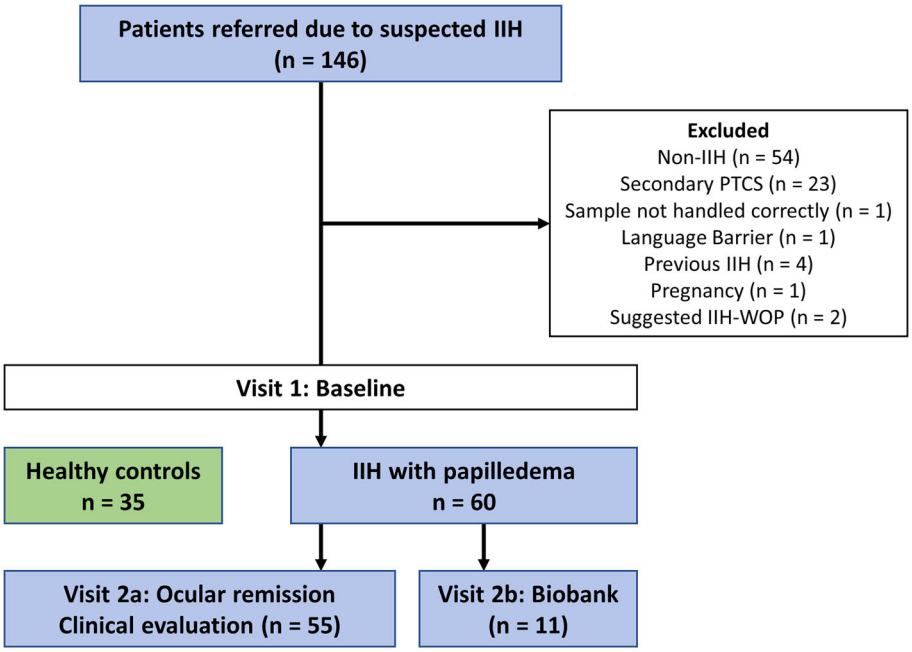

FIGURE 1: Inclusion of patients. Healthy controls were recruited on social media platforms and matched for age, sex, and BMI. Visit 2a (ocular remission) was a neuro-ophthalmological evaluation at time of ocular remission (no papilledema). Of 60 included patients, 55 were included at visit 2a (1 loss to follow-up moved abroad, 3 did not enter remission before conclusion of study, 1 patient excluded from ophthalmological review due to concomitant papilledema and significant optic disc drusen). Visit 2b was independent of visit 2a, and only included patients in whom a new lumbar puncture was done after significant, clinical improvement. The alternative diagnoses in the non-IIH and the secondary PTCS groups can be found in Table S1. Abbreviations: IIH = idiopathic intracranial hypertension; IIH-WOP = idiopathic intracranial hypertension without papilledema; PTCS = pseudotumor cerebri syndrome. [Color figure can be viewed at www.annalsofneurology.org]

Neuro-Ophthalmological Evaluation

Papilledema was graded according to Frisén[12] in 5 categories by a neuro-ophthalmologist (L.D.M., S.M.H., E.A.W.). Moderate–severe papilledema was defined as Frisén grade 3–5. Visual fields, with perimetric mean deviation (PMD), were performed by automated computer perimetry (Humphrey 24–2 Field Analyzer at OUH and 30–2 dynamic program, OCTOPUS 900; Haag-Streit in DHC). Visual fields were evaluated by a neuro-ophthalmologist and excluded in the case of sub-optimal compliance. Only visual fields with a reliability factor below 25% were included. OCT was used to image the posterior segment of the eye, and was done on Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany). Investigators repeated scans until sufficient quality was obtained (signal to noise ratio greater than 25 dB). Heidelberg Spectralis eye tracking and automated real-time averaging features were used (macula, optic nerve head). For the optic nerve head, high resolution imaging was done with at least 30 frames. Macular scans were in high-speed mode and $20^\circ \times 20^\circ$ with fovea in the center (5.9 mm), and scans were recorded with at least 9 frames. RNFL scans were 12.0° (3.5 mm) circle scans. GCL and RNFL measurements were done with "Heidelberg Eye Explorer" provided by the instrument manufacturer. Semi-automatic segmentation was used for GCL measurement[13] (visit 1, Fig 1). Optic nerve atrophy was concluded in cases with structural and functional indications of atrophy to the optic nerve: an appearance of pallor and sharp edges of the optic nerve head on fundus pictures supported by RNFL and/or GCL thinning. In cases of doubt or missing color fundus picture patients with RNFL and GCL thinning compared to normal values in the corresponding age group were concluded to have atrophy if this was in accordance with the corresponding visual fields. All neuro-ophthalmological outcomes, except for bilateral atrophy, were assessed by worst eye.

Clinical Correlations

To explore differentially expressed metabolites in serum and CSF, and their role in IIH pathophysiology, we investigated potential associations to disease severity and clinical phenotype (BMI) at baseline and ocular remission. Disease severity at baseline (visit 1, Fig 1) was defined by the following outcomes: Monthly headache days, headache severity (VAS score), OP, degree of papilledema (Frisén grade and RNFL), GCL and visual fields (PMD). Ocular outcomes (PMD, optic nerve atrophy, RNFL, GCL) were assessed ocular remission (visit 2a, Fig 1), where ocular remission was defined by complete resolution of papilledema.

Metabolomic Analysis

Serum and CSF underwent non-targeted ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) in negative and positive ion modes following monophasic extraction of metabolites. Data were assessed using MetaboAnalyst to assess both differential metabolite levels and differential metabolic pathways.[14] Outcome *p*-values were

corrected for multiple testing using the FDR method, resulting in q-values. Metabolites were called differential when $q < 0.05$. Full details are described in supplementary methods online.

Statistics

Clinical findings were described with means and standard deviations (continuous normally distributed data), interquartile range and medians (continuous non-normally distributed data), or frequencies and percentages (non-continuous data). Associations between clinical findings in patients and normalized levels of specific metabolites were investigated using the Welch T-test, the Wilcoxon rank sum test, or linear regression. Comparison of clinical findings between patients and controls were done using the Welch T-test, unpaired T-test, Wilcoxon rank sum test, and Mann–Whitney test. Diagnostic plots for regression analyses in R were evaluated to assess the fit of the model. Pearson's correlation or Spearman's correlation were used as appropriate to describe the strength of any linear association. The optimal cutoff points, area under the curve (AUC), sensitivity, and specificity for prediction of atrophy were calculated according to Youden's method. The upper limit of a standard issue manometer in Denmark is 50 cm CSF. In patients exceeding this ($n = 11$), OP was evaluated as the highest measurable value. Analyses were performed in R Studio (version 4.2.2), but figures and plots were made using GraphPad.

Associations between clinical findings and specific metabolites were not corrected for multiple testing to minimize the risk of type II errors, and because these were secondary outcomes of an exploratory nature. Sample size was based on previous studies.[6,15] Due to a technical error omics-data from 1 included patient were unavailable (excluded in relevant analyses). Full details are described in supplementary methods online.

Standard Protocol Approvals, Registrations, and Patient Consents

The study followed the Helsinki declaration, Danish law and was approved by the local ethical committee (Region of Southern Denmark, project ID: S-20170058). Participants provided written informed consent before participation.

Results

Clinical Findings

A total of 60 patients out of 146 candidates had IIH with papilledema and were eligible (Fig 1). Diagnoses of excluded patients are shown in Table S1. We included 35 healthy controls. Patients with IIH and controls were matched for age (28.5 ± 7.9 versus 29.1 ± 6.1 years, $p = 0.6$), sex (96.7% versus 97.1% female, $p = 1$), and BMI (36.9 ± 6.2 versus 36.8 ± 6.4, $p = 0.9$) (Table 1). In patients, mean OP was elevated (40.3 ± 10.5 cm CSF) compared with controls (19.5 ± 4.8 cm CSF, $p < 0.001$). Patients had a median PMD of −5 (4) dB, a mean RNFL of 236.7 (±110.3) µm and a mean GCL of 1.08 (±0.11) mm^3 at baseline (Table 1). Patients with IIH received treatment depending on disease severity and treatment response: 76.3% ($n = 45$) received only first-line treatment (acetazolamide, weight loss), 18.6% ($n = 11$) required second-line treatment (topiramate, furosemide), and in 5.1% ($n = 3$) third-line surgical treatment was necessary (CSF diversion procedures) (Table 1, Supplementary methods section).

We evaluated 55 of the 60 IIH patients at ocular remission (visit 2a, Fig 1, Table 1). Median PMD was −2.9 (3.2) dB, mean RNFL was 91.9 (±17.9) µm, mean GCL was 1.03 (±0.13) mm^3, and 44.4% ($n = 24$) had bilateral optic nerve atrophy. In a subset of patients ($n = 11$), additional samples were taken at visit 2b (clinical improvement). In these patients, mean OP was 22.6 ± 6.1 cm CSF, and 73% had complete remission of papilledema, while 27% had substantial improvement (Table S2). Outcomes for patients included at visit 2b versus patients not included at visit 2b were compared (BMI and weight loss at visit 2a, treatment, ocular outcome, and median follow-up time). There were no statistically significant differences between the 2 groups (Table S3).

Differential Metabolites

We found 5 metabolites to be differential between controls and IIH patients (Table 2, Fig 2). These were confirmed by LC–MS/MS fragmentation. Serum sphingosine 1-phosphate (d16:1-P) (S1P) was lower in patients by 0.46-fold ($q < 0.0001$). After significant clinical improvement (visit 2B, $n = 11$) S1P increased by 2.3-fold in patients ($q = 0.044$), effectively normalizing serum S1P levels to those in controls. In IIH, serum adenosine was lower by 0.25-fold ($q = 0.0048$), and serum glutamate was lower by 0.44-fold ($q < 0.0001$). In CSF, we found 2 annotated, differential metabolites between IIH and controls. These are the lysophosholipids stearoyl-lysophosphatidylcholine (LysoPC18) and 2-palmitoyl-lysophosphatidylcholine (LysoPC16), with a fold reduction of 0.42 ($q = 0.0025$) and 0.37 ($q < 0.0001$), respectively. Serum S1P was the only marker to change with clinical improvement. We found no similarities between the most differential serum and CSF metabolites.

Clinical Correlations

Normalized baseline CSF levels of Lyso-PC18 were higher in patients with moderate–severe papilledema, Frisén grade 3–5, compared to patients with mild papilledema, Frisén grade 1–2 (-0.02 ± 0.51 versus -0.38 ± 0.67 AU, $p = 0.022$). Baseline CSF Lyso-PC18 correlated with baseline RNFL ($p = 0.0012$, Pearson's $r = 0.42$) and was inversely correlated with baseline PMD ($p = 0.01$, Pearson's $r = -0.35$) (Fig 3A–C). Normalized, baseline serum levels of glutamate were inversely correlated with

TABLE 1. Demographics and Clinical Findings at Baseline and Ocular Remission

Baseline Demographics and Findings[a]	IIH (n = 60)	Controls (n = 35)	*p*-Value
Female no (%)	58 (96.7)	34 (97.1)	1
Age, years, mean (±SD)	28.5 (7.9)	29.1 (6.1)	0.6
BMI, mean (±SD)	36.9 (6.2)	36.8 (6.4)	0.9
Opening pressure, mean cm CSF (±SD)[b]	40.3 (10.5)	19.5 (4.8)	**<0.001**
Abducens nerve palsy n (%)	3 (5.2)	-	-
Symptoms			-
Monthly headache days, median (IQR)	30 (20)	1 (1–3)	-
Transient visual obscurations, n (%)	28 (47.5)	0	-
Pulsatile tinnitus, n (%)	31 (51.7)	0	-
Blurry vision, n (%)	31 (52.5)	0	-
Photopsia, n (%)	11 (18.6)	0	-
Dizziness, n (%)	25 (45.5)	0	-
Double vision, n (%)	44 (73.3)	0	-
Days from diagnosis to sample, mean (±SD)	1.02 (3.9)	0	-
Treatment with acetazolamide at sampling, n (%)	5 (8.3)	0	-
Number of days at sampling, mean (range)	3.8 (1–9)		
Perimetric MD, worst eye, median (IQR), db	-5 (4)	-	-
Papilledema, worst eye	60 (100)	0	-
Frisén grade 1, n (%)	9 (15)	-	-
Frisén grade 2, n (%)	22 (36.7)	-	-
Frisén grade 3, n (%)	14 (23.3)	-	-
Frisén grade 4–5, n (%)	15 (25)	-	-
RNFL, worst eye, µm, mean (±SD)	236.7 (110.3)	-	-
GCL, worst eye, mm^3, mean (±SD)	1.08 (0.11)	-	-
Treatment received[d]		-	-
1st line (ACT, weight loss programme), n (%)	45 (76.3)	-	-
ACT, maximum daily dose, mg, median (IQR)	1,500 (500)	-	-
2nd line (TOP, FUR)	11 (18.6)	-	-
Treated with TOP, n (%)	9 (15.3)	-	-
TOP maximum daily dose, mg, median (IQR)	75 (50)	-	-
Treated with FUR, n (%)	8 (13.6)	-	-
FUR maximum daily dose, mg, median (IQR)	40 (50)	-	-
3rd line (surgical treatment to lower ICP)[e], n (%)	3 (5.1)	-	-
Ocular remission, clinical data	**IIH (n = 55)**		
Time to remission, mean (±SD), days[c]	451 (258)	-	-
Perimetric MD, worst eye, median (IQR), db	−2.9 (3.2)	-	-
Bilateral optic nerve atrophy, n (%)	24 (44.4)	-	-
RNFL, worst eye, µm, mean (±SD)	91.9 (17.9)	-	-
GCL, worst eye, mm^3, mean (±SD)	1.03 (0.13)	-	-

Abbreviations: ACT = acetazolamide; BMI = body mass index; CSF = cerebrospinal fluid; FUR = furosemide; GCL = ganglion cell layer; ICP = intracranial pressure; IIH = idiopathic intracranial hypertension; IQR = interquartile range; MD = mean deviation; RNFL = retinal nerve fiber layer; SD = standard deviation; TOP = topiramate.

[a]Missing data for baseline: Abducens nerve palsy (IIH, n = 2), transient visual obscurations (IIH, n = 1), blurry vision (IIH, n = 1), photopsia (IIH, n = 1), dizziness (IIH, n = 5), headache days (IIH, n = 2), MD (IIH, n = 6), RNFL (IIH, n = 3), GCL (IIH, n = 8).

[b]In case of immeasurable high ICP the highest assessable value was used (IIH, n = 11).

[c]Missing data for remission: 1 loss to follow-up (moved abroad), 3 patients did not enter remission before conclusion of study, 1 patient excluded from ophthalmological review due to concomitant papilledema and significant optic disc drusen. Atrophy (n = 6), mean deviation (n = 6), RNFL (n = 5), GCL (n = 14).

[d]Missing answer for treatment, n = 1 (moved abroad shortly after diagnosis).

[e]Surgical treatment, n = 2 (ventriculo-peritoneal or AV shunt) and n = 1 (optic nerve sheath fenestration).

TABLE 2. Metabolites Differentially Expressed in Serum and CSF

HMDB ID	Common Name	Fold Change	*p* Adjusted	Comparison
Serum metabolites				
HMDB0060061	Sphingosine 1-phosphate (d16:1-P)	0.46647	3.20E-06	Ctl v IIH
		2.3902	0.044336	IIH baseline versus visit 2b
HMDB0000050	Adenosine	0.25758	0.0048426	Ctl v IIH
HMDB0060475	Glutamate	0.44445	4.50E-07	Ctl v IIH
HMDB0012238	Iodide	2963.5	0.0004603	Ctl v IIH
CSF metabolites				
HMDB11128	LysoPC(C18:0)	0.42538	0.0025157	Ctl v IIH
HMDB0240262	LysoPC(C16:0)	0.37615	5.11E-08	Ctl v IIH

Note: Significantly differential metabolites in serum and CSF based on MetaboAnalyst output. IIH baseline (N = 59), 1 patient excluded after technical errors in analyses. IIH visit 2b (N = 11) and controls (N = 35).
Abbreviations: CSF = cerebrospinal fluid; Ctl = control; HMDB ID = human metabolome database identification; IIH = idiopathic intracranial hypertension; LysoPC(C16:0) = 2-palmitoyl-lysophosphatidylcholine; LysoPC(C18:0) = stearoyl-lysophosphatidylcholine.

baseline RNFL in IIH ($p = 0.0026$, Pearson's r = −0.39) (Fig 3D). Normalized, baseline serum levels of S1P were higher (−0.29 ± 0.75 versus −0.07 ± 0.84 AU, $p = 0.018$) in patients with bilateral optic nerve atrophy at ocular remission with an excellent sensitivity of 91.7 (73–99%) and moderate specificity of 44.8 (26.4–64.3%), and AUC of 0.69 (Fig 4A,B). Normalized, baseline CSF levels of LysoPC-16 were lower in patients with any optic nerve atrophy at ocular remission (0.07 ± 0.31 versus −0.28 ± 0.67 AU, $p = 0.003$) with a sensitivity of 100 (89.7–100%), but specificity was 0 (0–17.6%) and AUC just 0.26 (Fig 4C,D). Remaining clinical findings at baseline and ocular remission were not associated with CSF LysoPC-16/18, serum glutamate, or serum S1P. Serum adenosine was not associated with any clinical findings (data not shown). No associations were found between the metabolites, and the headache findings or ICP. Likewise, there was no association between the level of the metabolites in serum or CSF and baseline BMI (linear regression model, R^2 adjusted was 0 for all metabolites and baseline BMI, *p*-values ranging from 0.29 to 0.74).

Pathway Analysis

To further interrogate the metabolic phenotypic differences between IIH patients and controls, we performed a functional metabolic pathway analysis (Tables SS4–S7). In serum, we identify 7 differential pathways associated with amino acid metabolism, including branch chain amino acid metabolism (q = 0.01) and amino group metabolism (q = 0.009). Multiple lipid biosynthetic pathways, vitamin E (q = 0.003) and vitamin B (q = 0.009) metabolism pathways were also found to be altered in new-onset IIH. Moreover, we identify 5 altered eicosanoid pathways and differential neuroprostane metabolism (q = 0.003) in the serum. Finally, glucocorticoid (q = 0.007) and sex steroid metabolism (q = 0.008) were also differential in the serum of IIH patients. In the CSF, we identified key, broad differences in carbohydrate metabolism as well as lipoic (q = 0.0008) and propanoate (q = 0.001) lipid metabolism. The serum and CSF share differences in lipid metabolism and inositol (serum, q = 0.01; CSF, q = 0.0007) metabolism. Moreover, multiple amino acid pathways perturbed in both the serum and CSF, with "glycine, serine, alanine and threonine metabolism" specifically being perturbed in both tissues (serum, q = 0.01; CSF, q = 0.02). Further details of all differential pathways can be found in the supplemental tables.

Discussion

We demonstrate that serum S1P is lower in IIH patients compared to matched, healthy controls and increases in patients after significant clinical improvement. Clinically, higher baseline levels of serum S1P in IIH is associated with a more severe outcome in the form of bilateral, optic nerve atrophy (Fig 5). Quantification is not possible given the method we used; therefore, normalized levels with arbitrary units are obtained. Accordingly, it was surprising that baseline serum S1P levels did in fact have excellent sensitivity and moderate specificity for predicting optic

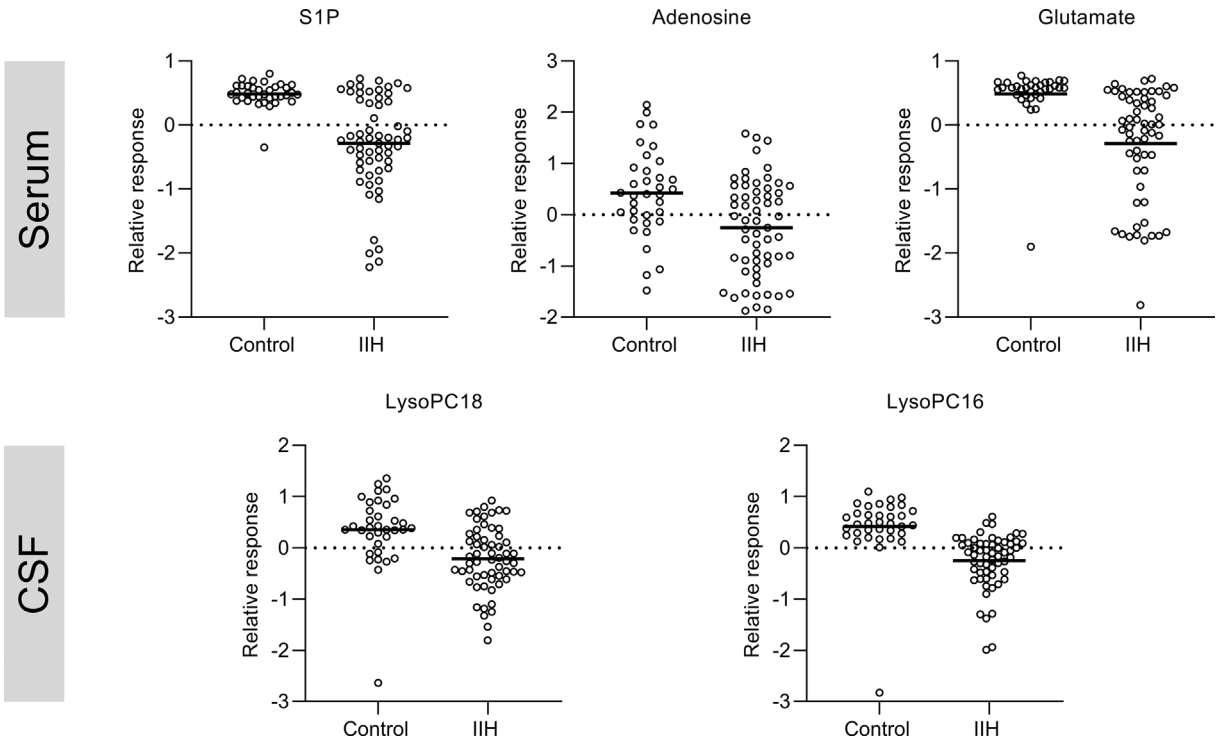

FIGURE 2: Boxplot of differential metabolites in serum and CSF. Abbreviations: CSF = cerebrospinal fluid; IIH = idiopathic intracranial hypertension; LysoPC16 = 2-palmitoyl-lysophosphatidylcholine; LysoPC18 = stearoyl-lysophosphatidylcholine; S1P = sphingosine 1-phosphate. Data presented as mean, where each dot represents a patient. Relative response is the quantile normalized and log10 transformed and Pareto scaled ion peak area to transform the data into a normal distribution.

nerve atrophy. S1P is the natural ligand of S1P receptors, a family of 5 G-protein coupled receptors.[16] In the context of ICP regulation, S1P receptors $S1P_{1–3}$ are expressed in the cerebral vasculature and the choroid plexus, all tissues highly involved in regulation of ICP.[16,17] Thus, S1P could affect ICP regulation. Moreover, S1P is considered vital in the maintenance of biological barriers including the blood-brain barrier, where inhibition of S1P receptors reduces the patency of biological barriers, including the blood-brain barrier.[18,19] The previous observation of a leaky blood-brain barrier in IIH could be partially explained by a reduction of S1P signaling.[20] Reduced serum S1P in new-onset IIH vs controls might reflect a protective mechanism which attenuates the effects of ICP-elevation (Fig 5). In line with our findings that lower serum S1P predicts a reduced likelihood of bilateral optic degeneration, S1P receptors are found within the retina. Reducing intraretinal S1P reduces retinal degeneration and preserves function in a rodent model of retinal degeneration.[21] Thus, reduced serum S1P in a pathological setting could protect the retina from ICP induced S1P increases, independently of any pressure modulating effects mediated by barrier patency changes. These findings make serum S1P a promising candidate biomarker. Given that serum S1P could rationally modify CSF dynamics and ICP, future functional assessments are imperative, to understand if this metabolite plays a role in the raised ICP in IIH, including whether it could be related to disease severity and prognosis. We have established a novel rodent model of IIH, and these clinical results provide candidates for further, translational research in this enigmatic disease.[22]

In the CSF analysis, we show decreased levels of 2 phospholipids, Lyso-PC18 and Lyso-PC16, in IIH. In the clinical correlation analyses, we find that patients with mild papilledema and good visual fields had low levels of CSF Lyso-PC18 at baseline, whereas patients with poorer ophthalmological status had intermediate levels of CSF Lyso-PC18 compared to the high levels seen in controls (Fig 5). Optic nerve atrophy (uni/bilateral) was associated with lower levels of the lipid metabolite CSF LysoPC-16. This finding was unspecific, but highly sensitive. Both lipids are from a family known to activate TRPV4 channels, which can promote CSF secretion in rats.[23,24] This suggests that decreased Lyso-PC18 in IIH could be a compensatory mechanism attenuating the effects of raised ICP at time of diagnosis (Fig 5). CSF LysoPC-16 is lower in IIH, but not related to baseline neuro-ophthalmological status; however, low baseline levels were associated with subsequent optic nerve atrophy at ocular remission, suggesting that these lipids are differentially involved in ICP-regulation.

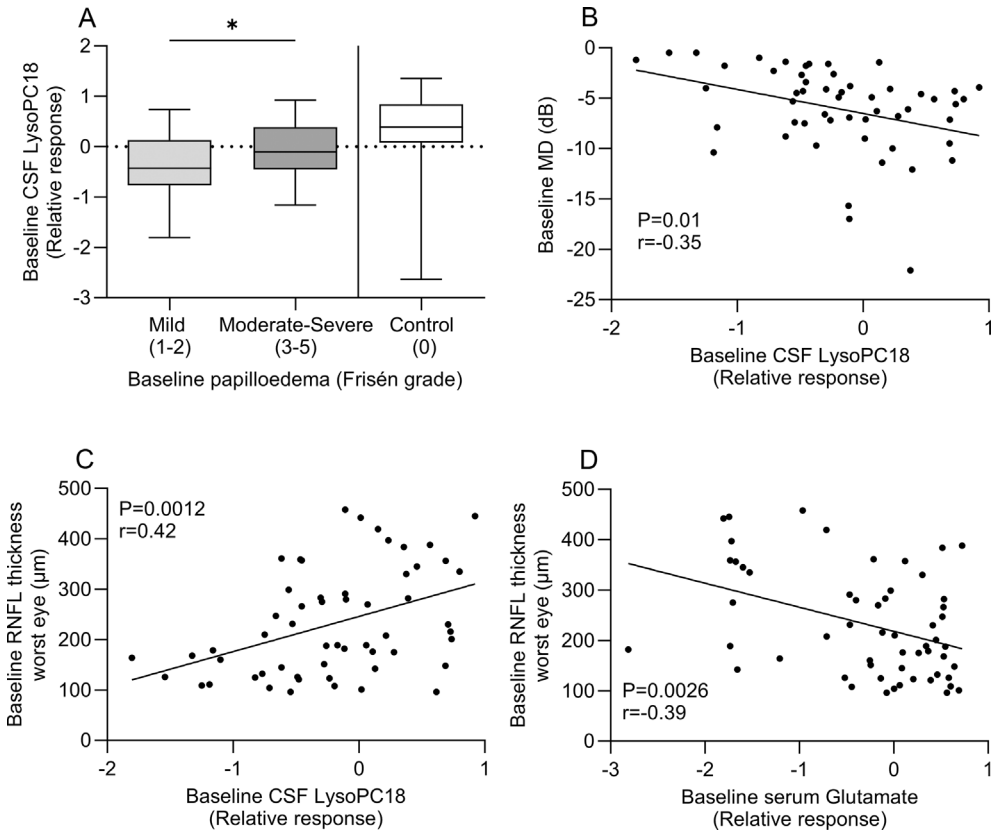

FIGURE 3: Associations between clinical findings at baseline and metabolites. (A) Baseline CSf LysoPC18 and baseline papilledema (Frisén grade). Note that the control column represents the control patients for visual comparison only, hence, the black line separating them. (B) Baseline CSF LysoPC18 vs. baseline MD. (C) Baseline CSF LysoPC18 vs. baseline RNFL thickness in the worst eye. (D) Baseline serum glutamate vs. baseline RNFL thickness in the worst eye. Pearson's correlation for (B–D). Unpaired t-test for A. * = $p < 0.05$. Abbreviations: CSF = cerebrospinal fluid; dB = decibel; LysoPC18 = stearoyl-lysophosphatidylcholine; MD = perimetric mean deviation; RNFL = retinal nerve fiber layer. Relative response is the quantile normalized and log10 transformed and Pareto scaled ion peak area to transform the data into a normal distribution.

Thus, reduced serum S1P and CSF LysoPC-18 could represent compensatory mechanisms directly related to reduction of CSF secretion, while CSF Lyso-PC16 and serum S1P are promising prognostic markers (Fig 5). Although elevated BMI is a well-known risk factor for IIH, we found no link between BMI and the disturbed metabolites, supporting the hypothesis that the identified metabolites are related to compensatory mechanisms and ICP regulation, rather than directly coupled to the pathophysiological changes occurring specifically in IIH. These findings may be useful, not only in IIH, but also in other disorders associated with ICP elevation, for example, acute head injury, stroke, or hydrocephalus. Further work is required to test this hypothesis, and patients with secondary intracranial hypertension were not included in this study.

Adenosine is a ubiquitous signaling molecule which signals via several G protein coupled receptors, which we find to be lower in the serum of IIH patients, where its receptors are linked to CSF dynamics. Caffeine, a pan-antagonist of adenosine receptors, has been demonstrated to modify CSF dynamics in rodents. Acutely administered caffeine lowers CSF secretion and ICP, in contrast chronically administered caffeine increases CSF secretion and induces hydrocephalus.[25,26] Consequently, a lowering of serum adenosine in IIH, which is pharmacologically analogous to administering caffeine, could either represent a physiological compensation to raised ICP or a potential contributor to the raised ICP in IIH. Further research is required to understand the role of adenosine in IIH pathophysiology and ICP dynamics in general.

Glutamate, a major excitatory neurotransmitter and amino acid, was found to be lower in IIH patients. Determining the consequences of the lower serum glutamate in IIH is difficult due to the plethora of tissues that metabolize and have receptors for glutamate. Further work is required to understand the potential role of glutamate in the pathophysiology of IIH and ICP dynamics in general. We cannot comment if any of these metabolites are a cause or consequences of the underlying metabolic disease of IIH, which likely precedes the classic neuro-

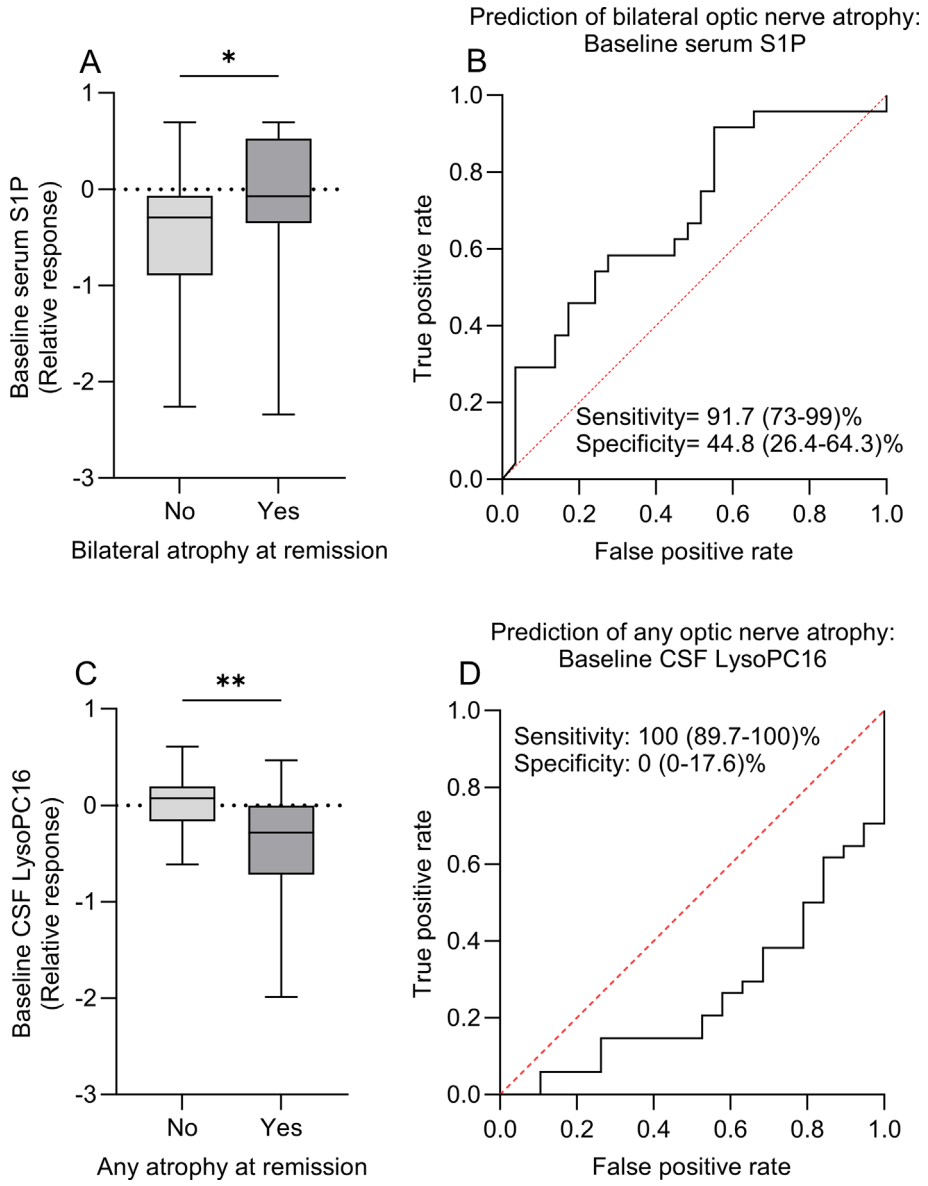

FIGURE 4: Associations between clinical findings at ocular remission and metabolites. (A) Baseline serum S1P and bilateral atrophy at ocular remission. (B) ROC curve of baseline serum S1P and bilateral atrophy at remission. (C) Baseline CSF LysoPC16 and any atrophy at ocular remission. (D) ROC curve of baseline CSF LysoPC16 and any atrophy at ocular remission. * = $p < 0.05$, ** = $p < 0.01$. Mann–Whitney test for A and C. Abbreviations: CSF = cerebrospinal fluid; LysoPC16 = 2-palmitoyl-lysophosphatidylcholine; LysoPC18 = stearoyl-lysophosphatidylcholine; MD = perimetric mean deviation; dB = decibel; RNFL = retinal nerve fiber layer; S1P = sphingosine 1-phosphate. Relative response is the quantile normalized and log10 transformed and Pareto scaled ion peak area to transform the data into a normal distribution. [Color figure can be viewed at www.annalsofneurology.org]

ophthalmological disease, due to the poor understanding of the pre-diagnostic natural history of IIH.

Although serum iodide was found to be differential (fold change 2,963, q = 0.0004), this is an artifact. IIH subjects had diagnostic CT scans with an iodide-based contrast agent prior to sample collection, whereas controls did not. Consequently, iodide became an inadvertent positive spike control for the method.

All the identified metabolites in IIH are not IIH specific as they have been identified as being differential in other diseases, including diagnostic differentials of IIH and primary causes of raised ICP. However, the direction of change is differential. Both serum glutamate and adenosine have been identified as being raised in chronic migraine, a differential diagnosis for IIH, whereas we find these metabolites to be lower in IIH serum.[27,28] LysoPC18 has been found to be increased in subarachnoid hemorrhage (associated with acute ICP elevation), in contrast to the lower LysoPC18 we find in IIH.[29] In another condition of chronic ICP elevation, medulla blastoma,

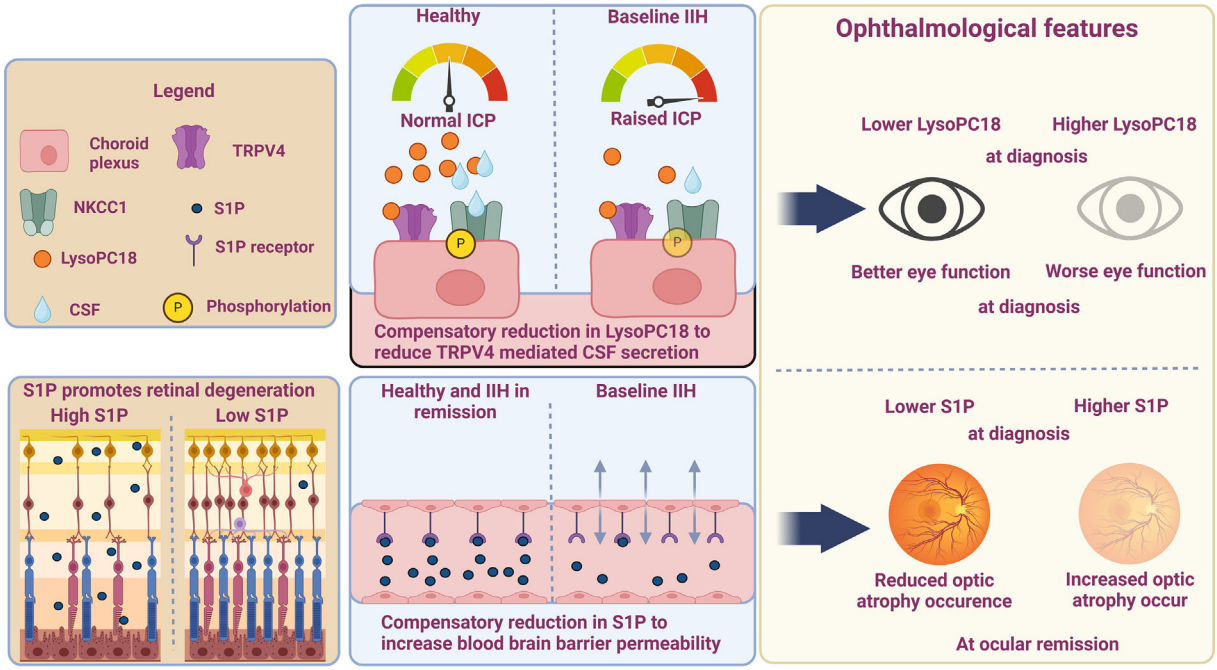

FIGURE 5: Potential compensatory mechanism of LysoPC-18 and S1P in IIH. A figure describing the hypothesized compensatory mechanisms of LysoPC-18 and S1P. In healthy individuals with normal ICP, CSF LysoPC18 acts, via a single metabolite, to agonise TRPV4 at choroid plexus, promoting CSF secretion. In IIH, where ICP is raised, there could be a compensatory reduction in CSF LysoPC18. This would reduce CSF secretion and could explain why, at baseline, IIH patients with lower CSF LysoPC18 have better eye function. In healthy individuals and IIH in remission, serum S1P is high likely contributing maintaining low blood-brain barrier permeability. In contrast, lower S1P in new onset IIH likely contributes to increased blood-brain barrier permeability. This potential compensatory mechanism could act as a measure to increase CSF drainage, thus providing an explanation as to why lower serum S1P at baseline predicts a reduced likelihood of bilateral optic atrophy. Abbreviations: IIH = idiopathic intracranial hypertension; ICP = intracranial pressure; S1P = sphingosine 1-phosphate; LysoPC18 = stearoyl-lysophosphatidylcholine). [Color figure can be viewed at www.annalsofneurology.org]

LysoPC16 is lower in the CSF just like IIH, suggesting that this metabolic change is related to the chronic ICP elevation rather than being specific disease (eg, compensatory).[30] There is no indication of what S1P does in the context of IIH differential diagnoses or in other diseases of raised ICP. The direction of change in some of our metabolites thus may help to differentiate IIH from IIH differentials and secondary causes of raised ICP.

Two previous studies have investigated the metabolomic phenotype in IIH, a UHPLC–MS non-targeted metabolomics study by Alimajstorovic et al and an NMR-based metabolomics study by Grech et al.[6,15] Here, both studies report differences in serum and CSF amino acid levels compared to controls, corroborating our pathway analysis, which identifies many differential amino acid pathways.[6,15] Moreover, the Alimajstorovic study reports broad differences in the levels of lipids in both the serum and the CSF, this is also in line with our pathway analysis of highly differential lipid pathways.[15]

There are however key differences between our and the previous studies. Neither previous study reported differences in metabolic pathways between IIH patients and controls; this is in contrast to our findings of multiple differential pathways between IIH patients and controls. Moreover, there are no shared differential metabolites between our study and the previous studies.

These differences cannot be attributed solely to methodological differences alone, as the Alimajstorovic et al. study used the same analytical methods as our study. However, the Grech et al. study used a quantitative method which yields far fewer metabolites, and so provides a methodological bias to which metabolites it could investigate. It is likely that demographic differences underlie most of the differences in results between the studies.

Our study has a similar sized IIH cohort but a substantially larger control cohort than the previous studies. In the present study, we used newly diagnosed IIH patients rather than patients who had undergone disease modifying treatment, for example, weight loss, and were on average 1 year into diagnosis in the Alimajstorovic study.[15,31] This difference in "new" vs "old" IIH could confer different metabolic profiles. Indeed, the longer an IIH patient is into their disease the more likely they are to develop metabolic complications (eg, type 2 diabetes), which may drive further differential metabolite levels compared to healthy individuals.[8] Only a small subset of our

patients (8.3%) had received acetazolamide treatment (mean duration 3.8 days) (Table 1) compared to the previous study (29%) with an indeterminate treatment length.[15,31] Acetazolamide has been shown to modify the metabolome, so we aimed to avoid it in this study.[32] It should also be noted that the Alimajstorovic et al. study's controls were significantly older than the IIH patients, which could introduce heterogeneity into the results, where age is an additional differentiator that could influence the results.[15] Some of their results could be age differences rather than disease differences. In contrast, our IIH patients and controls were well matched for age, sex, and BMI. The Grech et al study used a demographically similar, small control cohort and almost the same IIH cohort as the Alimajstorovic et al study, conferring the same types of heterogeneity previously discussed.[6] Another difference between the 2 studies is that our cohort was not fasted, as samples were taken in a subacute setting at time of diagnosis, preventing pre-visit planning, whereas the previous study included fasted patients as part of a planned trial visit. Fasting state is well known to modify the metabolome.[33] Future studies, combining an appropriate fasting state, appropriately sized, well-matched control cohorts with patients who are treatment naïve, combined with a multiomic approach will help further our understanding of IIH.

Our pathway analysis highlights multiple differential pathways between IIH patients and controls. We identified differential androgen and glucocorticoid metabolism; these results are corroborated by previous studies which found these to be in excess and be implicated in IIH pathophysiology.[9,34] Both androgens and glucocorticoids have been linked to CSF dynamics in humans and rodents, where androgens increase ICP in a rodent model of obesity.[9,25,34,35] Consequently, altered androgens may contribute to the raised pressure seen in IIH. Moreover, we found differential amino acid and lipid metabolism, features that have been shown to be systematically disturbed in the adipose tissue and serum in previous studies of IIH.[7,15] Shared dysregulation of amino acid, carbohydrate, and lipid metabolism in both the serum and CSF suggest broad central and system metabolic dysfunction. We also highlighted a dysregulation of neuroprostane metabolism. Neuroprostanes are prostanoid-like lipids that are activated by reactive-oxygen species rather than cyclooxygenase. Neuroprostanes have been demonstrated to be increased in neurodegenerative and neuroinflammatory diseases such as Rett syndrome, multiple sclerosis, Alzheimer disease, and Huntington disease.[36,37] Given that neuroinflammation has been demonstrated in the brains of IIH patients, and that they have increased levels of neurofilament light chain (a marker of neuronal degradation) in CSF, and documented cognitive disturbances,[3] an alteration of neuroprostanes is in keeping with findings in other neurological diseases of neuroinflammatory or neurodegenerative origin.[38] Thus, our findings raise again the question of neurodegeneration and neuroinflammation in IIH. Vitamin E metabolism was also found to be modified in our IIH patients. Vitamin E and its derivatives are fat soluble antioxidants. Altered antioxidants in the context of increased neuroprostanes is suggestive of increased metabolic stress in newly diagnosed IIH. Altered redox is also in keeping with the demonstration of the presence of disturbed mitochondria in the brain of IIH patients.[39] Oxidative stress is also both a cause and consequence of neuroinflammation and neurodegeneration. As such, it is interesting to see the constellation of neurodegeneration and the phenotypic features of oxidative stress in IIH. Future work investigating oxidative and metabolic stress in IIH patients is needed.

Strengths and Limitations

The primary strength of this study is the well-described, large prospective cohort of patients and matched controls. Misdiagnosis of IIH is well described,[40] complicating retrospective studies, and matching controls for BMI is central due to the metabolic effects of simple obesity. Patients with IIH may experience chronic disease, spontaneous remission or relapses, and it is unknown whether the mechanisms driving these outcomes are the same as those driving disease development. Long-term treatment with acetazolamide and weight loss could drive changes in the metabolome.[6] Moreover, both acetazolamide and topiramate are endocrine disruptors and, thus, alter the metabolome.[41] Considering this and the fact that the prediagnostic natural history of IIH is unknown, and the clinical presentation at diagnosis varies considerably, we argue that time of diagnosis is the most optimal way to describe changes associated with active, treatment-naïve IIH. Therefore, it is a strength that our samples are taken at time of diagnosis in treatment-naïve patients. We included a subset of patients for evaluation of metabolites after significant clinical improvement. Although these analyses are exploratory and done in a minority of the cohort, any inclusion of long-term biomarker follow-up is an important strength. The untargeted mass spectrometry approach, inclusion of 2 types of tissue, as well as polar and non-polar metabolites are also strengths.

The numbers in the present study may be relatively low for a metabolomics study but given that IIH is a rare disease collection of 60 well-described patients adds strength. The long-term follow-up of neuro-ophthalmological outcome is also a significant strength as

it allows for further hypothesis-generating analyses to help identify which metabolites are candidate biomarkers.

The study is limited to single analysis as no proteomics or RNA-sequencing to identify upstream signaling pathways was performed. Given the non-quantitative nature of our study, future targeted prospective studies are required to validate our findings. Tissue collection is cross-sectional with limited longitudinal sampling. Because of inclusion at time of diagnosis, in an acute clinical setting, we could not control for specific circumstances, for example, fasting, or include 24 hour urine samples. This means that composition of the last meal may alter the results, but on the other hand, it reflects the standard clinical diagnostic process. Given that controls were headache free, analgesic use in IIH patients could have conferred a specific metabolic profile to the IIH patients. The patient cohort was included in Denmark and, therefore, ethnically homogenous. It is unknown whether our findings are applicable to other ethnicities. As the protocol did not allow follow-up samples purely for research purposes, only a minority of the cohort was included for follow-up CSF and serum samples at visit 2a ($n = 11$). We compared outcome in patients included at visit 2b to patients not included at visit 2b. There were no statistically significant differences, and the population included at visit 2b generally reflected the population of IIH patients at our tertiary care center. Nevertheless, we cannot rule out selection bias as the included number of patients at visit 2b was low, and these patients were selected based on the clinical need for a new lumbar puncture. This is an important limitation, consequently, results relating to visit 2b are purely exploratory and must be confirmed in a separate cohort.

Conclusion

We present a novel, prognostic case–control study in which the metabolome of new-onset IIH is investigated in CSF and serum compared to matched controls. We show that a sphingolipid in serum (S1P) and a phospholipid in CSF (LysoPC-16) are downregulated in IIH, and baseline levels are associated with subsequent optic nerve atrophy. Androgen, glucocorticoid, amino acid, and lipid metabolism show differential expression in IIH confirming previous findings and establishing IIH as a neurometabolic disorder. Longitudinal studies are needed to confirm our findings and investigate the clinical implications.

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Author Contributions

J.J.K., R.H.J., and C.S.J.W. conceived and designed the study. All authors were involved in the acquisition and/or the analysis of data. J.J.K. and C.S.J.W. drafted significant portions of the manuscript and figures. All authors read the final manuscript.

Potential Conflicts of Interest

Nothing to report.

Data Availability

De-identified data can be shared with qualified researchers who provide a methodologically sound proposal. It is a legal requirement by Danish law that a data processing agreement is signed and approved. Raw neuro-ophthalmological data are shared locally for technical and legal reasons. Data are available 2 years after publication. Proposals can be directed at the corresponding author.

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