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Association of Circulating Metabolites in Plasma or Serum and Risk of Stroke

Meta-analysis From 7 Prospective Cohorts

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Abstract

Objective

To conduct a comprehensive analysis of circulating metabolites and incident stroke in large prospective population-based settings.

Methods

We investigated the association of metabolites with risk of stroke in 7 prospective cohort studies including 1,791 incident stroke events among 38,797 participants in whom circulating metabolites were measured by nuclear magnetic resonance technology. The relationship between metabolites and stroke was assessed with Cox proportional hazards regression models. The analyses were performed considering all incident stroke events and ischemic and hemorrhagic events separately.

Results

The analyses revealed 10 significant metabolite associations. Amino acid histidine (hazard ratio [HR] per SD 0.90, 95% confidence interval [CI] 0.85, 0.94; $p = 4.45 \times 10^{-5}$), glycolysis-related metabolite pyruvate (HR per SD 1.09, 95% CI 1.04, 1.14; $p = 7.45 \times 10^{-4}$), acute-phase reaction marker glycoprotein acetyls (HR per SD 1.09, 95% CI 1.03, 1.15; $p = 1.27 \times 10^{-3}$), cholesterol in high-density lipoprotein (HDL) 2, and several other lipoprotein particles were associated with risk of stroke. When focused on incident ischemic stroke, a significant association was observed with phenylalanine (HR per SD 1.12, 95% CI 1.05, 1.19; $p = 4.13 \times 10^{-4}$) and total and free cholesterol in large HDL particles.

Conclusions

We found association of amino acids, glycolysis-related metabolites, acute-phase reaction markers, and several lipoprotein subfractions with the risk of stroke. These findings support the potential of metabolomics to provide new insights into the metabolic changes preceding stroke.

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Glossary

BMI = body mass index; **CI** = confidence interval; **DILGOM** = Dietary, Lifestyle and Genetic Determinants of Obesity and Metabolic Syndrome; **EGCUT** = Estonian biobank; **FHS** = Framingham Heart Study; **FINRISK97** = FINRISK study 1997; **HDL** = high-density lipoprotein; **HR** = hazard ratio; **ICD** = International Classification of Diseases; **LDL** = low-density lipoprotein; **NMR** = nuclear magnetic resonance; **PROSPER** = Prospective Study of Pravastatin in the Elderly at Risk.

Stroke is a leading cause of death and serious long-term disability worldwide.[1] The majority of strokes are of the ischemic type, while the hemorrhagic type occurs less often but is associated with higher mortality risk.[1,2] Stroke risk is determined by various modifiable risk factors such as hypertension, diabetes mellitus, cardiovascular disease, smoking, and obesity, whereas the association of stroke with cholesterol and its subfractions has shown inconsistent results.[1–5] Opportunities for therapeutic interventions in patients with stroke depend on the type of stroke and rely on brain imaging techniques.[6] Despite advances in brain imaging techniques, costs are still high, availability is limited, and not all patients show a relevant lesion on neuroimaging.[6,7] New technology is needed to identify high-risk patients, to understand the etiology of stroke, and to develop future prevention strategies. Detailed profiling of metabolic status can provide insights into metabolic changes that lead to a higher risk of stroke. Because the metabolome reflects both genome and exposome, including exposures to risk factors that determine the risk of stroke, this new -omics technology may open new avenues for stroke prevention. To date, only a few studies have analyzed metabolic disturbances in stroke and identified various metabolites to be associated with stroke.[8–10] These studies are based on relatively small samples or were performed on participants of non-European ancestry.[11] The most comprehensive study to date was a nested case-control study conducted within the China Kadoorie Biobank including 1,146 patients with ischemic stroke and 1,138 patients with intracerebral hemorrhage.[11] The study reported an association between lipids and lipoprotein particles of various sizes with ischemic stroke but not with hemorrhage.[11] Furthermore, the study identified glycoprotein acetyls, ketone bodies, glucose, and docosahexaenoic acid to be associated with both ischemic and hemorrhagic stroke.[11]

Because large metabolomics studies of stroke in persons of European origin are lacking and data from well-established prospective cohort studies are limited, the aim of our study is to conduct a comprehensive analysis of circulating metabolites and incident stroke in large prospective population-based settings involving 1,791 incident stroke events among 38,797 participants of European origin.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Each of the participating studies received approval by local ethical committees or institutional review boards (data available from Dryad [additional Methods in Additional Information file), doi.org/10.5061/dryad.kh1893239). All participants provided written informed consent.

Study Population

Our study population included 38,797 participants from 7 cohorts, including the Rotterdam Study, Whitehall II study (Whitehall II), the national FINRISK study 1997 (FINRISK97), Dietary, Lifestyle and Genetic Determinants of Obesity and Metabolic Syndrome (DILGOM), Prospective Study of Pravastatin in the Elderly at Risk (PROSPER), Estonian biobank (EGCUT), and the Framingham Heart Study (FHS). A description of participating studies is available from Dryad (additional Methods in Additional Information file, doi.org/10.5061/dryad.kh1893239).

Stroke Assessment

Details on stroke assessment are available from Dryad (additional Methods in Additional Information file, doi.org/10.5061/dryad.kh1893239). The incident stroke events were assessed through follow-up of health records, while in some studies, additional periodic visits to research centers were used (e.g., Rotterdam Study, FHS). Participants of the Rotterdam Study were monitored for incident stroke through an automated linkage of medical records from general practitioners with the study database.[12] Incident stroke events in the Whitehall II study were ascertained through linkage to electronic records from hospitalizations due to stroke and national statistics death registries,[13,14] whereas in the FINRISK and DILGOM studies, linkage to national health registries was used (biorxiv.org/content/early/2018/03/12/280677). Ascertainment of incident stroke events in EGCUT was also performed through linkage to electronic records from multiple databases (thl.fi/publications/morgam/cohorts/full/estonia/est-esta.htm), while information regarding domiciliary visits or hospitalizations associated with possible cardiovascular events, including stroke, and on all deaths was used for classification of study endpoints in PROSPER.[15] In the FHS, incident clinical stroke was identified as part of ongoing clinic and hospital surveillance with additional stroke surveillance by annual phone health updates and collaboration with primary care physicians and local emergency departments.[16,17] Participants with a history of stroke at baseline were excluded from the analyses.

Baseline Clinical Characteristics

The baseline clinical characteristics included assessment of blood pressure, plasma glucose levels, smoking status, weight, and height. Hypertension was defined as a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg,

or use of antihypertensive medication or based on general practitioner diagnosis, medication reimbursement or ICD diagnosis (I10/401/491, ICD-10/9/8) from hospital discharge register or cause of death register, or self-report. Diabetes was defined as fasting plasma glucose levels >7 mmol/L or use of medication indicated for the treatment of diabetes. Body mass index (BMI) was calculated as weight in kilograms divided by square of heights in meters.

Metabolite Quantification

Circulating metabolites were quantified with a high-throughput nuclear magnetic resonance (NMR) technology. In all participating studies except the FHS, the Nightingale Health metabolomics platform (Helsinki, Finland) was used for simultaneous quantification of a wide range of metabolites, including routine lipids, 14 lipoprotein subclasses and their lipids (esterified cholesterol, free cholesterol, total cholesterol, triglycerides, phospholipids, and total lipids), fatty acids, amino acids, ketone bodies, and various glycolysis precursors. A detailed description of the methodology has been provided previously.[18,19] The Nightingale quality control procedures were applied to the data from each cohort. The Nightingale automated data processing and quality control procedures included safety checks for unexpected metabolite signals. For each sample, integrated quality procedures verified the sample quality by reporting signs of degradation and contamination issues. If a metabolite concentration was below the limit of quantification, due to either biological reasons or external compounds interfering with quantification, but above the limit of detection, the metabolite value was presented as 0. The Nightingale quality control procedure did not have an upper limit for quantified concentrations except for high lactate and high pyruvate, which were established irregularities arising from suboptimal sample collection procedure. Other high values were reported as they are, meaning that high concentrations were not excluded because in some context high biomarker concentration may be biologically and physiologically relevant and provide valuable molecular insight into a disease or an outcome. Every metabolite that has been reported in the results file has passed this strict quality control procedure. No additional single-cohort quality control was applied except for the EGCUT cohort in which metabolites that were detected in the large majority (at least 95%) of the cohort were included while individuals with missing values in >10% of the metabolites were excluded from the analyses. In the FHS, lipoprotein subclasses were measured by proton NMR spectroscopic assay (LipoScience, Raleigh, NC).[20,21] Blood samples were collected after overnight fasting in all studies except for FINRISK97, in which the samples were collected after 4 hours of fasting (semifasting state).[22,23] The sample material was EDTA-plasma in the Rotterdam Study, FHS, and EGCUT, whereas the serum was used in FINRISK97, DILGOM, PROSPER, and Whitehall II.[22–25] The EDTA-plasma/serum samples were stored at either −80°C or −70°C. The duration of sample storage ranged from 2 to 9 years in EGCUT and 8 years in DILGOM to 11 years in Rotterdam Study, 15 years in FINRISK97, and 15 to 20 years in PROSPER. There were 147 primary nonderived metabolite measurements quantified in absolute concentration units that were further analyzed in this study (data available from Dryad, additional table 1, doi.org/10.5061/dryad.kh1893239). The descriptive statistics of metabolites were coherent across the cohort (cohort-specific descriptive statistics are available from Dryad (additional table 2).

Statistical Analyses

To obtain an approximately normal distribution, all metabolites measurements were natural logarithmic transformed before the analyses. Because some of the metabolite values in our datasets were below the limit of quantification and therefore presented as 0, 1 was added to all values of the metabolites before the transformation. The metabolite measurements were subsequently scaled to SD units (mean 0, SD 1) to enable comparison of results for measures with different units and across wide ranges of concentrations. The relationship between metabolites and stroke was assessed with Cox proportional hazards regression models. The analyses were performed while adjusted for age, sex, BMI, lipid-lowering medication, and study-specific covariates if needed (model 1). The associations were further adjusted for smoking status, diabetes, and hypertension (model 2). Proportional hazard assumption was tested in EGCUT and FINRISK97. Violation of this assumption was observed for 7 metabolites (noted with an asterisk in additional table 1, doi.org/10.5061/dryad.kh1893239). None of these metabolites showed statistically significant association with incident stroke in our analyses. To overcome the problem of false positives due to differences in study design, sampling, storage, or metabolite assessment, we did not pool and analyze the data of different studies jointly. Rather, we have analyzed individual studies and combined the findings using the meta-analysis. The summary statistics results of participating studies were combined using inverse variance-weighted fixed-effect meta-analysis in METAL.[26] The heterogeneity of effects was assessed by I^2, which indicates the percentage of variance in the meta-analysis attributable to study heterogeneity.[26,27] All hazard ratios (HRs) of continuous variables are expressed per 1 SD of the transformed variable. The analyses were performed considering all incident stroke events and ischemic and hemorrhagic events separately.

Because most of the 147 metabolite measures are highly correlated, we estimated the number of independent tests in the correlation matrix using the previously described method of Li and Ji.[28] Subsequently, the number of independent tests was used for calculation of Bonferroni-corrected p value (p = 0.05/30 independent metabolites = 1.7×10^{-3}).

Data Availability

Data are available on request. Interested researchers may contact the corresponding author.

Results

The baseline descriptive characteristics of study participants are shown in table 1. In total, 1,791 incident stroke events

Table 1 Descriptive Statistics of Study Population

Variable[a]	Rotterdam Study		Whitehall II[b]		FINRISK97		DILGOM		PROSPER		EGCUT		FHS	
	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls
No.	257	2,308	197	5,792	474	6,384	107	4,424	197	4,627	308	10,268	251	3,203
Age, y	76.9 (6.2)	75.0 (6.1)	59.4 (5.9)	55.6 (6)	59.6 (10.4)	47.0 (12.9)	62.0 (10.4)	51.9 (13.5)	75.9 (3.7)	75.2 (3.3)	66.3 (12.5)	44.5 (17.1)	58.1 (9.0)	51.7 (10.1)
Women, %	54.1	58	25.4	29.1	38.6	52.6	42.1	53.7	54	52.2	54.9	63.3	47.4	51.4
Current smoking, %	15.2	13	15.7	9.4	24.5	23.7	21.5	17.4	28.9	27.1	17.5	29.9	24.9	24.6
Diabetes, %	17.9	14.3	8.6	4.4	16.9	4.9	15.9	8.9	18.3	10.6	35.4	7.7	15.9	5.0
Hypertension, %	85.6	81.0	41.1	28.0	48.9	21	43	16.5	58.9	62.5	66.2	24.4	60.2	34.2
Systolic blood pressure, mmHg	156.8 (23.9)	151.4 (20.1)	127.4 (16.3)	122.9 (16.5)	147.7 (22.3)	134.7 (19.2)	149.8 (23.7)	136.4 (20.2)	157.1 (21.9)	154.5 (21.8)	142.8 (18.8)	125.7 (16.9)	137.8 (20.8)	126.2 (18.5)
Diastolic blood pressure, mmHg	79.7 (12.4)	79.2 (11.1)	78.3 (10.2)	77.5 (10.5)	86.1 (11.9)	81.9 (11.2)	83.1 (13.6)	79.3 (11.0)	84.6 (11.8)	83.7 (11.4)	83.4 (10.9)	77.6 (10.7)	81.6 (10.4)	78.9 (9.9)
Antihypertensive medication, %[c]	51	47.1	21.8	12.3	27.4	11.5	34.6	22.1	70.6	74.4	69.5	24.3	36.3	16.4
BMI, kg/m^2	27.2 (3.5)	27.4 (4.2)	26.2 (4.2)	26.0 (3.9)	28.4 (4.8)	26.5 (4.5)	28.0 (5.0)	27.2 (4.8)	26.5 (4.1)	26.9 (4.2)	29.1 (5.7)	26.4 (5.4)	27.6 (5.1)	26.7 (4.8)
Follow-up time, y	5.7 (3.5)	9.8 (3.5)	12.5 (4.9)	18.2 (3.0)	15.0 (4.2)	16.9 (3.0)	7.25 (1.5)	7.75 (0.7)	1.9 (1.0)	3.3 (0.5)	6.9 (3.1)	8.9 (1.8)	14.7 (7.0)	22.4 (6.0)
Total cholesterol, mmol/L	5.5 (1.0)	5.6 (1.0)	5.8 (1.1)	5.9 (1.1)	5.8 (1.1)	5.52 (1.1)	5.23 (1.0)	5.28 (1)	5.64 (0.9)	5.68 (0.9)	6.0 (1.2)	5.7 (1.2)	5.6 (1.1)	5.3 (1.0)
HDL cholesterol, mmol/L	1.4 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.3 (0.3)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.3 (0.3)	1.3 (0.4)	1.5 (0.4)	1.6 (0.5)	1.2 (0.4)	1.3 (0.4)
LDL cholesterol, mmol/L	NA	NA	3.8 (1.0)	3.9 (0.9)	3.7 (0.9)	3.5 (0.9)	3.1 (0.8)	3.2 (0.9)	3.8 (0.8)	3.8 (0.8)	2.5 (0.7)	2.3 (0.6)	3.6 (1.0)	3.4 (0.9)
Triglycerides, mmol/L	NA	NA	1.3 (0.8)	1.4 (0.9)	1.8 (1.1)	1.5 (1.0)	1.5 (0.8)	1.4 (0.9)	1.6 (0.7)	1.5 (0.7)	1.9 (1.0)	1.6 (0.9)	1.7 (1.2)	1.4 (1.2)
Lipid-lowering medication, %	21.4	20.6	5.1	3.0	7.8	3.1	25.2	14.7	52.3	49.5	13.6	4.7	6.0	3.7
Coronary heart disease, %	13.6	10.8	11.7	5.9	9.1	1.9	5.6	2.9	16.8	13.1	35.1	9.2	12.4	5.7
Stroke														
Hemorrhagic, No (%)	32 (12.5)	—	48 (24.4)	—	69 (14.6)	—	23 (21.5)	—	—	—	45 (14.6)	—	30 (12)	—

Continued

Table 1 Descriptive Statistics of Study Population *(continued)*

Variable[a]	Rotterdam Study		Whitehall II[b]		FINRISK97		DILGOM		PROSPER		EGCUT		FHS	
	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls	Incident Cases	Controls
Ischemic, No (%)	183 (71.2)	—	126 (64.0)	—	405 (85.4)	—	84 (78.5)	—	—	—	261 (84.7)	—	219 (87.3)	—
Not defined, No (%)	42 (16.3)	—	23 (11.6)	—	—	—	—	—	—	—	11 (3.6)	—	2 (0.8)	—

Abbreviations: BMI = body mass index; DILGOM = Dietary, Lifestyle and Genetic Determinants of Obesity and Metabolic Syndrome; EGCUT = Estonian biobank; FHS = Framingham Heart Study; FINRISK97 = FINRISK study 1997; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NA = not applicable; PROSPER = Prospective Study of Pravastatin in the Elderly at Risk.

[a] Values are means ± SD for continuous variables and percentages for dichotomous variables.

[b] While all other cohorts included participants of European ancestry, 87.3% of Whitehall II study cases were of European ancestry, 6.6% of Asian, 5.1% of African American, and 1% of other.

[c] The percentage of participants taking antihypertensive medication is greater than the percentage of hypertension in some cohorts because study participants might use antihypertensive medication for other reasons than hypertension such as β-blockers for heart rhythm problems.

were observed among 38,797 participants across the 7 cohorts. The mean follow-up time ranged from 2 years in PROSPER, 6 years in the Rotterdam Study, and 7 years in EGCUT and DILGOM to 13 years in Whitehall II and 15 years in FINRISK97 and FHS.

The association analysis between circulating metabolites and all incident stroke revealed 27 significant metabolite associations ($p < 1.7 \times 10^{-3}$) in model 1, which are shown in table 2. After further adjustment for hypertension status, diabetes, and smoking, 7 metabolite associations remained significant after correction for multiple testing (table 2 and figure). These included the amino acid histidine (HR 0.90, 95% confidence interval [CI] 0.85, 0.94; $p = 4.45 \times 10^{-5}$) and cholesterol in high-density lipoprotein (HDL) 2 (HR 0.91, 95% CI 0.87, 0.97; $p = 1.41 \times 10^{-3}$), which were associated with a lower risk of stroke, and glycolysis-related metabolite pyruvate (HR 1.09, 95% CI 1.04, 1.14; $p = 7.45 \times 10^{-4}$) and acute-phase reaction markers glycoprotein acetyls (HR 1.09, 95% CI 1.03, 1.15; $p = 1.27 \times 10^{-3}$), which were associated with higher risk of stroke, and several lipoprotein particles, including HDL and low-density lipoprotein (LDL) subfractions (table 2). Cholesterol in medium HDL was associated with lower risk (HR 0.92, 95% CI 0.87, 0.97; $p = 1.35 \times 10^{-3}$) whereas triglycerides in medium and large LDL were associated with a higher risk (HR 1.09, 95% CI 1.03, 1.14; $p = 1.67 \times 10^{-3}$ and HR 1.09, 95% CI 1.03, 1.14; $p = 1.19 \times 10^{-3}$, respectively; table 2) of stroke. The direction of effect across the cohorts showed no evidence of a single cohort driving the associations (data available from Dryad [additional figure 1 in Additional Information file, doi.org/10.5061/dryad.kh1893239). Whereas the Whitehall II study showed the opposite direction of effect for apolipoprotein A, HDL, and HDL2 cholesterol, the findings showed a general spread for most HDL subfractions.

When we stratified the analysis by stroke type, we observed differences between ischemic and hemorrhagic stroke events (table 3). Amino acid histidine and cholesterol in HDL2 were associated with decreased risk of ischemic but not hemorrhagic incident stroke (table 3). Differences were also observed for glycolysis-related metabolite pyruvate and acute-phase inflammation marker glycoprotein acetyls, which were associated with increased risk of ischemic but not hemorrhagic stroke (table 3). Associations between incident stroke events and LDL and HDL particles of various sizes were observed only in the overall analysis, suggesting contributions from both stroke subtypes (table 3).

Furthermore, a significant association was observed between phenylalanine levels and increased risk of incident ischemic stroke (HR 1.12, 95% CI 1.05, 1.19; $p = 4.13 \times 10^{-4}$). We also observed association of circulating levels of cholesterol (HR 0.89, 95% CI 0.84, 0.95; $p = 9.00 \times 10^{-4}$) and free cholesterol in large HDL cholesterol (HR 0.89, 95% CI 0.82, 0.95; $p = 1.33 \times 10^{-3}$) with decreased risk of ischemic stroke. No metabolite surpassed the significant threshold in the analysis for hemorrhagic stroke.

Table 2 Results of Association Analysis Showing the Significant Metabolite Associations With Overall Incident Stroke

	Model 1						Model 2					
Metabolite	No.	No. Cases	HR	CI	I^{2b}	*p* Value	No.	No. Cases	HR	CI	I^2	*p* Value
Phenylalanine	35,091	1,527	1.11	1.06, 1.17	44.4	4.88E-05	35,036	1,524	1.08	1.03, 1.14	17	3.36E-03
Histidine[a]	35,017	1,526	0.89	0.84, 0.93	40.7	7.94E-06	34,962	1,523	0.90	0.85, 0.94	30.2	4.45E-05
Plasma-ApoA1	35,107	1,529	0.91	0.86, 0.96	18.3	7.14E-04	35,052	1,526	0.94	0.88, 0.99	30.3	1.79E-02
HDL-cholesterol	35,107	1,529	0.89	0.84, 0.94	65.8	2.89E-05	35,052	1,526	0.92	0.87, 0.97	68.3	3.20E-03
HDL2-cholesterol[a]	35,107	1,529	0.88	0.84, 0.93	66.3	9.13E-06	35,052	1,526	0.91	0.87, 0.97	69.1	1.41E-03
IDL-triglycerides	38,561	1,780	1.10	1.05, 1.16	38.6	6.06E-05	38,494	1,775	1.07	1.02, 1.12	44	9.91E-03
LDL-triglycerides	35,107	1,529	1.12	1.06, 1.18	2.5	3.93E-05	35,052	1,526	1.08	1.03, 1.14	20.1	2.47E-03
Glucose	34,980	1,524	1.15	1.10, 1.20	13.7	7.81E-11	34,925	1,521	1.06	1.01, 1.11	0	1.87E-02
Lactate	35,100	1,529	1.12	1.07, 1.18	56.7	1.11E-05	35,045	1,526	1.08	1.02, 1.13	29.1	5.09E-03
Pyruvate[a]	24,423	1,205	1.13	1.08, 1.18	48.6	1.37E-07	24,368	1,202	1.09	1.04, 1.14	13.6	7.45E-04
Glycoprotein acetyls[a]	35,101	1,529	1.15	1.09, 1.21	43.2	1.25E-07	35,046	1,526	1.09	1.03, 1.15	38.9	1.27E-03
HDL-diameter	35,107	1,529	0.89	0.84, 0.94	49	3.05E-05	35,052	1,526	0.92	0.87, 0.98	61.6	6.73E-03
S-HDL-triglycerides	35,108	1,529	1.11	1.06, 1.17	48.4	6.80E-05	35,053	1,526	1.07	1.01, 1.12	53.5	1.97E-02
M-HDL-cholesterol[a]	38,560	1,780	0.89	0.85, 0.94	56	2.07E-05	38,493	1,775	0.92	0.87, 0.97	50.4	1.35E-03
M-HDL-cholesterol esters	35,106	1,529	0.90	0.85, 0.95	60.1	2.05E-04	35,051	1,526	0.92	0.87, 0.97	57.8	3.73E-03
M-HDL-free cholesterol	35,106	1,529	0.91	0.86, 0.96	56	7.33E-04	35,051	1,526	0.93	0.88, 0.98	50.1	8.24E-03
L-HDL-cholesterol	38,555	1,780	0.89	0.84, 0.94	63	2.13E-05	38,488	1,775	0.92	0.88, 0.98	66.6	5.50E-03
L-HDL-cholesterol esters	35,101	1,529	0.90	0.84, 0.95	69.1	2.03E-04	35,046	1,526	0.93	0.88, 0.99	72.4	1.37E-02
L-HDL-free cholesterol	35,101	1,529	0.89	0.84, 0.94	67.6	1.25E-04	35,046	1,526	0.92	0.87, 0.98	70.8	9.96E-03
L-HDL-total lipids	35,101	1,529	0.90	0.85, 0.95	69	2.12E-04	35,046	1,526	0.93	0.88, 0.99	71.8	1.70E-02
L-HDL-phospholipids	35,101	1,529	0.90	0.85, 0.96	71.1	6.29E-04	35,046	1,526	0.94	0.89, 1.00	72.2	3.49E-02
L-HDL concentration	35,101	1,529	0.90	0.85, 0.96	69.4	8.53E-04	35,046	1,526	0.94	0.89, 1.00	71.2	4.21E-02
XL-HDL-free cholesterol	35,099	1,527	0.91	0.86, 0.96	0	8.31E-04	35,044	1,524	0.94	0.89, 1.00	11.7	3.55E-02
S-LDL-triglycerides	35,107	1,529	1.10	1.05, 1.16	44.6	1.97E-04	35,052	1,526	1.07	1.01, 1.12	51.3	1.58E-02

Continued

Table 2 Results of Association Analysis Showing the Significant Metabolite Associations With Overall Incident Stroke *(continued)*

	Model 1						Model 2					
Metabolite	No.	No. Cases	HR	CI	I^{2b}	*p* Value	No.	No. Cases	HR	CI	I^2	*p* Value
L-LDL-triglycerides[a]	35,107	1,529	1.12	1.06, 1.17	12.2	3.00E-05	35,052	1,526	1.09	1.03, 1.14	27.5	1.67E-03
M-LDL-triglycerides[a]	35,106	1,529	1.12	1.06, 1.18	0	1.68E-05	35,051	1,526	1.09	1.03, 1.14	1.4	1.19E-03
XL-VLDL-triglycerides	38,284	1,769	1.09	1.04, 1.14	75.9	1.56E-04	38,217	1,764	1.05	1.00, 1.10	73.9	4.66E-02

Abbreviations: ApoA1 = apolipoprotein A1; CI = confidence interval; HDL = high-density lipoprotein; HR = hazard ratio; IDL = intermediate-density lipoprotein; I^2 = heterogeneity parameter; L = large; LDL = low-density lipoprotein; M = medium; *p* = *p* value for association of metabolite and stroke; S = small; VLDL = very-low-density lipoprotein; XL = very large.
Model 1 adjusted for age, sex, body mass index, lipid-lowering medication, and study-specific covariates if needed. Model 2 additionally adjusted for smoking status, diabetes, and hypertension.
[a] Associations that surpassed significance threshold in model 2.
[b] Random-effect meta-analysis was performed in the case of statistical heterogeneity, defined as I^2 > 50%. Subsequently, the correlation between effect estimates (HR) derived from fixed- and random-effect meta-analysis was checked. The correlation coefficient between these 2 estimates was 0.99.

Discussion

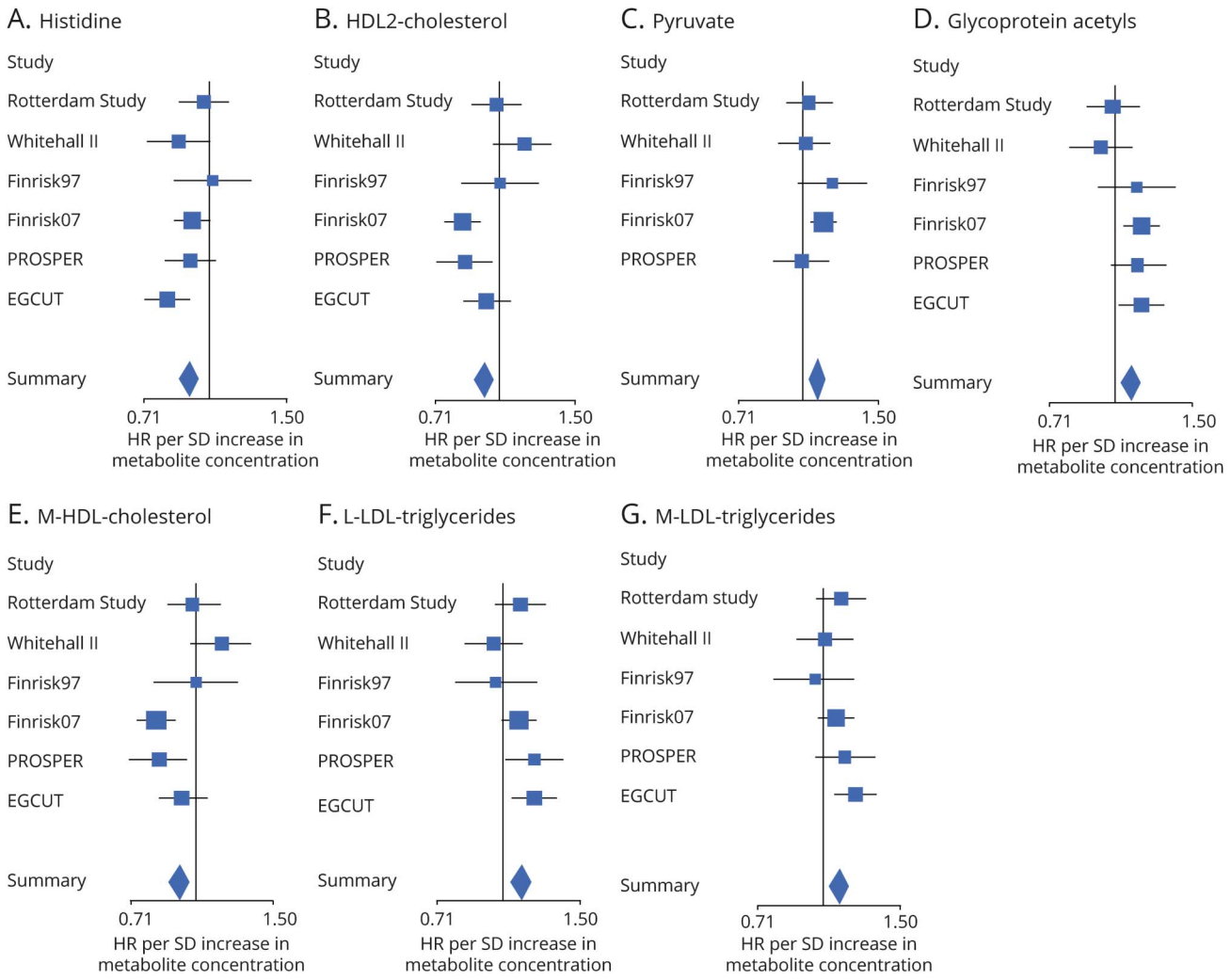
In this study, we identified 10 metabolites associated with the risk of stroke. These include amino acid histidine and cholesterol in HDL2, which were associated with decreased risk of stroke overall and ischemic stroke subtype, and glycolysis-related metabolite pyruvate and acute-phase reaction markers glycoprotein acetyls, which were associated with increased risk of stroke overall and ischemic stroke. Cholesterol in medium HDL and triglycerides in medium and large LDL particles were associated with stroke overall, while amino acid phenylalanine and HDL subfractions, including cholesterol and free cholesterol in large HDL, were associated with ischemic but not with hemorrhagic stroke. This pattern of results was independent of traditional risk factors, including hypertension, diabetes, smoking, and BMI.

The strongest association was found between amino acid histidine and risk of stroke. We observed that a 1-SD increase in concentration of histidine was associated with 10% lower risk of stroke. The effect was similar across studies, with only the FINRISK97 study showing no effect. Even though the same direction of effect was observed for both ischemic and hemorrhagic stroke subtypes, the association was driven mainly by ischemic stroke. Histidine is a semiessential amino acid; adults generally produce it while children may not. Histidine can be converted to histamine, which shows a strong effect on vasodilatation and functions as a neurotransmitter in the brain.[29,30] Previous studies reported that oral administration of histidine can reduce blood pressure.[31–33] Plasma concentrations of histidine have been inversely associated with inflammation and oxidative stress in patients with chronic kidney disease and obese women with metabolic syndrome.[34,35] Recent animal studies reported that histidine treatment alleviated the infarction induced by middle cerebral artery occlusion[36] and showed long-term neuroprotection after cerebral ischemia with decreased infarct volume and improved neurologic function.[37] Even though our findings support the results of previous studies, in the most comprehensive study of stroke to date within the China Kadoorie Biobank, histidine was not associated with ischemic or hemorrhagic stroke. However, in that study, a nominal association was found with myocardial infarction.[11] This might be explained by either environmental or ethnic differences of studied populations or differences in the confounders for which the investigators adjusted. In the present study, we adjusted for a more comprehensive set of potential confounders, including BMI, lipid-lowering medication, diabetes, and hypertension.

We also found the glycolysis-related metabolite pyruvate to be associated with increased risk of stroke. The analyses of stroke subtypes suggested that this association was driven by ischemic incident stroke events. Our findings suggested that a 1-SD increase in pyruvate concentration was associated with 13% higher risk of ischemic stroke. Pyruvate is the end-product of glycolysis, and it is critical for supplying energy to the cell.[38] Pyruvate has previously been shown to protect against experimental stroke possibly by blocking inflammation.[39,40] In this light, our finding seems to contrast previously described effects of pyruvate. However, in a combined study of myocardial infarction and stroke using the same metabolomics platform as the present study, higher levels of pyruvate were also associated with a higher risk of cardiovascular disease.[41] The mechanisms linking circulating levels of pyruvate to stroke and cardiovascular disease risk are still to be elucidated.

Acute-phase marker glycoprotein acetyls, mainly α-1 glycoprotein, were associated with higher risk of stroke. Our analyses suggested that the association was strongest for the ischemic subtype, for which we found that an increase of 1 SD in the circulating compound was associated with 13% higher risk of ischemic stroke. Our results confirmed the association of glycoprotein acetyl with ischemic stroke that was observed

Figure Forest Plots for Metabolites Associated With Overall Incident Stroke



(A–G) Associations were significant after adjustment for age, sex, body mass index, lipid-lowering medication, smoking status, diabetes, and hypertension. EGCUT = Estonian biobank; FINRISK97 = FINRISK study 1997; HDL = high-density lipoprotein; L = large; HR = hazard ratio; LDL = low-density lipoprotein; M = medium; PROSPER = Prospective Study of Pravastatin in the Elderly at Risk.

in individuals in the China Kadoorie Biobank.[11] Circulating levels of glycoprotein acetyls have previously been associated with cardiovascular diseases and dementia but also inflammatory disease, cancer, and mortality.[41–43]

Analyses focused on stroke subtypes revealed the association of essential amino acid phenylalanine with increased risk of ischemic stroke. A 1-SD increase in concentration of phenylalanine was associated with 12% higher risk of ischemic stroke. Phenylalanine is a precursor for tyrosine and catecholamines, including dopamine, epinephrine, and norepinephrine. Phenylalanine has previously been associated with increased risk of cardiovascular disease and diabetes.[41,44,45] However, the association with phenylalanine remained after adjustment for diabetes. Phenylalanine was not associated with risk of hemorrhagic stroke.

The majority of circulating biomarkers measured by NMR metabolomics technology belong to lipid concentrations and the composition of 14 lipoprotein subparticles. This provides an excellent opportunity for a comprehensive investigation of lipoprotein particles in stroke because analyses of cholesterol and cholesterol subfractions have shown inconsistent results.[3–5] Previous metabolomic and lipidomic studies of stroke reported associations of various molecular species with ischemic stroke, including ceramides, diacylglycerol, docosatrienoic acid, hydroxyeicosatetraenoic acid, hydroxyoctadecadienoic acid, lysophosphatidylcholines, and triacylglycerols.[46] Furthermore, lipidomics approach provided insights into the metabolism of stroke induced by small vessel disease because associations with glucosylceramide, phosphatidylethanolamine, free fatty acid, and triacylglycerol were observed.[47] In our study population, we observed associations of cholesterol in medium HDL with decreased risk of stroke and of triglycerides in large and medium LDL particles with increased risk of stroke. None of these lipoprotein measurements were found to be associated with stroke in the China Kadoorie Biobank.[11] However, the

Table 3 Associations for Incident Stroke Events When Classified by Stroke Type

		Model 1					Model 2				
Metabolite	**Type**	**No.**	**No. Cases**	**HR**	**CI**	***p* Value**	**No.**	**No. Cases**	**HR**	**CI**	***p* Value**
Phenylalanine	Hemorrhagic Ischemic[a]	30,144 30,290	214 1,051	0.94 1.16	0.81, 1.09 1.09, 1.23	3.90E-01 3.15E-06[b]	30,092 30,236	214 1,049	0.91 1.12	0.78, 1.05 1.05, 1.19	2.00E-01 4.13E-04[b]
Histidine	Hemorrhagic Ischemic[a]	30,070 30,216	214 1,050	0.95 0.88	0.82, 1.1 0.82, 0.94	4.78E-01 1.18E-04[b]	30,018 30,162	214 1,048	0.96 0.89	0.83, 1.11 0.84, 0.95	6.16E-01 4.94E-04[b]
ApoA1	Hemorrhagic Ischemic	30,155 30,301	216 1,051	1.03 0.89	0.89, 1.19 0.83, 0.95	7.32E-01 5.87E-04[b]	30,103 30,247	216 1,049	1.04 0.92	0.9, 1.2 0.86, 0.98	6.17E-01 1.43E-02
HDL-cholesterol	Hemorrhagic Ischemic	30,155 30,301	216 1,051	1.04 0.86	0.9, 1.21 0.81, 0.92	5.63E-01 1.82E-05[b]	30,103 30,247	216 1,049	1.07 0.9	0.92, 1.24 0.84, 0.96	3.97E-01 1.89E-03
HDL2-cholesterol	Hemorrhagic Ischemic[a]	30,155 30,301	216 1,051	1.04 0.85	0.9, 1.21 0.8, 0.91	5.75E-01 2.85E-06[b]	30,103 30,247	216 1,049	1.07 0.89	0.92, 1.24 0.83, 0.95	3.90E-01 5.29E-04[b]
IDL-triglycerides	Hemorrhagic Ischemic	33,609 33,755	246 1,270	0.92 1.13	0.81, 1.06 1.07, 1.2	2.63E-01 6.91E-06[b]	33,545 33,689	246 1,266	0.89 1.09	0.78, 1.02 1.03, 1.15	1.02E-01 2.01E-03
LDL-triglycerides	Hemorrhagic Ischemic	30,155 30,301	216 1,051	1.02 1.14	0.88, 1.18 1.07, 1.21	8.27E-01 2.89E-05[b]	30,103 30,247	216 1,049	0.99 1.1	0.85, 1.14 1.04, 1.17	8.62E-01 1.82E-03
Glucose	Hemorrhagic Ischemic	30,033 30,179	214 1,048	1.13 1.17	0.99, 1.28 1.12, 1.23	7.07E-02 5.37E-11[b]	29,981 30,125	214 1,046	1.09 1.07	0.96, 1.24 1.01, 1.13	2.20E-01 2.13E-02
Lactate	Hemorrhagic Ischemic	30,153 30,299	216 1,051	1.06 1.16	0.92, 1.22 1.09, 1.24	4.04E-01 1.11E-06[b]	30,101 30,245	216 1,049	1.04 1.1	0.9, 1.19 1.04, 1.17	6.13E-01 1.98E-03
Pyruvate	Hemorrhagic Ischemic[a]	19,481 19,627	167 778	0.99 1.17	0.84, 1.16 1.11, 1.23	8.70E-01 2.86E-10[b]	19,429 19,573	167 776	0.96 1.13	0.81, 1.12 1.07, 1.19	5.94E-01 1.93E-05[b]
Glycoprotein acetyls	Hemorrhagic Ischemic[a]	30,154 30,300	216 1,051	1.02 1.2	0.88, 1.18 1.13, 1.28	8.06E-01 8.55E-09[b]	30,102 30,246	216 1,049	0.96 1.13	0.83, 1.11 1.06, 1.2	6.28E-01 2.17E-04[b]
Mean diameter of HDL	Hemorrhagic Ischemic	30,155 30,301	216 1,051	1.03 0.86	0.89, 1.2 0.8, 0.92	6.98E-01 1.28E-05[b]	30,103 30,247	216 1,049	1.07 0.9	0.92, 1.24 0.84, 0.96	4.08E-01 2.93E-03
S-HDL-triglycerides	Hemorrhagic Ischemic	30,156 30,302	216 1,051	1 1.14	0.86, 1.15 1.08, 1.22	9.71E-01 1.99E-05[b]	30,104 30,248	216 1,049	0.96 1.09	0.83, 1.11 1.02, 1.16	5.84E-01 8.32E-03
M-HDL-cholesterol	Hemorrhagic Ischemic	33,608 33,754	246 1,270	1 0.88	0.87, 1.15 0.83, 0.93	9.88E-01 3.11E-05[b]	33,544 33,688	246 1,266	1.02 0.91	0.88, 1.17 0.85, 0.97	8.27E-01 1.95E-03
M-HDL-cholesterol esters	Hemorrhagic Ischemic	30,154 30,300	216 1,051	0.99 0.89	0.86, 1.14 0.83, 0.95	8.84E-01 4.60E-04[b]	30,102 30,246	216 1,049	1 0.91	0.87, 1.16 0.86, 0.98	9.72E-01 6.99E-03
M-HDL-free cholesterol	Hemorrhagic Ischemic	30,154 30,300	216 1,051	1.02 0.9	0.88, 1.18 0.84, 0.96	8.25E-01 1.75E-03	30,102 30,246	216 1,049	1.02 0.92	0.88, 1.18 0.86, 0.98	7.79E-01 1.56E-02
L-HDL-cholesterol	Hemorrhagic Ischemic[a]	33,603 33,749	246 1,270	1.07 0.85	0.93, 1.24 0.8, 0.91	3.19E-01 2.03E-06[b]	33,539 33,683	246 1,266	1.11 0.89	0.96, 1.28 0.84, 0.95	1.56E-01 9.00E-04[b]
L-HDL-cholesterol esters	Hemorrhagic Ischemic	30,149 30,295	216 1,051	1.07 0.86	0.92, 1.24 0.8, 0.92	3.88E-01 4.28E-05[b]	30,097 30,241	216 1,049	1.1 0.9	0.95, 1.28 0.84, 0.97	2.13E-01 3.59E-03

Continued

Table 3 Associations for Incident Stroke Events When Classified by Stroke Type *(continued)*

		Model 1					Model 2				
Metabolite	**Type**	**No.**	**No. Cases**	**HR**	**CI**	***p* Value**	**No.**	**No. Cases**	**HR**	**CI**	***p* Value**
L-HDL-free cholesterol	Hemorrhagic Ischemic[a]	30,149 30,295	216 1,051	1.09 0.85	0.93, 1.26 0.79, 0.91	2.81E-01 1.04E-05[b]	30,097 30,241	216 1,049	1.12 0.89	0.96, 1.3 0.82, 0.95	1.44E-01 1.33E-03[b]
L-HDL-total lipids	Hemorrhagic Ischemic	30,149 30,295	216 1,051	1.06 0.87	0.91, 1.23 0.81, 0.93	4.71E-01 5.91E-05[b]	30,097 30,241	216 1,049	1.09 0.91	0.93, 1.27 0.84, 0.97	2.77E-01 6.00E-03
L-HDL-concentration	Hemorrhagic Ischemic	30,149 30,295	216 1,051	1.09 0.87	0.94, 1.27 0.81, 0.93	2.73E-01 1.30E-04[b]	30,097 30,241	216 1,049	1.12 0.91	0.96, 1.3 0.85, 0.98	1.44E-01 1.04E-02
L-HDL-phospholipids	Hemorrhagic Ischemic	30,149 30,295	216 1,051	1.08 0.87	0.93, 1.26 0.81, 0.93	3.21E-01 9.62E-05[b]	30,097 30,241	216 1,049	1.11 0.91	0.95, 1.29 0.85, 0.98	1.85E-01 9.32E-03
XL-HDL-free cholesterol	Hemorrhagic Ischemic	30,147 30,293	216 1,049	1.07 0.88	0.93, 1.24 0.82, 0.94	3.52E-01 3.46E-04[b]	30,095 30,239	216 1,047	1.09 0.92	0.94, 1.26 0.86, 0.99	2.38E-01 1.75E-02
S-LDL-triglycerides	Hemorrhagic Ischemic	30,155 30,301	216 1,051	1.00 1.12	0.87, 1.16 1.06, 1.19	9.84E-01 1.18E-04[b]	30,103 30,247	216 1,049	0.97 1.08	0.84, 1.12 1.02, 1.15	6.99E-01 1.10E-02
L-LDL-triglycerides	Hemorrhagic Ischemic	30,155 30,301	216 1,051	1.01 1.13	0.87, 1.17 1.07, 1.2	9.12E-01 4.39E-05[b]	30,103 30,247	216 1,049	0.98 1.1	0.85, 1.13 1.03, 1.17	7.87E-01 2.20E-03
M-LDL-triglycerides	Hemorrhagic Ischemic	30,154 30,300	216 1,051	1.04 1.14	0.9, 1.2 1.07, 1.21	5.70E-01 2.84E-05[b]	30,102 30,246	216 1,049	1.02 1.1	0.88, 1.17 1.04, 1.17	8.35E-01 1.80E-03
XL-VLDL-triglycerides	Hemorrhagic Ischemic	33,352 33,499	242 1,263	0.98 1.12	0.85, 1.12 1.06, 1.18	7.66E-01 2.00E-05[b]	33,288 33,433	242 1,259	0.96 1.07	0.83, 1.1 1.01, 1.13	5.23E-01 1.41E-02

Abbreviations: ApoA1 = apolipoprotein A1; CI = confidence interval; HDL = high-density lipoprotein; HR = hazard ratio; IDL = intermediate-density lipoprotein; I^2 = heterogeneity parameter; LDL = low-density lipoprotein; M = medium; L = large; *p* = *p* value for association of metabolite and stroke; S = small; VLDL = very-low-density lipoprotein; XL = very large.
Metabolites that showed significant association with overall incident stroke in table 2 were included.
Model 1 adjusted for age, sex, body mass index, lipid-lowering medication, and ethnicity if needed. Model 2 additionally adjusted for smoking status, diabetes, and hypertension.
[a] Associations that surpassed the significance threshold in model 2.
[b] Associations that passed the threshold for multiple testing.

association of cholesterol in medium HDL with decreased risk of stroke was previously observed in the cohort of Japanese men and women.[48] Furthermore, previous studies also showed inverse association of coronary heart disease with medium-sized HDL particles.[49] Alterations in specific HDL particles provide additional evidence about the heterogeneity of HDL lipoprotein particles. However, mechanisms through which specific HDL particles might protect against stroke are not well understood yet. Previous studies reported that smaller HDL particles have a larger capacity to remove cholesterol from membranes of peripheral cells such as macrophage foam cell.[48,50] This potentially antiatherogenic effect is one possible mechanism underlying association of medium HDL cholesterol and stroke. This is also in line with our finding that individuals with higher cholesterol levels in medium HDL particle subclasses may be protected against stroke. Another explanation for the HDL protective effect could be its antioxidant function, which differs across subfractions. Previous studies showed that antioxidant properties of HDL were enriched in the smallest and densest HDL particles, including medium HDL.[51,52] Similarly, anti-apoptotic and anti-inflammatory properties of HDL may also play a role in protection against stroke. Sphingosine-1-phosphate and other lipids carried on HDL particles are possible molecular mediators of this anti-inflammatory effect. Previous studies reported enrichment of sphingosine-1-phosphate in small and dense HDL particles and its inverse correlation endothelial cell apoptosis.[52,53] Furthermore, specific LDL particles such as triglycerides in large and medium LDL were associated with increased risk of stroke in our study. Previous studies reported high LDL triglycerides levels to be associated with incident stroke and coronary heart disease.[54] Because higher LDL triglycerides levels were also reported to be associated with increased high-sensitivity C-reactive protein level and white blood cell count, one possible mechanism underlying association of LDL triglycerides and cerebrovascular disease may be inflammation.[54,55] Interestingly, the China Kadoorie

Biobank reported associations of very-low-density lipoprotein, intermediate-density lipoprotein, and LDL with ischemic stroke.[11] However, we were not able to confirm these results in our study population. Lack of replication might be explained by environmental and ethnic differences of studied populations or the confounders that were adjusted for.

The strengths of our study include its large sample size, prospective study design with detailed data collection over a long period of follow-up, and the same experimental NMR setup for metabolite quantification across multiple studies. Our study also has several limitations. With new, improved methods available, many additional metabolites can be measured, which can be of importance for stroke.[56] Included studies showed heterogeneity in terms of types of samples used across the cohorts and different times and types of sample storage. Even though different sample material was used in cohorts, a previously published article illustrated for NMR-based lipoprotein subclass measures that most metabolite levels are identical in plasma and serum samples, in particular lipid measures.[57] For some metabolites, the absolute levels are slightly different with constant offset, but the relation is linear. This would suggest that metabolite variability in different types of samples is not problematic for cohort-specific analysis followed by meta-analysis, as illustrated in many published articles that have combined analyses of plasma and serum samples in meta-analysis[58,59] and showed consistent results between the cohorts with different blood specimens. Furthermore, highly consistent epidemiologic results have been observed between studies that differ in terms of the time of sample storage. It is well known that storage slightly modifies the lipoprotein composition of serum/plasma samples; however, these effects are due to biological changes in the actual samples and are not related to the analysis method as such. These are minor compared to interindividual differences in metabolite concentrations if the samples are handled according to standard clinical laboratory practices and stored at least in −70°C. Deelen et al.[58] and van der Lee et al.[25] reported biomarker association with mortality or cognition using the same population-based studies as in our project, and the metabolite associations showed consistency even though there were differences in how long the samples have been stored. There was also heterogeneity in the methods to ascertain cases of incident stroke across the cohorts. Most of the cohort studies used electronic health registries, which may have limited sensitivity and subsequently influenced power to identify novel significant associations. Statistical power was reduced in analyses of stroke subtypes because some of the cohorts were unable to distinguish between them. Limited sample size for the analysis of hemorrhagic stroke reduced our ability to detect novel associations for this stroke type. Finally, reported associations may represent signals due to other metabolites or other factors. Therefore, future studies should explore whether these metabolites play a causal role.

We found an association between 10 metabolites and risk of stroke in 1,791 incident stroke events observed among 38,797 individuals from 7 prospective cohort studies. The biological mechanisms underlying these associations should be the subject of further studies.

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Disclosure

Dr. Dina Vojinovic, Ms. Marita Kalaoja, Dr. Stella Trompet, Dr. Krista Fischer, Mr. Martin J. Shipley, Dr. Shuo Li, Mr. Aki S. Havulinna, Dr. Markus Perola, Dr. Veikko Salomaa, Dr. Qiong Yang, Dr. Naveed Sattar, Dr. Pekka Jousilahti, Dr. Najaf Amin, Dr. Claudia L. Satizabal, Ms. Nele Taba, Dr. Behnam Sabayan, Dr. Ramachandran S. Vasan, Dr. M. Arfan Ikram, Dr. David J. Stott, Dr. Mika Ala-Korpela, Dr. J. Wouter Jukema, PhD, Dr. Sudha Seshadri, Dr. Mika Kivimaki, Dr. Tonu Esko, and Dr. Cornelia M. van Duijn report no disclosures. Dr. Johannes Kettunen possesses a modest amount of stock options of Nightingale Health Ltd, a company offering NMR metabolomics profiling. Go to Neurology.org/N for full disclosures.

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Name	Location	Contribution
Dina Vojinovic, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Design and conceptualized study; analyzed and interpreted the data; drafted the manuscript for intellectual content
Marita Kalaoja, MSc	University of Oulu and Biocenter Oulu, Finland	Analyzed the data; revised the manuscript for intellectual content
Stella Trompet, PhD	Leiden University Medical Center, the Netherlands	Analyzed and interpreted the data; revised the manuscript for intellectual content
Krista Fischer, PhD	University of Tartu, Estonia	Analyzed the data; revised the manuscript for intellectual content
Martin J. Shipley, MSc	UCL, London, UK	Contributed to acquisition of data; analyzed and interpreted the data; revised the manuscript for intellectual content
Shuo Li, PhD	Boston University, MA	Analyzed the data; revised the manuscript for intellectual content
Aki S. Havulinna, PhD	Finnish Institute for Health and Welfare, Helsinki, Finland	Contributed to acquisition of data; revised the manuscript for intellectual content
Markus Perola, PhD	Finnish Institute for Health and Welfare, Helsinki, Finland	Contributed to acquisition of data; revised the manuscript for intellectual content
Veikko Salomaa, PhD	Finnish Institute for Health and Welfare, Helsinki, Finland	Contributed to acquisition of data; revised the manuscript for intellectual content
Qiong Yang, PhD	Boston University, MA	Interpreted the data; revised the manuscript for intellectual content
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Najaf Amin, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Interpreted the data; revised the manuscript for intellectual content
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Nele Taba, MSc	University of Tartu, Estonia	Interpreted the data; revised the manuscript for intellectual content
Behnam Sabayan, PhD	Northwestern University, Chicago, IL	Revised the manuscript for intellectual content
Ramachandran S. Vasan, MD	Framingham Heart Study, MA	Interpreted the data; revised the manuscript for intellectual content

Continued

Appendix *(continued)*

Name	Location	Contribution
M. Arfan Ikram, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Interpreted the data; revised the manuscript for intellectual content
David J. Stott, MD	University of Glasgow, UK	Contributed to acquisition of data; revised the manuscript for intellectual content
Mika Ala-Korpela, PhD	University of Oulu and Biocenter Oulu, Finland	Interpreted the data; revised the manuscript for intellectual content
J. Wouter Jukema, PhD	Leiden University Medical Center, the Netherlands	Contributed to acquisition of data; revised the manuscript for intellectual content
Sudha Seshadri, MD	UT Health San Antonio, TX	Interpreted the data; revised the manuscript for intellectual content
Johannes Kettunen, PhD	University of Oulu and Biocenter Oulu, Finland	Interpreted the data; revised the manuscript for intellectual content
Mika Kivimaki, FMedSci	UCL, London, UK	Contributed to acquisition of data; interpreted the data; revised the manuscript for intellectual content
Tonu Esko, PhD	University of Tartu, Estonia	Interpreted the data; revised the manuscript for intellectual content
Cornelia M. van Duijn, PhD	University of Oxford, UK	Design and conceptualized study; interpreted data; drafted the manuscript for intellectual content

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