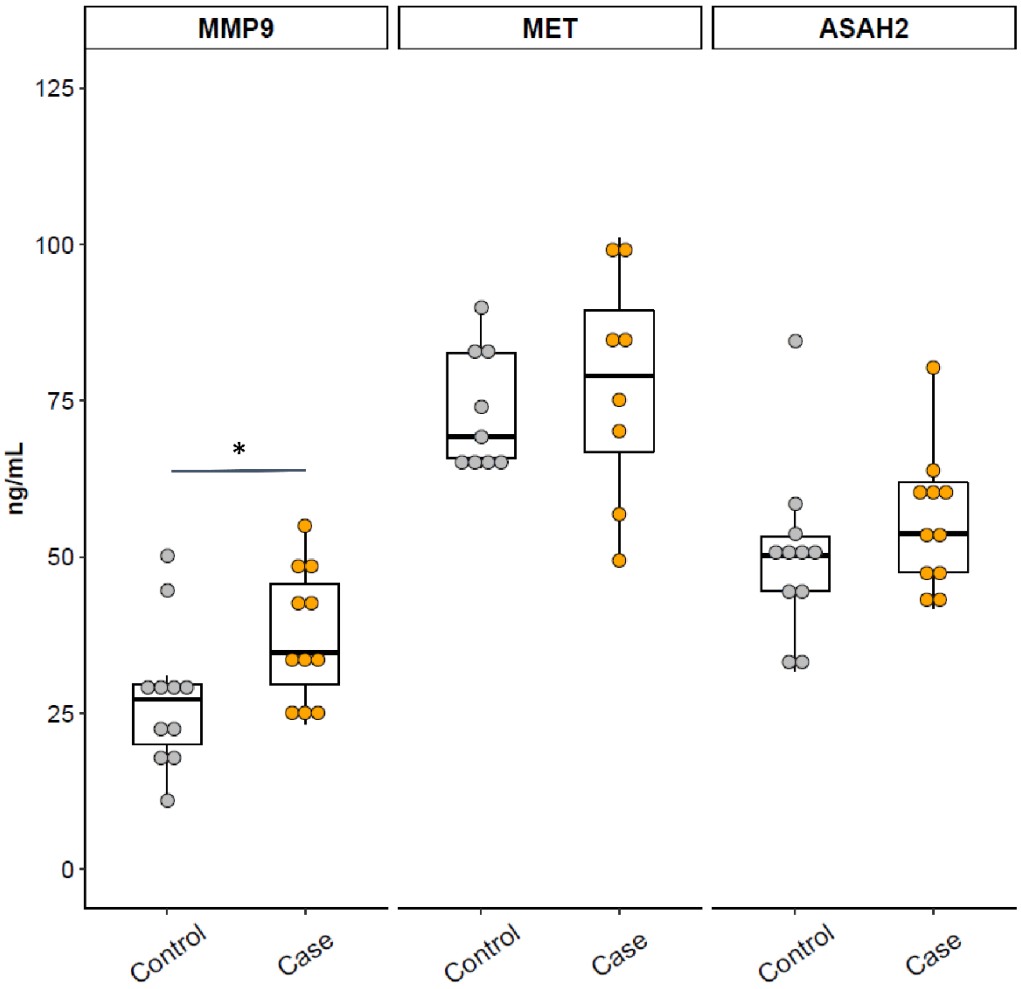
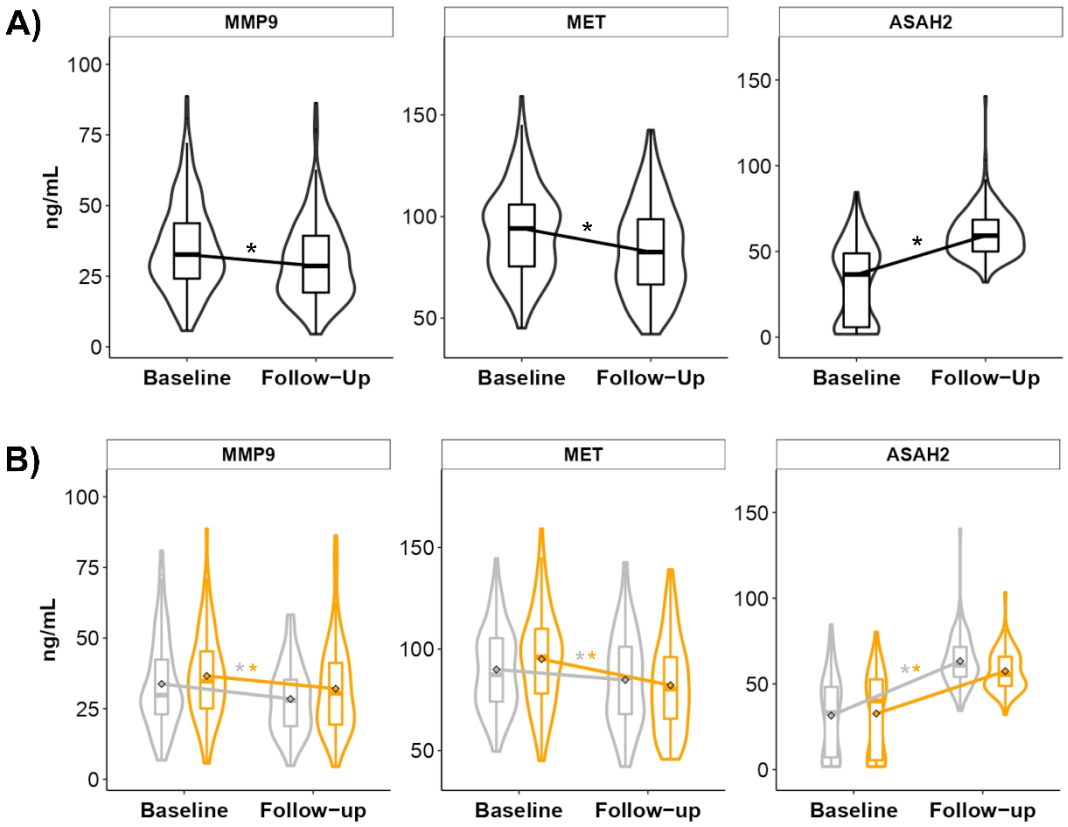


Supplementary Figure 1. Boxplots representing baseline biomarker concentrations of the replication phase in subjects who additionally belonged to the discovery study (N=22).

Y axis represents baseline concentrations in biomarkers tested in the replication phase. X axis represents the group (cases versus controls). Each panel represents one protein (MMP9, MET and ASAH2). Results can be compared with those of figure 2-B.

Missing data: 3 out of 11 pairs missed baseline MET concentration in the replication phase.

*: *p*-value<0.05.



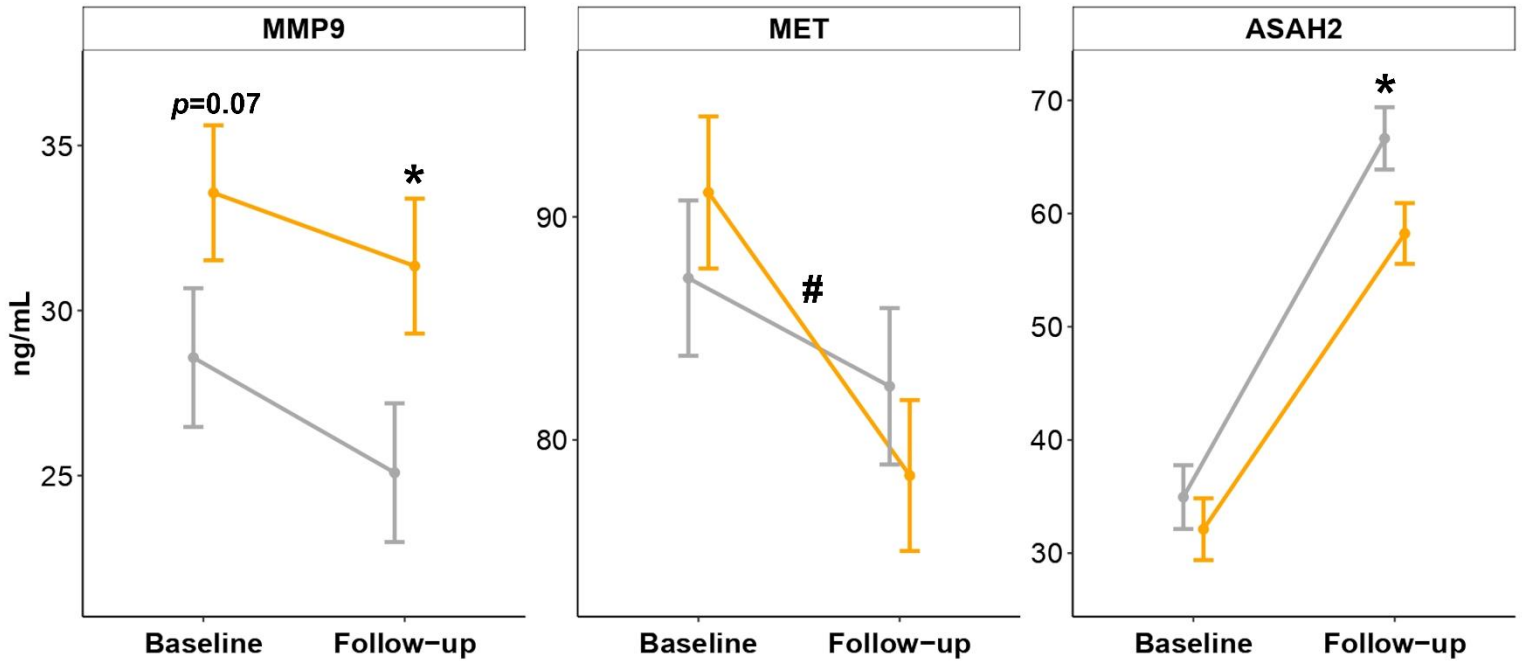
Supplementary figure 2. Boxplots showing the temporal profile of biomarkers from the replication phase.

Violin plots show the distribution (empirical density function) of those biomarkers chosen in the replication phase, while boxplots display the median and the inter-quartile range (N=160). Lines represent the changes in the concentration (Y axis) from baseline to follow-up visit (X axis). Row A shows the change in MMP9, MET and ASAH2 in the whole sample, while in row B these results are stratified by group (controls in grey, and cases in orange). In row B, the small diamonds represent the mean of each group at each time.

Missing data: 13 samples were missing considering MET (7 were baseline samples and 6 were follow-up samples) and 12 for ASAH2 (all baseline samples).

*: Repeated measures *p*-value<0.01.

Key: ASAH2, neutral ceramidase; MET, hepatocyte growth factor receptor; MMP9, matrix metalloproteinase-9; RFU, relative fluorescence units.



Supplementary Figure 3. **Sensitivity analysis representing biomarker concentrations in pairs of subjects having equal WMH burden (N=110).** Lines represent adjusted means from baseline to the follow-up visit in cases (orange) and controls (grey). Error bars represent the standard error of the adjusted mean. These adjusted means have been extracted from mixed models corrected for: baseline age, sex, visit, change in systolic blood pressure and baseline WMH.

After excluding pairs of subjects showing differences in baseline WMH, we obtained equivalent results as in the whole sample (see Figure 3).

*: *p*-value<0.05 between groups at the baseline visit or at the follow-up visit.

#: group and visit interaction *p*-value<0.05. This interaction term represents the difference in the rate of changes between groups in biomarker concentration.

Supplementary table 1. Characteristics of the discovery phase sample by cases and controls

	Controls (n = 12)	Cases (n = 12)	*p*-value
Baseline Age, years	64.0 (62.3-67.8)	63.5 (62.0-67.0)	0.909
Sex	6 (50.0)	6 (50.0)	1
Time between visits, years	4.0 (3.8;4.2)	3.9 (3.8;4.7)	0.453
Vascular risk factors			
Diabetes mellitus	3 (25.0)	0 (0)	0.220
Active Smoker	0 (0)	3 (25.0)	0.220
SBP, mmHg	145.3 (11.3)	138.3 (15.2)	0.208
ΔSBP, mmHg	**-3.4 (11.5)**	**11.1 (16.8)**	**0.024**
DBP, mmHg	78.2 (10.6)	77.3 (9.6)	0.834
ΔDBP, mmHg	-3.5 (7.4)	-0.5 (7.9)	0.361
Cholesterol, mg/dL	214.3 (42.3)	222.7 (44.5)	0.639
ΔCholesterol, mg/dL	8.9 (44.3)	-14.2 (31.4)	0.156
HDL Cholesterol, mg/dL	49.6 (14.9)	51.5 (13.0)	0.742
ΔHDL Cholesterol, mg/dL	2.8 (0.0;5.3)	4.4 (2.0;10.2)	0.441
Baseline Cerebral Small Vessel Disease			
Extensive PVH	3 (25.0)	3 (25.0)	1
Extensive DWMH	2 (16.7)	4 (33.3)	0.640
Silen brain infarct	0 (0)	3 (25.0)	0.220

Values represent mean (SD), median (interquartile range) or number (%). Bolded variables are those which show significant differences between cases and controls (P-value<0.05).

Key: DBP, diastolic blood pressure; DWMH, deep white matter hyperintensities; HDL, high density lipoprotein; PVH, periventricular hyperintensities; SBP, systolic blood pressure.

Supplementary table 2. Proteins differentially expressed in the discovery study

Gene name	Uniprot Code	logFC	*t*-statistic	*p*-value	*q*-value
MET	P08581	-0.211	-2.867	0.0084	0.9366
ASAH2	Q9NR71	-0.382	-2.858	0.0085	0.9366
IDS	P22304	-0.395	-2.838	0.0090	0.9366
MMP9	P14780	0.482	2.796	0.0099	0.9366
CD200R1	Q8TD46	-0.268	-2.756	0.0108	0.9366
IL5RA	Q01344	-0.263	-2.740	0.0113	0.9366
F2	P00734	-0.324	-2.707	0.0122	0.9366
ADGRE2	Q9UHX3	-0.279	-2.695	0.0125	0.9366
SEMA3E	O15041	-0.497	-2.673	0.0131	0.9366
GPC3	P51654	-0.326	-2.598	0.0156	0.9366
CGA TSHB	P01215 P01222	-0.579	-2.516	0.0188	0.9366
SEMA6A	Q9H2E6	-0.440	-2.491	0.0198	0.9366
CLEC7A	Q9BXN2	0.278	2.482	0.0203	0.9366
CD109	Q6YHK3	-0.390	-2.477	0.0205	0.9366
PGK1	P00558	-0.258	-2.435	0.0225	0.9366
DCN	P07585	-0.211	-2.395	0.0245	0.9366
CADM1	Q9BY67	-0.309	-2.373	0.0258	0.9366
A2M	P01023	-0.255	-2.370	0.0260	0.9366
COLEC12	Q5KU26	-0.392	-2.347	0.0273	0.9366
MPO	P05164	0.352	2.321	0.0289	0.9366
BRF1	Q92994	0.227	2.302	0.0301	0.9366
CST5	P28325	-0.619	-2.262	0.0328	0.9366
BMPER	Q8N8U9	0.203	2.261	0.0329	0.9366
NR1D1	P20393	0.281	2.252	0.0335	0.9366
CCL26	Q9Y258	0.202	2.244	0.0341	0.9366
C4A C4B	P0C0L4, P0C0L5	0.119	2.243	0.0342	0.9366
ITGAV ITGB5	P06756, P18084	0.367	2.240	0.0344	0.9366
NTRK3	Q16288	-0.253	-2.232	0.0349	0.9366
PROC	P04070	0.163	2.222	0.0357	0.9366
FTH1 FTL	P02794 P02792	-0.569	-2.218	0.0361	0.9366
IL1R1	P14778	-0.537	-2.185	0.0386	0.9366
SLITRK5	O94991	-0.427	-2.157	0.0410	0.9366
BOC	Q9BWV1	-0.426	-2.147	0.0418	0.9366
SGTA	O43765	-0.520	-2.146	0.0419	0.9366
EDA	Q92838	-0.295	-2.145	0.0420	0.9366
POMC	P01189	0.175	2.141	0.0423	0.9366
PLXNB2	O15031	-0.133	-2.106	0.0456	0.9366
SPINT2	O43291	-0.241	-2.096	0.0465	0.9366
GDNF	P39905	0.133	2.094	0.0468	0.9366
C5	P01031	0.275	2.086	0.0475	0.9366
LAG3	P18627	-0.317	-2.071	0.0490	0.9366

Proteins differentially expressed in the discovery study. LogFC (log fold change) and *t*-statistic correspond to $\text{mean}_{\text{cases}} - \text{mean}_{\text{controls}}$ contrast. Adjusted *q*-values have been calculated with false discovery rate correction.

Supplementary table 3: Enriched Gene Sets in the discovery phase.

ID	Name	Size	*p*-value	FDR *q*-value
REAC:R-HSA-6803157	Antimicrobial peptides	97	<0.0001	<0.0001
GO:0019199	Transmembrane receptor protein kinase activity	82	<0.0001	0.0222
GO:0004714	Transmembrane receptor protein tyrosine kinase activity	64	<0.0001	0.0278
GO:0004888	Transmembrane signaling receptor activity	1244	<0.0001	0.1439
REAC:R-HSA-1442490	Collagen degradation	64	0.0029	0.1528
GO:0004252	Serine-type endopeptidase activity	247	<0.0001	0.1735
GO:1901343	Negative regulation of vasculature development	35	<0.0001	0.1752
GO:0140110	Transcription regulator activity	2068	0.0201	0.1796
REAC:R-HSA-6798695	Neutrophil degranulation	477	<0.0001	0.1813
GO:0008134	Transcription factor binding	654	0.0300	0.1815
GO:0019838	Growth factor binding	139	0.0091	0.1863
GO:1990837	Sequence-specific double-stranded DNA binding	848	0.0377	0.1869
GO:0017171	Serine hydrolase activity	274	<0.0001	0.1873
GO:0003712	Transcription coregulator activity	561	0.0334	0.1898
GO:0001012	RNA polymerase II regulatory region DNA binding	758	0.0388	0.1899
GO:0000977	RNA polymerase II regulatory region sequence-specific DNA binding	751	0.0606	0.1911
REAC:R-HSA-3781865	Diseases of glycosylation	138	0.0028	0.1952
GO:0043565	Sequence-specific DNA binding	1131	0.0206	0.199
GO:0001540	Amyloid-beta binding	75	0.0168	0.2019
GO:0044212	Transcription regulatory region DNA binding	919	0.0242	0.2046
REAC:R-HSA-1630316	Glycosaminoglycan metabolism	124	0.0015	0.2087
GO:0000978	RNA polymerase II proximal promoter sequence-specific DNA binding	513	0.0718	0.2192
GO:0000987	Proximal promoter sequence-specific DNA binding	527	0.0901	0.2208
GO:0004713	Protein tyrosine kinase activity	182	0.0024	0.2212
GO:0003690	Double-stranded DNA binding	938	0.0335	0.2269
GO:0001067	Regulatory region nucleic acid binding	921	0.0205	0.2328
GO:0016525	Negative regulation of angiogenesis	32	<0.0001	0.2347

GO:0038023	Signaling receptor activity	1462	0.0023	0.2443

Enriched pathways (*q*-value<0.25) obtained from the GSEA analysis in the discovery phase (N=24, panel background=1305 proteins). Values represent the size of the complete pathway, and the *p*-values and *q*-values of the overlap.

Supplementary table 4: Enriched gene sets involving the biomarkers candidates

MMP9	MET
Collagen Degradation (REAC)	Signaling receptor activity (GO)
Neutrophil degranulation (REAC)	Transmembrane signaling receptor activity (GO)
Serine hydrolase activity (GO)	Transmembrane receptor protein kinase activity (GO)
Serine-type endopeptidase activity (GO)	Transmembrane receptor protein tyrosine kinase activity (GO)
	Protein tyrosine kinase activity (GO)

Enriched pathways including the biomarker candidates. Parentheses indicate whether the pathway was obtained from the gene-ontologies (GO) or reactome (REAC) databases. None of the 28 enriched pathways included ASAH2.

Supplementary Table 5. Characteristics of the replication phase in subjects showing no differences in baseline WMH

	Controls (N=55)	**Cases (N=55)**
Baseline Age, years	64 (61;67)	64 (62;66)
Sex	31 (56.4)	31 (56.4)
Time between visits, years	4.0 (3.8;4.1)	4.0 (3.8;4.3)
Vascular risk factors		
Diabetes mellitus	9 (16.4)	8 (14.6)
Active Smoker	5 (9.1)	6 (10.9)
SBP, mmHg	144.5 (16.2)	139.9 (18.0)
ΔSBP, mmHg	**3.1 (13.8)**	**9.8 (18.0)**
DBP, mmHg	78.3 (9.4)	79.1 (9.5)
ΔDBP, mmHg	-3.5 (8.8)	-1.0 (10.2)
Cholesterol, mg/dL	217.1 (43.3)	211.6 (43.9)
ΔCholesterol, mg/dL	-3.7 (40.5)	-7.5 (40.5)
HDL Cholesterol, mg/dL	46.1 (39.1;60.2)	47.5 (41.9;57.6)
ΔHDL Cholesterol, mg/dL	3.9 (-0.3;9.0)	3.4 (-1.6;7.8)
Baseline Cerebral Small Vessel Disease		
Extensive PVH	9 (16.4)	8 (14.6)
Extensive DWMH	3 (5.5)	8 (14.6)
Silent brain infarct	7 (12.7)	15 (27.3)

Values represent mean (SD), median (interquartile range) or number (%). Bolded variables are those which show significant differences between cases and controls (P-value<0.05).

Key: DBP, diastolic blood pressure; DWMH, deep white matter hyperintensities; HDL, high density lipoprotein; PVH, periventricular hyperintensities; SBP, systolic blood pressure.