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TEMPORAL ORDERING OF BIOMARKERS IN DUTCH-TYPE HEREDITARY CEREBRAL AMYLOID ANGIOPATHY

Chapter 11 | Temporal ordering of biomarkers in Dutch-type hereditary cerebral amyloid angiopathy

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Abstract

Background: The temporal ordering of biomarkers for cerebral amyloid angiopathy (CAA) is important for their use in trials and for the understanding of the pathological cascade of CAA. We investigated presence and abnormality of most common biomarkers in the largest (pre)symptomatic Dutch-type hereditary (D-CAA) cohort so far.

Methods: We included cross-sectional data from participants with (pre)symptomatic D-CAA and controls without CAA. We investigated CAA-related cerebral small vessel disease markers on 3T-MRI, cerebrovascular reactivity with functional 7T-MRI (fMRI) and amyloid-β40 and amyloid-β42 levels in CSF. We calculated frequencies and plotted biomarker abnormality according to age to form scatterplots and survival curves.

Results: We included 68 participants with D-CAA (59% presymptomatic, mean age 50y [range 26-75], 53% women), 53 controls (mean age 51y, 42% women) for CSF analysis and 36 controls (mean age 53y, 100% women) for fMRI analysis. Decreased CSF amyloid-β40 and amyloid-β42 levels were the earliest biomarkers present: all D-CAA participants had lower levels of amyloid-β40 and/or amyloid-β42 compared to controls (youngest participant 30y). Markers of non-hemorrhagic injury (>20 enlarged perivascular spaces in the centrum semiovale and/or white matter hyperintensities Fazekas score ≥2, present in 83%) and markers of impaired cerebrovascular reactivity (abnormal BOLD amplitude, time to peak and/or time to baseline, present in 56%) were present from the age of 30. Finally, markers of hemorrhagic injury were present in 64% and only appeared after the age of 41 (first micro- and macrobleeds followed by cortical superficial siderosis).

Conclusions: Our results suggest that amyloid biomarkers in CSF are the first to become abnormal in CAA, followed by MRI biomarkers for cerebrovascular reactivity and non-hemorrhagic injury and lastly hemorrhagic injury. This temporal ordering probably reflects the pathological stages of CAA and should be taken into account when future therapeutic trials targeting specific stages are designed.

Introduction

Cerebral amyloid angiopathy (CAA) is one of the most prevalent causes of intracerebral hemorrhage (ICH), cognitive decline and dementia in the elderly. 1 The disease is caused by a range of pathologic changes following the deposition of the amyloid-β protein in the vessel wall of cortical and leptomeningeal arteries. 2 The mechanisms that cause amyloid-β accumulation, the subsequent pathological cascade of brain damage and associated clinical symptoms are still not fully understood. Furthermore, it is unclear which biomarkers are present at different stages of this pathological cascade.

Research in the field of the pathophysiology underlying CAA development and progression is challenging. First, it is not possible to diagnose definite CAA during life, as this requires post-mortem brain examination. Only a diagnosis of possible or probable CAA can be made during life based on the clinical and radiological Boston criteria 2.0. 3 Second, most patients develop symptoms at an advanced age, when age related brain changes and co-morbidities may obscure or overlap with CAA-related pathology. This presentation in a relatively advanced stage of the disease furthermore complicates investigation of the earlier, presymptomatic phases.

One way to overcome these obstacles is to study hereditary CAA. Hereditary Dutch-type CAA (D-CAA) is one of the most common and best phenotyped hereditary variants of CAA. 4 Pathological and biochemical changes, MRI findings and clinical symptoms are similar to sporadic CAA (sCAA), except for an earlier onset and faster disease progression in D-CAA. 5 DNA testing can identify mutation carriers with 100% certainty enabling research from early to advanced CAA. Studies in presymptomatic mutation carriers have identified several early biochemical and imaging biomarkers. 6-9 The progression of these biomarkers over time is thought to reflect different disease stages similar to sporadic CAA and is essential information for future clinical trials. 10

We investigated the presence and/or abnormality of currently known CSF and MRI biomarkers for CAA according to age in the largest (pre)symptomatic D-CAA cohort so far to increase our insight in their temporal ordering and interrelation.

Methods

For this cross-sectional study, we included participants from the ongoing prospective D-CAA natural history study (AURORA) of the Leiden University Medical Center (LUMC). 11 Symptomatic (defined as a history of ≥1symptomatic ICH) and

presymptomatic mutation carriers with D-CAA aged >18y were recruited via the clinic and outpatient clinic of the LUMC. We included participants who had the causal APP mutation for D-CAA or who had a history of symptomatic ICH on CT or MRI suspect for CAA and at least one first-degree relative with D-CAA. As part of the AURORA study participants underwent yearly (presymptomatic participants every two years) research visits which included a 3 and 7 Tesla (T) MRI, functional MRI (fMRI), blood and CSF collection, neurological and cognitive tests, as well as questionnaires on demographics and medical history, all performed on the same day. For the present study the AURORA baseline data were used. The ethics committee of the LUMC approved the study and written informed consent was obtained from all participants prior to enrollment. For this study we investigated the following biomarkers in CSF and on (f)MRI: amyloid-β40 and amyloid-β42, thought to reflect vascular amyloid deposition, measures of cerebrovascular reactivity at functional 7T-MRI (time to peak, time to baseline and amplitude), measures of non-hemorrhagic injury (white matter hyperintensities and enlarged perivascular spaces at the centrum semiovale) and hemorrhagic injury (microbleeds, macrobleeds and cortical superficial siderosis) at susceptibility weighted 3T-MRI. We chose these biomarkers as we assume that they reflect the steps of our recently published pathophysiological framework for CAA. 10

CSF and MRI biomarkers

We investigated changes in CSF amyloid-β40 and amyloid-β42 levels in participants with D-CAA by comparing them with controls without CAA from the outpatient clinic of the Radboud University Medical Center (RUMC). 7 In the controls, CSF was obtained as part of a diagnostic workup during which central nervous system disorders were excluded. 12 Controls were divided into age <55y and age ≥55y for comparison with (pre)symptomatic D-CAA groups respectively. This cut-off point was chosen based on the mean age of the first symptomatic ICH in D-CAA. 13 For details see supplements. 12

With functional 7T-MRI (fMRI) we investigated visually stimulated blood-oxygen-level-dependent (BOLD) amplitude, time to peak (TTP) and time to baseline (TTB) as measures of cerebrovascular reactivity. 8,14 To investigate visually stimulated BOLD amplitude, TTP and TTB on 7T-fMRI in D-CAA we included control participants from the WHISPER study. 8 The WHISPER study is a 7T-MRI study of the LUMC which includes women between 40 and 60y with a history of ischemic stroke and women without neurological disorders or systemic diseases associated with demyelinating or inflammatory white matter brain lesions. For the present study we only included

the control participants. WHISPER Participants were retrieved via the outpatient clinic of the LUMC and via advertising and were scanned at the same scanner and using the same protocol as participants of the AURORA study. BOLD parameters from the high temporal resolution scans were obtained according to previously published methods. 14 For details see supplements.

The following 3T-MRI markers were scored according to the Standards for Reporting Vascular Changes on neuroimaging (STRIVE) criteria 2.0: cerebral microbleeds (CMB), macrobleeds, cortical superficial siderosis (cSS), CSO-EPVS, periventricular and deep WMH. 15 CMB count, macrobleed count and cSS focality were scored on susceptibility weighted images (SWI). 16 CSO-EPVS were scored on T2-weighted images and classified into the following categories; no EPVS, 1-10 EPVS, 11-20 EPVS, 21-40 EPVS, >40 EPVS. 17 Deep and periventricular WMH were graded with the Fazekas score on FLAIR images. 18 On each FLAIR image white matter spots were counted (defined as small circular or ovoid hyperintense lesions in the bilateral subcortical white matter), and presence of a multi-spot pattern (defined as >10 white matter spots) was assessed. 3 Based on the diffusion tensor imaging (DTI), peak width of skeletonized mean diffusivity (PSMD) was calculated according to previously published methods. 19 PSMD is a marker used for the interpretation of white matter integrity, and is related to neurovascular injury and cognitive performance in CAA. 19,20 For each participant the CAA cerebral small vessel disease (cSVD) score was calculated according to previously published methods using the following markers; lobar CMB, cSS, CSO-EPVS, and WMH. 21 All structural MRI markers were scored by one observer (E.A.K >5 years of experience in the field), apart from white matter spots, which were scored by R.v.d.Z (2 years of experience in the field). In case of doubt, findings were discussed with a third observer with >15 years of experience in the field (M.A.A.v.W.) for the final decision. For details see supplements.

Statistics

Descriptive statistics were used to calculate frequency, medians and means of baseline characteristics, CSF amyloid-β40 and amyloid-β42 levels and MRI markers. Proportions were calculated with 95% confidence intervals (CI). Results were reported in temporal order by presenting those biomarkers first with the highest frequency at the youngest age of the participants. We considered a participant with D-CAA to have abnormal levels of amyloid-β40 and/or amyloid-β42 if these were lower than in any of the controls. Similar, we considered a participant with D-CAA to have abnormal cerebrovascular reactivity if TTP and/or TTB were higher and/

or amplitude lower than the highest/lowest control. For details see supplements. We created scatterplots illustrating amyloid-β40 and amyloid-β42 levels, BOLD parameters, PSMD, CAA cSVD score and counts of CMB and macrobleeds, and their correlation with age at time of lumbar puncture or MRI. We considered a participant with D-CAA to have abnormal levels of amyloid-β40 and/or amyloid-β42 if these were lower than in any of the controls. Similar, we considered a participant with D-CAA to have abnormal cerebrovascular reactivity if TTP and/or TTB were higher and/or amplitude lower than the highest/lowest control. For amyloid-β40 and amyloid-β42 levels and BOLD parameters we estimated the age of divergence between D-CAA mutation carriers and controls, according to previously published methods: for each marker we used linear mixed-effect models to assess the longitudinal effects of D-CAA carriage with age, and assessed the distribution of parameter estimates across iterations to generate 95% credible intervals of the model fit. Age of divergence was then determined as the point where the 95% credible intervals of the difference distribution did not overlap. 22 We considered a participant with D-CAA to have non-hemorrhagic injury on MRI if they had >20 CSO-EPVS, >10 white matter spots and/or WMH Fazekas score ≥2 (deep and/or periventricular) on MRI. Finally, we considered a participant with D-CAA to have signs of hemorrhagic injury if they had any CMB, macrobleeds, and/or cSS on MRI. We used Kaplan Meier survival analysis to visualize the probability of biomarker presence or abnormality according to age at MRI using survival curves. The survival curves were truncated once <10% of the participants remained at risk.

Data sharing statement

Further information about the dataset is available from the corresponding author upon reasonable request.

Results

We included 68 participants (age range 26-75 years) with D-CAA; 37 presymptomatic (mean age 43 years, 62% women) and 31 symptomatic (mean age 59 years, 42% women). For baseline characteristics see Supplemental Table 1, for a flowchart of participant inclusion see Figure 1. Of the 68 participants with D-CAA, 3T MRI was available in 64 (94%), CSF was available in 23 (34%) and 7T-fMRI was available in 32 (47%) participants.

Frequency of biomarkers

Presymptomatic and symptomatic participants with D-CAA had lower median levels of amyloid-β40 and amyloid-β42 compared to (age-category matched) controls (Table 1). All participants with presymptomatic D-CAA except three had amyloid-β40 levels below the lowest control (cut-off 2889ng/mL, youngest participant below cut-off 30 years old, Figure 2A). Amyloid-β42 levels were lower in all participants with D-CAA compared to any of the controls (cut-off 233ng/mL, youngest participant below cut-off 30 years old, Figure 2B). The age of convergence for D-CAA mutation carriers and controls was -5.6 years for amyloid-β40 and -115.3 years for amyloid-β42 (supplemental Figure 1).

Median TTP and TTB were higher, and BOLD amplitude lower, in presymptomatic and symptomatic participants with D-CAA compared to controls (Table 1, Figure 2). The youngest participant with D-CAA with a longer TTP compared to all controls was 30 years old, the youngest participant with D-CAA with a longer TTB compared to any of the controls was 31 years old. The youngest participant with D-CAA who had a lower BOLD amplitude compared to any of the controls was 48 year old. We determined the age of convergence for D-CAA mutation carriers and controls: for TTP this was 15.4 years, for TTB 19.2 years and for BOLD amplitude 31.5 years (supplemental Figure 1).

Table 1. CSF amyloid-β40 and β42 markers and 7T-fMRI BOLD parameters in (pre)symptomatic D-CAA and controls.

CSF data	Presymptomatic D-CAA (n=11)	Symptomatic D-CAA (n=12)	Controls <55y (n=32)	Controls ≥55y (n=21)
Mean age in years (range)	39 (30-53)	60 (49-71)	44 (27-54)	65 (55-75)
Women (n,%)	7 (64)	5 (42)	16 (50)	6 (29)
Median CSF Aβ40 (ng/mL) (range)	2184 (832-3752)	1733 (910-2702)	7551.5 (2889-14874)	9036 (3905-16305)
Median CSF Aβ42 (ng/mL) (range)	1733 (910-2702)	76 (41-106)	629 (233-1578)	681 (326-1265)

7T-fMRI data	Presymptomatic D-CAA (n=23)	Symptomatic D-CAA (n=9)	Controls (n=36)	
Mean age in years (range)	39 (26-55)	61 (49-75)	53 (41-60)	
Women (n,%)	17 (74)	6 (67)	36 (100)	
Median time to peak in seconds (range)	7.6 (4.2-29.2)	15.4 (8.2-23.1)	6.5 (3.9-8.4)	
Median time to baseline in seconds (range)	12.2 (5.9-22.8)	16.0 (8.7-23.7)	9.2 (5.0-15.5)	
Median BOLD amplitude (% BOLD change)	1.2 (0.1-3.9)	0.3 (0.0-0.9)	1.3 (0.4-2.8)	

BOLD: Blood-oxygen-level-dependent. D-CAA: hereditary Dutch-type cerebral amyloid angiopathy.

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Figure 1. Flowchart detailing participant inclusion.
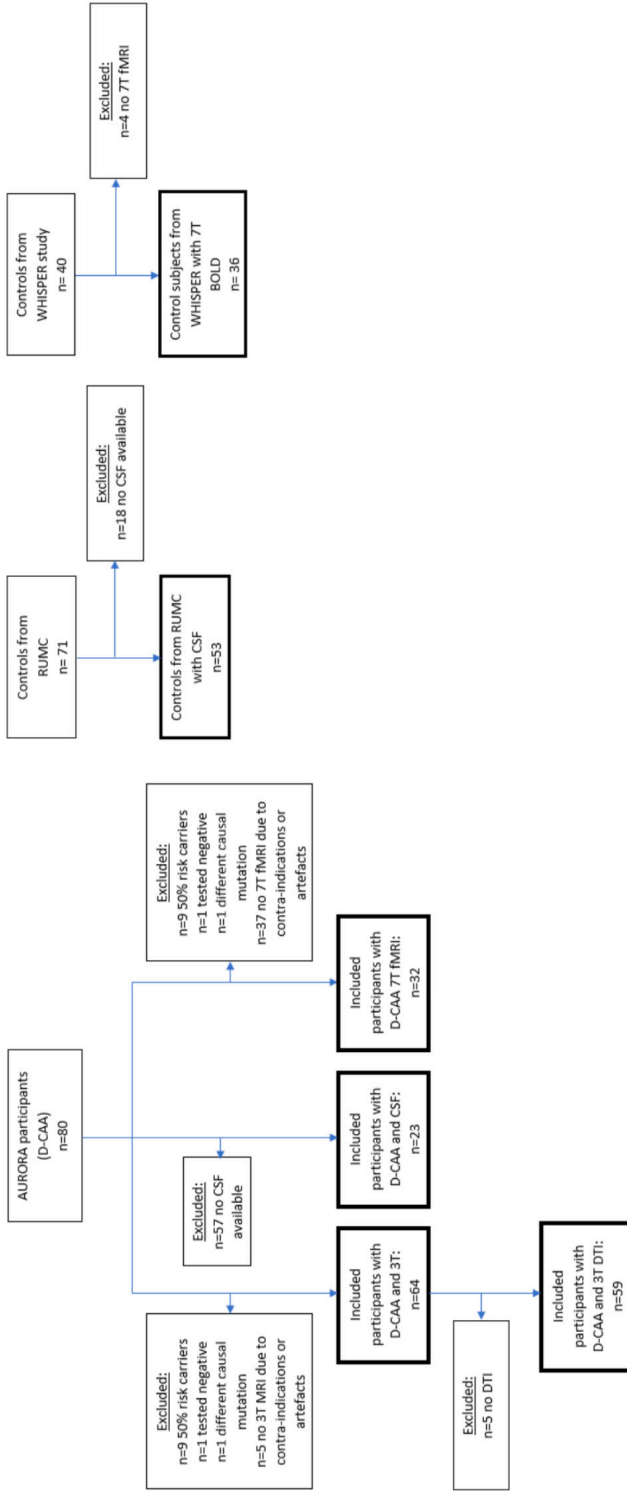
Flowchart detailing participant inclusion from the AURORA and WHISPER studies and from the RUMC. 50% risk carries are participants without a clinical history of D-CAA but with a positive family history of D-CAA in a first degree relative, who did not undergo genetic testing.

Markers of non-hemorrhagic injury (>20 CSO-EPVS, >10 white matter spots and/or Fazekas score ≥2) were visible in 71% (95%CI 54-85) of presymptomatic, and 100% (95%CI 88-100) of symptomatic participants (Table 2). The youngest participant with >20 CSO-EPVS was 30 years old, the youngest participant with >10 WMH spots was 31 years old and the youngest participant with WMH Fazekas score ≥2 was 37 years old. Median PSMD scores were higher in symptomatic compared to presymptomatic participants (2.9 vs 5.1mm2/s x 10-4, Figure 3). Development of >20 CSO-EPVS seemed to occur prior to development of >10 WMH spots and WMH Fazekas score ≥2 (Figure 4A).

Markers of hemorrhagic injury (defined as presence of any CMB, macrobleeds and/or cSS) were present in 34% (95%CI 19-52) of presymptomatic and 100% (95%CI 88-100) of symptomatic participants (Table 2). CMB and macrobleeds seemed to occur simultaneously (Table 2, Figure 3, Figure 4B). The youngest participant with CMB was 44 years old, the youngest participant with macrobleeds was 41 years old. In our cohort, cSS seemed to occur the latest: the youngest presymptomatic participant with cSS was 50 years old and the youngest symptomatic participant with cSS was 47 years old (Figure 4B). The youngest participant with a marker reflecting hemorrhagic injury was approximately 11 years older than the youngest participant with a marker reflecting non-hemorrhagic injury (>20 CSO-EVS in a 30 year old participant, compared to a single macrobleed in a participant of 41 years old).

Table 2: 3T-MRI markers in (pre)symptomatic participants with D-CAA.

	Presymptomatic D-CAA (n=35)	Symptomatic D-CAA (n=29)
Mean age in years (range)	42 (26-69)	58 (47-75)
Women (%)	22 (63)	13 (45)
Markers of non-hemorrhagic brain injury		
Deep WMH (n, %)	24 (69)	29 (97)
Fazekas score (median, range)	1 (0-3)	2 (0-3)
Periventricular WMH (n, %)	18 (51)	29 (100)
Fazekas score (median, range)	1 (0-3)	3 (1-3)
WMH Fazekas score ≥1* (n,%)	25 (71)	29 (100)
Age youngest participant with Fazekas ≥1	26	47
Age oldest participant without Fazekas ≥1	56	-
WMH Fazekas score ≥2* (n,%)	11 (31)	26 (90)
Age youngest participant with Fazekas ≥2	37	47
Age oldest participant without Fazekas ≥2	56	52
1-5 white matter spots (n, %)	10 (29)	0
6-10 white matter spots (n, %)	3 (9)	0
>10 white matter spots (n,%)	16 (46)	29 (100)
Age youngest participant with >10 spots	31	47
Age oldest participant with <10 spots	48	-
1-10 CSO-EPVS (n, %)	5 (14)	1 (3)
11-20 CSO-EVS (n, %)	6 (17)	2 (7)
>20 CSO-EPVS (n, %)	24 (67)	26 (90)
Age youngest participant with >20 CSO-EPVS	30	47
Age oldest participant with ≤20 CSO-EPVS	55	75
PSMD (mm^2/s x10^{-4}) (median, range)[†]	2.9 (2.0-5.6)	5.1 (2.8-9.5)
≥1 non-hemorrhagic lesion (n,%)[‡]	25 (71)	29 (100)
Age youngest participant with non-hemorrhagic lesions	30	47
Age oldest participant without non-hemorrhagic lesions	45	-

	Presymptomatic D-CAA (n=35)	Symptomatic D-CAA (n=29)
Markers of hemorrhagic brain injury		
CMB (n, %)	11 (31)	29 (100)
Count (median, range)	0 (0-397)	205 (5-1906)
Age youngest participant with CMB	44	47
Age oldest participant without CMB	48	-
Macrobleeds (n, %)	7 (20)	29 (100)
Count (median, range)	0 (0-26)	27 (4-86)
Age youngest participant with macrobleeds	41	47
Age oldest participant without macrobleeds	56	-
cSS (n, %)[§]	2 (6)	24 (83)
Age youngest participant with cSS	50	47
Age youngest participant with cSS	68	60
≥1 hemorrhagic lesion (n,%)[‖]	12 (34)	29 (100)
Age youngest participant with hemorrhagic lesions	41	47
Age oldest participant without hemorrhagic lesions	48	-
CAA cSVD score (median, range)	1 (0-5)	5 (3-6)

D-CAA: hereditary Dutch-type CAA. WMH: white matter hyperintensities. CSO-EPVS: enlarged perivascular spaces in the centrum semiovale. PSMD: Peak width of skeletonized mean diffusivity. CMB: cerebral microbleeds. cSS: cortical superficial siderosis. cSVD: cerebral small vessel disease. *Deep WMH and/or periventricular WMH. [†]n=59, no DTI performed in n=5 participants. [‡] Any WMH (Fazekas score ≥2) and/or >20 CSO-EPVS. [§]Includes cSS < 3 sulci of a symptomatic ICH. [‖]Any microbleeds and/or macrobleeds and/or cSS.

Figure 2. CSF amyloid-β40 and amyloid-β42 levels and BOLD parameters at 7T-fMRI in (pre)symptomatic participants with D-CAA and controls.
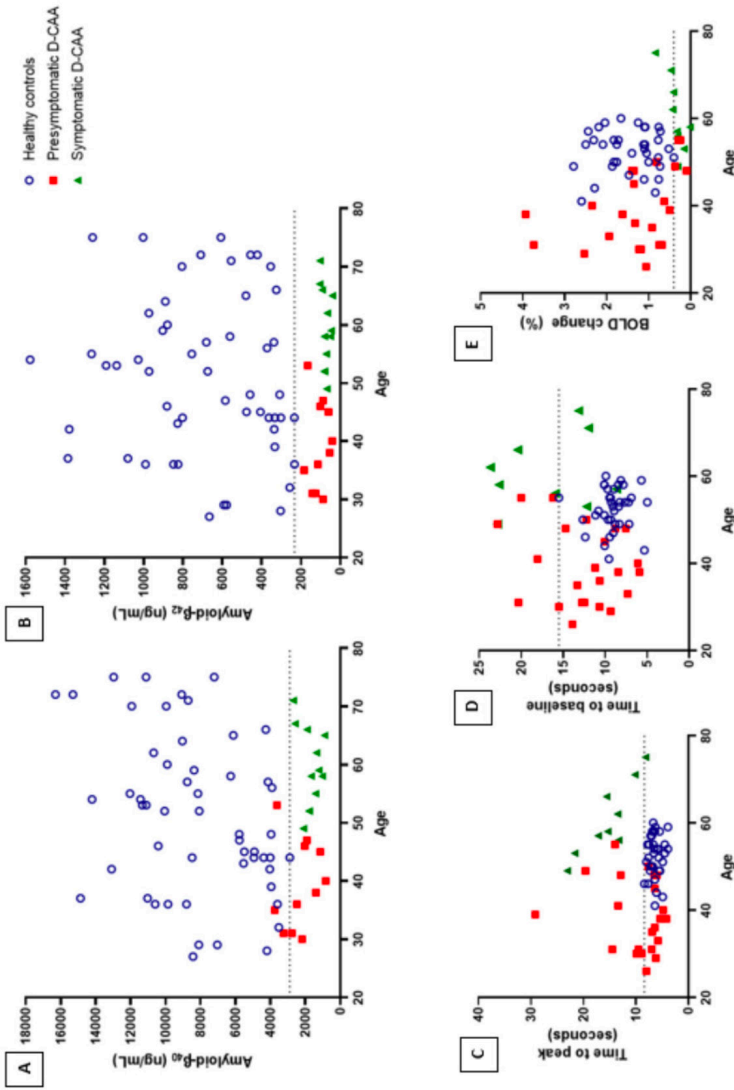
In presymptomatic participants with D-CAA (n=9, green triangles) and healthy controls (n=36, blue circles): (A). CSF amyloid-β40 levels (dotted line at cut-off 2889ng/m), (B). CSF amyloid-β42 levels (dotted line at cut-off 233ng/mL) levels, (C). BOLD 7T-fMRI time to peak in seconds (dotted line at cut-off 8.4s), (D). BOLD 7T-fMRI Time to baseline in seconds (dotted line at cut-off 15.5s) and (E). BOLD 7T-fMRI Amplitude in BOLD change (%) (dotted line at cut-off 0.4% BOLD change).

Figure 3. Hemorrhage counts, CAA cSVD sum scores and PSMD at 3T-MRI in (pre)symptomatic participants with D-CAA.
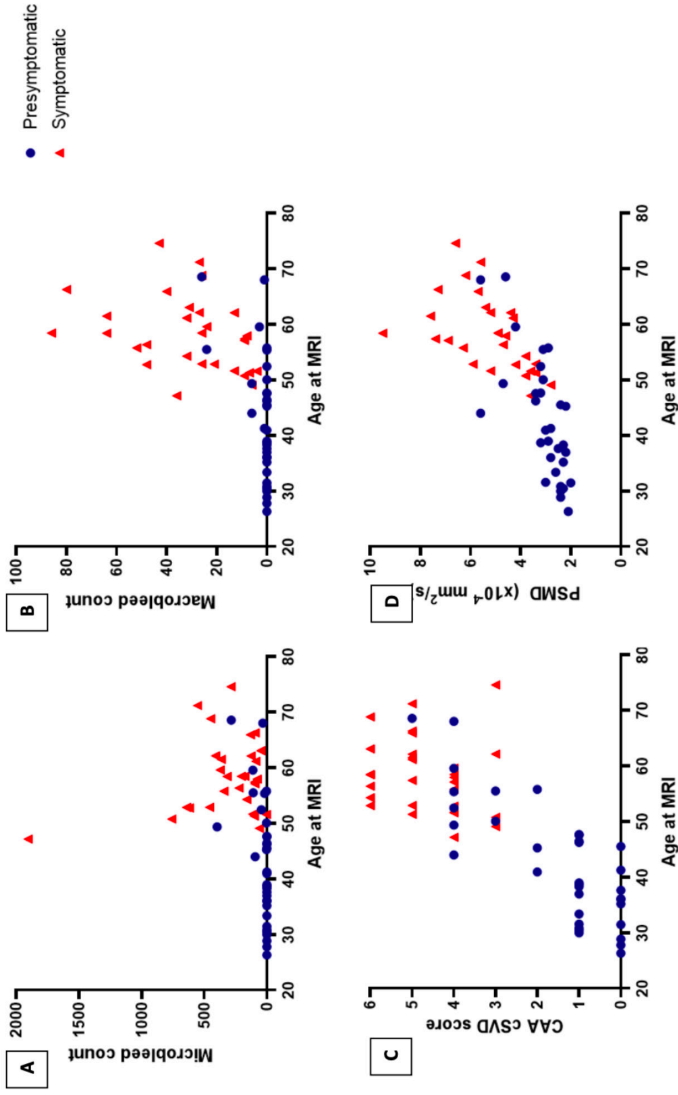
Microbleed count (A), macrobleed count (B), CAA cSVD score (C) and PSMD (D) in participants with presymptomatic D-CAA (n=35 for A, B and C, n=31 for D) (blue circles) and symptomatic D-CAA (n=29 for A, B and C, n=28 for D) (red triangles) according to age at MRI.

Temporal ordering of the biomarkers

Levels of amyloid-β40 and/or amyloid-β42 in CSF were abnormal in 100% of the presymptomatic and symptomatic participants of our cohort (youngest participant with abnormal values was 30y). Abnormal cerebrovascular reactivity was present in 44% of presymptomatic and 89% of symptomatic participants (youngest participant with abnormal values was 30y). Thirteen participants had both available CSF and fMRI. Of those 13, four (all presymptomatic) had abnormal CSF amyloid-β40 and/or amyloid-β42 levels, whilst having normal cerebrovascular reactivity biomarkers. Sixteen (89%) participants with abnormal cerebrovascular reactivity also had signs of non-hemorrhagic brain injury on MRI. Fourteen (40%) of the presymptomatic participants with D-CAA had signs of non-hemorrhagic brain injury without hemorrhagic brain injury, and nine (26%) did not have any signs of either non-hemorrhagic or hemorrhagic brain injury on MRI. All participants with signs of hemorrhagic injury had at least one feature of non-hemorrhagic injury (88% >20 CSO-EPVS, 95% >10% white matter spots and 83% Fazekas score ≥2). The youngest participant with a history of symptomatic ICH was 47 years old.

Based on these data, we speculated upon the temporal ordering of the biomarkers in this cohort, and suggest that in this cohort of participants with D-CAA levels of amyloid-β40 and/or amyloid-β42 in CSF were probably the first biomarkers to become abnormal, followed by markers of impaired cerebrovascular reactivity and non-hemorrhagic brain injury and lastly markers of hemorrhagic brain injury.

Figure 4. Explorative analyses of the probability of non-hemorrhagic and hemorrhagic biomarker abnormality at 3T-MRI in participants with D-CAA.
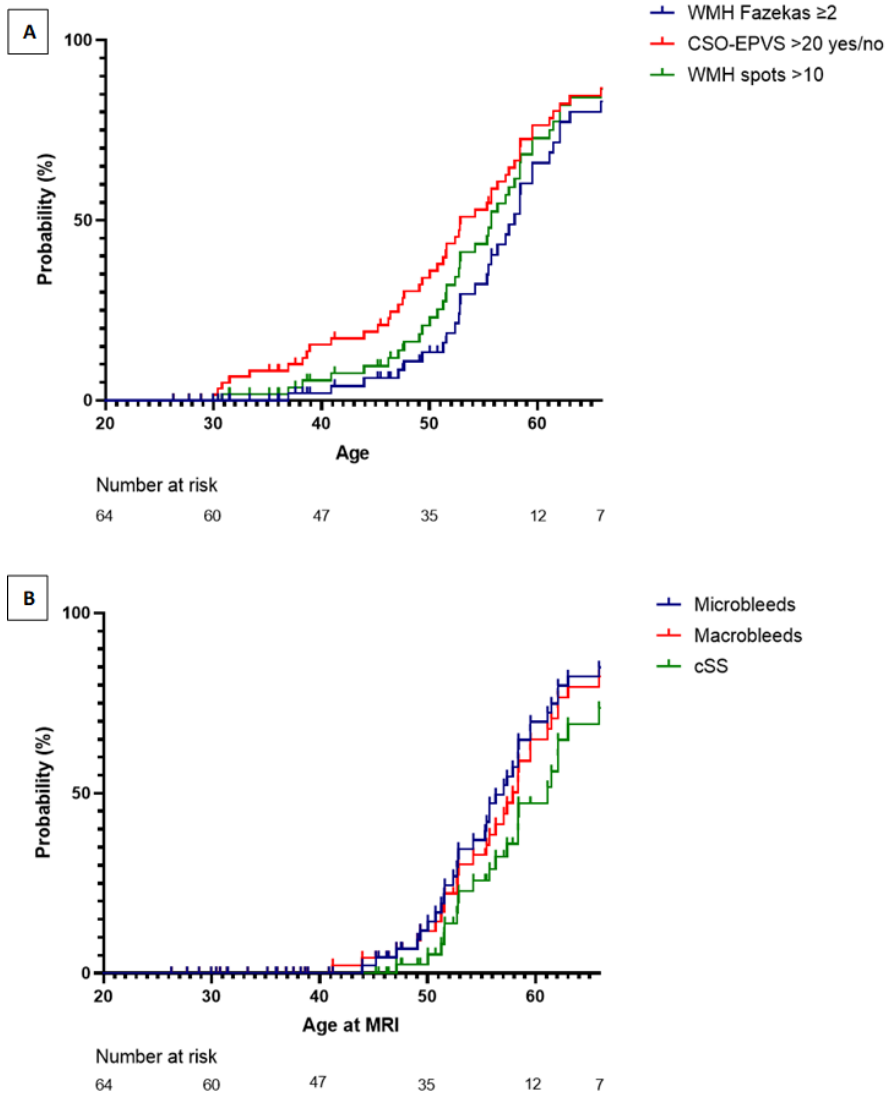
Survival curves illustrating the probability of presence of biomarkers according to age at 3T-MRI. Survival curves were truncated once <10% of participants remained at risk (A). Presence of non-hemorrhagic brain injury at 3T-MRI: WMH Fazekas score ≥2 (blue), >20 CSO-EPVS (red) and >10 WMH spots (green) according to age at MRI (n=64). (B). Presence of hemorrhagic brain injury at 3T-MRI: microbleeds (blue), macrobleeds (red), cSS (green) according to age at MRI (n=64).

Discussion

In this cohort of participants with D-CAA we found that CSF amyloid-β40 and amyloid-β42 levels seemed to be the earliest biomarkers to become abnormal with lower levels in all participants, followed by impaired vascular reactivity (expressed by increased TTP and/or TTB and/or decreased amplitude) and the occurrence of non-hemorrhagic brain injury (expressed by >20 CSO-EPVS, >10 white matter spots and/or WMH Fazekas score ≥2) in most participants from the age of thirty on, and finally hemorrhagic brain injury (CMB and macrobleeds, followed by the development of cSS and lastly symptomatic ICH) appearing from the age of 40.

Our results seem to be in line with our recently proposed pathophysiological framework for CAA. 10 In this framework we proposed four steps: initial vascular amyloid deposition (step 1), alteration of cerebrovascular physiology (step 2), non-hemorrhagic brain injury (step three), and finally appearance of hemorrhagic brain lesions (step four). 10 The framework was based on cohort studies in sCAA and small studies in D-CAA, in which mostly one single biomarker at the time was investigated. Our current study includes the largest cohort of D-CAA mutation carriers thus far with novel data on a large set of different biomarkers all measured at the same time point. A limitation is that the D-CAA subjects were recruited from the prospective AURORA cohort, whereas controls are derived from two other studies; the RUMC study (for CSF) and the WHISPER study (for 7T-fMRI). However, all data were collected prospectively, MRI scanners and protocols from the WHISPER and AURORA studies were identical as were CSF processing procedures in the LUMC and RUMC. In this way we tried to minimize differences in MRI and sample handling to optimize comparison between the D-CAA and control cohorts. Definite validation of our previous proposed framework would require longitudinal data of both a large group of participants with D-CAA and controls.

We found that the earliest biomarkers for CAA pathology are abnormal in mutation carriers in their mid-twenties and early thirties, decades prior to the age at which in general the first ICH occurs (the youngest age of onset of first ICH in our cohort was 47 years). We did not enroll children/adolescents in our study and therefore, we could not investigate the exact age at which the amyloid levels in CSF and the vasoreactivity start to become abnormal.

Although in the cohort the biomarkers seemed to occur in a fixed temporal ordering, we found a striking variability in the timing of which the different biomarkers become abnormal in individual patients. As our data are cross-sectional, it is unclear how this variation evolves over time. It is for example unclear whether patients who develop abnormal biomarkers relatively 'early' in life are also the first ones to

develop the biomarkers associated with the advanced stages of CAA. This individual variability in clinical and radiological phenotype is also present in sporadic CAA, and suggests that there are (risk) factors that influence disease onset and course. Possible influential factors are hypertension, the use of antithrombotic/coagulating drugs and APOE genotype, although the role of this last factor has not been fully elucidated in D-CAA. [23-26] Apart from these known factors, several other, (epi)genetic, biological, lifestyle or environmental factors might exist that influence D-CAA disease course. [27]

Our results have important implications for future clinical research. The stepwise progression of the biomarkers as suggested in our current study can help identify timing and type of candidate treatments for future clinical trials in CAA. Our results suggest that in CAA there is significant lag time of 20 to 30 years between the first CSF and MRI signs of the disease and the development of clinical symptoms. This time lag offers a window of opportunity for both prevention and disease modifying drugs: in the early non-hemorrhagic phases of the disease, tissue injury could hypothetically still be prevented, whereas in the late hemorrhagic stage disease progression or onset of ICH can be delayed but cerebral injury will probably be irreversible. Treatment in the late stage mainly has to target triggers of ICH. Inclusion criteria for trials should take the different steps and their associated biomarkers into account to time when in the disease process the drug of interest would take action and which disease mechanisms would be targeted.

Our results on the interrelation of biomarkers may also have implications for the diagnosis of CAA (mimics). We showed that all patients with D-CAA who had hemorrhagic markers also had non-hemorrhagic markers on their MRI. Although the new Boston 2.0 criteria emphasize the importance of non-hemorrhagic markers, the diagnosis possible CAA can still be based on the presence of clinical symptoms and hemorrhagic markers only. Our findings however suggest that it is unlikely to have no non-hemorrhagic signs in the hemorrhagic stage of CAA. Therefore, in patients with suspicion of CAA based on CMB, macrobleeds or cSS only without presence of WMH or CSO-EPVS, alternative diagnoses such as multiple small cavernomas or reversible vasoconstriction syndrome should be considered. In our cohort, cSS was the last biomarker to become abnormal, suggesting that it is a sign of late-stage CAA. cSS is strongly related to leptomeningeal vessel remodeling (Vonsattel grade III: concentric splitting of the vessel wall) at histopathology, as opposed to cerebral microbleeds which are related to cortico-subcortical vessel remodeling (Vonsattel grade IV: replacement of the arteriolar wall with fibrinoid material). [28,29] It is possible that leptomeningeal vessels are affected later in the disease, or that grade III occurs in later, more severe stages than grade IV. [28]

Our study has several limitations. First, we used a cross-sectional study design: data on longitudinal progression of biomarkers in individual patients are not yet available in our cohort and the exact time-points of the first appearance of markers could therefore not be assessed. Within the D-CAA cohort, there was substantial sample loss, especially regarding the CSF data, which complicates interpretation. Second, we use categorical variables to measure WMH (Fazekas score and multi-spot pattern), presence of multiple white matter spots and CSO-EPVS which, are subject to ceiling effects. [17,18] Furthermore, our cut-off for CSO-EPVS (>20), spots (>10) and WMH (Fazekas score ≥2) remain semi-artificial. [3] Unfortunately, quantitative measures for CSO-EPVS, spots and WMH are not yet available for our cohort. In the future the availability of quantitative measures might enable a better distinction of differences in temporal ordering of the non-hemorrhagic steps of the cascade. For PSMD, a quantitative marker for diffuse brain injury, we unfortunately had no available control participants to investigate abnormality. However, compared with data from previously published, population-based cohorts, the PSMD values in our participants with CAA seemed to be on average higher compared with healthy, age-matched controls (Figure 3D). [30,31] A third limitation is that due to the inclusion of only women in the WHISPER study we were not able to include male control subjects for the 7T-fMRI data. Another limitation is that although CSF amyloid-β40/42 levels and BOLD fMRI parameters are present in the early phases of D-CAA, there is no universal established cut-off for when the levels of these markers are abnormal. Therefore, they are not very suitable for determining disease stages in individual patients. [7,8] By using a cut-off based on the lowest threshold of controls without CAA, we could only infer reference-based and no absolute abnormality. The cut-off points requiring a threshold lower (or higher) than all controls however was very strict by definition. Last, although D-CAA is a useful model for sporadic CAA, there are also differences which might limit generalizability. All D-CAA mutation carriers eventually develop a symptomatic ICH. In sporadic CAA, there seems to be more variation in different pathophysiological and clinical phenotypes, perhaps due to vascular or Alzheimer comorbidity, and not all patients suffer from symptomatic ICH. [27] Strengths of our current study are the relatively large cohort of participants with this pure, hereditary form of CAA, and the use of different modalities for investigating biomarker abnormality (CSF, MRI, fMRI), all obtained on the same day. Our results have important implications for future clinical trial design, aiding in the identification and timing of candidates for disease-modifying treatments and the choice for the appropriate biomarkers to monitor treatment effect.

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CHAPTER 11

Supplemental material

Supplemental methods

CSF acquisition

The CSF samples from the healthy control group of the RUMC cohort obtained as part of diagnostic work-up were coded and used with full consent from the participants. CSF samples were obtained from AURORA participants and from the controls of the RUMC under standardized conditions. 1 CSF amyloid-β40 and amyloid-β42 levels were quantified at the RUMC using Lumipulse® G fully automated immunoassays (Fujirebio, Ghent, Belgium).

Image acquisition 3T

AURORA participants were scanned using a whole body 3 Tesla (3T) MRI system (Philips Healthcare, Best, The Netherlands). The following sequences were performed using a standard 32-channel head coil: Three-dimensional T1 weighted images (3DT1) (repetition time (TR)/ echo time (TE) = 9.7/4.6ms, flip angle 7 degrees, 130 slices with no interslice gap, field of view (FOV) 217x172x156mm, voxel size 1.2x1.2x1.2mm), T2 weighted images (TR/TE = 4744/80ms, flip angle 90 degrees, 48 slices with no interslice gap, FOV 220x176x144mm, voxel size 0.5x0.6x3mm). Three dimensional Fluid Attenuated Inversion Recovery (FLAIR) images (TR/TE = 4800/280ms, inversion time (TI) 1650ms, 321 slices with no interslice gap, FOV of 250x250x180mm, voxel size 1.1x1.1x0.6 mm), susceptibility weighted images (SWI) (TR/TE = 31/7.2ms, flip angle 17 degrees, 130 slices and an FOV of 230x190x130 mm, voxel size of 0.6x0.6x1mm). Diffusion images (DTI) (TR/TE = 8194/76 ms, voxel size 1.72x1.72x2.5 mm, flip angle = 90 degrees, 48 slices and FOV of 220x220x120mm, 45 gradient directions with a b-value of 1200 s/mm2 and one baseline image with b-value 0 s/mm2).

Image acquisition 7T

Participants of the AURORA study and the WHISPER study underwent MRI scanning on a on a whole body human 7T MR system (Philips, Best, the Netherlands). For AURORA participants this 7T MRI was performed on the same day as the 3T and lumbar puncture. A quadrature transmit and 32-channel receive head coil (Nova Medical, Wilmington, MA, USA) was used and all participants were scanned according

to a protocol which has been published in previous studies. [2,3] The protocol included a 3DT1 weighted scan (scan parameters: repetition time (TR) 4.146 ms, echo time (TE) 1.86 ms, flip angle 7°, field of view (FOV) 240 x 255 x 246 mm, 250 slices, slice thickness 0.9 mm, matrix size 288 x 288, and scan duration 142 s) and a coronal single slice high temporal resolution fMRI scan through the occipital cortex (scan parameters: TR 100 ms, TE 22 ms, flip angle 20°, FOV 100 x 141 mm, slice thickness 1.5 mm, matrix size 128 x 128, 3360 dynamics, and scan duration 336 s) with a visual stimulus of seven 48s blocks consisting of 3 seconds flashing black and white radial checkerboard pattern at 8 Hz, followed by 45 seconds of grey screen.

Acknowledgements

Supplemental Table 1: Baseline characteristics of the participants with D-CAA.

	All (n=68)	Presymptomatic D-CAA (n=37)	Symptomatic D-CAA (n=31)
Age in years (mean, range)	50 (26-75)	43 (26-69)	59 (47-75)
Women (n, %)	36 (53)	23 (62)	13 (42)
Symptomatic ICH (n,%)	31 (47)	0	31 (100)
Hypertension (n, %)	16 (24)	8 (22)	8 (26)
Hypercholesterolemia (n, %)	15 (22)*	4 (11)	11 (36)[a]
Diabetes mellitus type 2 (n, %)	3 (4)	1 (3)	2 (7)
Smoking, ever (n, %)[†]	43 (63)	23 (62)	20 (65)

D-CAA: hereditary Dutch-type CAA. ICH: intracerebral hemorrhage.

*Data missing in n=1 participant. [†]Defined as having smoked ≥1 cigarette/day for the duration of at least one year.

Supplemental Figure 1. Ages of divergence between D-CAA mutation carriers and controls for CSF and fMRI BOLD parameters
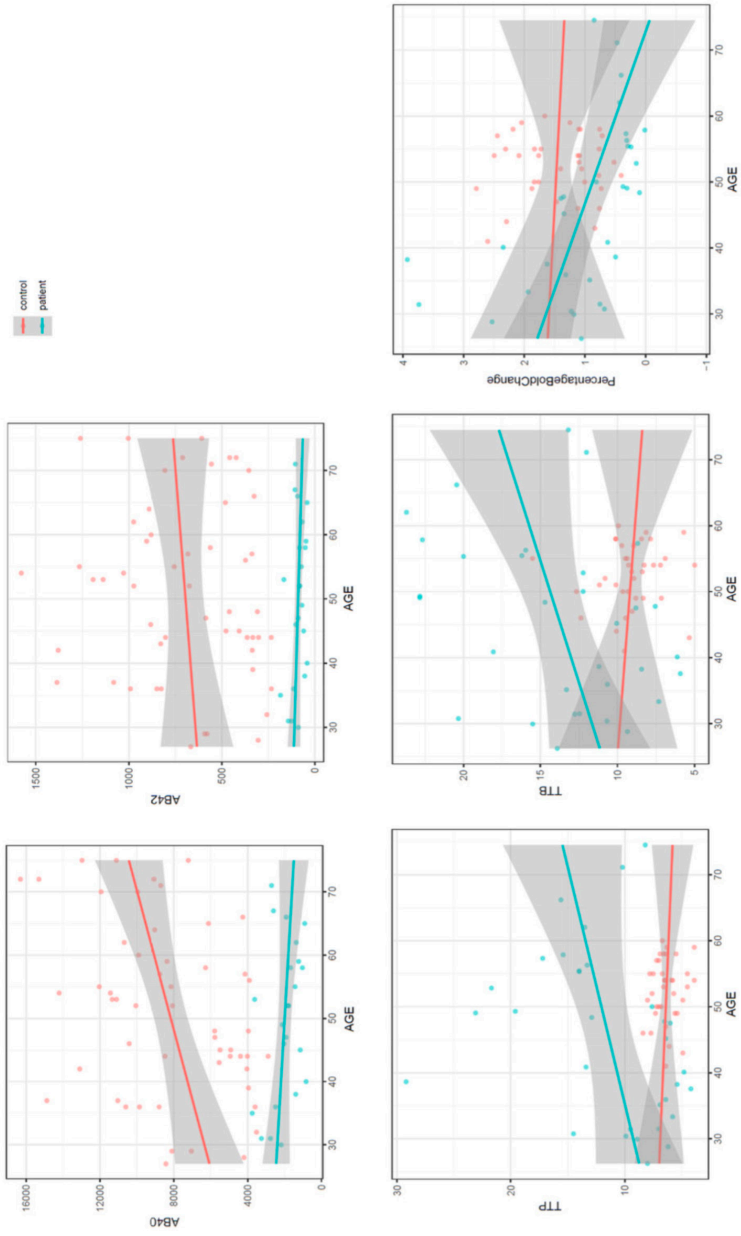
Age of convergence for patients and controls calculated by determining the intersection point of the 95% confidence intervals. Determined for A. amyloid-β40 levels in ng/ml, age of convergence -5.6 years. B. amyloid-β42 levels in ng/ml, age of convergence -115.3 years. C. Time to peak in seconds, age of convergence 19.2 years. E. Amplitude in BOLD change %, age of convergence 31.5 years.

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