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## Staging cerebral amyloid angiopathy: from marker to model

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A watercolor illustration of a brain, showing the cerebral cortex and underlying structures. The brain is rendered in warm tones of orange, yellow, and red. There are several dark blue, irregular shapes scattered across the brain, representing amyloid plaques. Some of these shapes have small white dots on them, possibly representing neurofibrillary tangles. The overall style is artistic and scientific.

# 3

SEX DIFFERENCES IN ONSET AND PROGRESSION  
OF CEREBRAL AMYLOID ANGIOPATHY

## Chapter 3 | Sex differences in onset and progression of cerebral amyloid angiopathy

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## Abstract

**Background:** Cerebral Amyloid Angiopathy (CAA) disease course is highly variable even in hereditary forms. Sex may be a possible modifying factor. We investigated biological sex differences in clinical disease course and MRI-markers in sporadic (sCAA) and Dutch-type hereditary CAA (D-CAA).

**Methods:** Patients with D-CAA and sCAA were included from hospital and research databases of the Leiden University Medical Center (2012-2020) and Massachusetts General Hospital (1994-2012). Key outcomes were: sex differences in symptomatic intracerebral hemorrhage (sICH) onset, recurrence and survival (analyzed using Kaplan Meier survival and regression analyses), and sex differences in MRI-markers in D-CAA (explored using scatterplots), and in sCAA (investigated using regression analysis).

**Results:** We included 136 patients with D-CAA (mean age 57 years, 56% female, 64% with previous sICH) and 370 patients with sCAA (mean age 76 years, 51% female, all with previous sICH). Males and females with D-CAA did not differ for sICH onset (median age 54 in males and 56 in females ( $p=0.13$ )). Males with D-CAA had a slightly higher number of sICH compared to females (median 2 versus 1, adjusted RR 1.5, 95%CI:1.1-1.9) and a shorter interval between the first and second sICH (median 1.8 years for males and 3.1 years for females,  $p=0.02$ ). Males with sCAA had their first sICH at an earlier age (median 75 versus 78 years respectively,  $p=0.003$ ) and more lobar microbleeds (median 1 vs 0,  $p=0.022$ ) compared with females with sCAA. No substantial differences were found in the other MRI markers. Survival after first sICH was comparable between sexes for D-CAA ( $p=0.12$ ) and sCAA ( $p=0.23$ ).

**Conclusions:** Males with CAA seem to have an earlier onset (sCAA) and more hemorrhagic disease course (sCAA and D-CAA) compared to females. Future studies are necessary to confirm these findings and determine the underlying role of sex-related factors.

## Introduction

Cerebral Amyloid Angiopathy (CAA), caused by accumulation of the protein amyloid- $\beta$  in the walls of the small cortical and leptomeningeal arteries, is thought to be present in 23% of the general population and in 48% of patients with Alzheimer's disease.<sup>1</sup> It is the cause of >50% of all lobar intracerebral hemorrhage (ICH) in the elderly, with the highest ICH recurrence rate and highest all-cause mortality of all primary ICH etiologies.<sup>2,1, 3-5</sup> Patients with CAA show a striking variability in clinical symptoms and disease course; some patients present with cognitive decline and have multiple lobar microbleeds on MRI, while others suffer from transient neurological episodes or (repeated) lobar located symptomatic ICH (sICH).<sup>2</sup> Even in Dutch-type hereditary CAA (D-CAA), an autosomal dominant variant of sporadic CAA (sCAA), there is a large variation in phenotype. Although patients carry the same causal mutation, the age at first sICH in patients with D-CAA ranges between 39 and 70 years and the number of sICH recurrences varies from one to 10.<sup>6,7</sup>

One possible contributing factor for this disease variability in CAA could be a modulating effect of sex. Until now, limited research has been performed investigating sex differences in CAA: a study in D-CAA from the early nineties reported higher mortality rates in females compared to males but these findings were never replicated.<sup>8</sup> One study on primary ICH found that females have a higher risk of lobar ICH.<sup>9</sup> However, in this study it was not clear whether the ICH was caused by CAA.

In contrast to CAA, the role of sex has been extensively examined in Alzheimer's disease (AD). AD is also caused by amyloid- $\beta$  accumulation and many AD patients show co-existent CAA pathology.<sup>10</sup> Several studies have shown that males with AD or mild cognitive impairment have more lobar microbleeds (a hallmark of CAA) compared to females.<sup>11</sup> In contrast, AD occurs more frequently in females and females with AD have higher levels of total amyloid- $\beta$  load at histopathological examination.<sup>12</sup> Some observational studies have suggested that estrogen may be protective against AD dementia and that its depletion could exacerbate AD progress in post-menopausal females.<sup>12-14</sup> Based on these previous findings in D-CAA and AD we hypothesize that sex might impact pathogenic pathways for amyloid- $\beta$  deposition.

This study aims to explore the effect of biological sex in CAA on symptomatic ICH onset, sICH recurrence, survival and the occurrence of CAA associated MRI markers in (pre)symptomatic D-CAA mutation carriers and patients with sCAA.

## Methods

We conducted an observational study in two cohorts; one with patients with D-CAA and one with patients with sCAA. Both cohorts contain clinical follow-up data and MRI data. Further details regarding the recruitment, inclusion and follow-up of the participants can be found in the supplemental methods and supplemental figure 1.

### *Data availability*

Further information about the dataset can be obtained from the corresponding author upon reasonable request.

### *Standard Protocol Approvals, Registrations, and Patient Consents*

This study was approved by the local ethics review boards of the LUMC and the MGH, who waived the need for written informed consent from individual participants for the retrospective part of the study. The participants of the AURORA study and the patients from MGH who consented to longitudinal follow-up and gave written informed consent for their participation in these studies.

### *MRI*

In the patients with D-CAA who participated in the prospective AURORA study, MRI scans of the brain were performed on a whole body human 3 Tesla (3T) MRI. The sCAA patients who were included in the MGH cohort were scanned with a 1.5T MRI.<sup>4</sup> Further information regarding the scanners and MRI protocol can be found in the supplemental methods.

### *Image analysis*

The following MRI markers were scored according to the Standards for Reporting Vascular Changes on Neuroimaging recommendations (STRIVE) criteria: lobar cerebral microbleeds (CMB), lobar macrobleeds, cortical superficial siderosis (cSS), enlarged perivascular spaces in the centrum semiovale (CSO-EPVS) and white matter hyperintensities (WMH).<sup>15</sup> CMB were counted and scored on SWI or  $T_2^*$ -weighted images. Macrobleeds were also counted and scored on SWI or  $T_2^*$ -weighted images, T1 and T2-weighted images. cSS was scored on either SWI or  $T_2^*$ -weighted images using both the focality score and the hemisphere score, according to previously published classifications.<sup>16, 17</sup> CSO-EPVS were



scored on T2-weighted images and classified into the following categories; no EPVS, 1-10, 11-20, 21-40, >40.<sup>15, 18</sup> WMH was graded separately for deep and periventricular white matter with the Fazekas score on FLAIR images.<sup>15, 19</sup> The total MRI brain burden of CAA related small vessel disease score (CAA-CSVD) was calculated for each participant according to previously published methods.<sup>20</sup>

### *Statistics*

Statistical analysis were performed using the software R (R foundation for statistical computing, Vienna, Austria; [www.R-project.org](http://www.R-project.org)) and the Statistical Package for Social science (IBM SPSS). Figures were created using GraphPad Prism. Only participants with available data were used for the analysis, we did not perform data imputation. We used descriptive statistics to calculate means, medians and frequencies of the baseline characteristics of all participants. In patients with D-CAA, data from birth until date of medical file perusal (October 29<sup>th</sup>, 2020) were included. This approach was chosen due to the genetic character of the disease and the uncertainty regarding the exact onset of the disease process. For this group we used age at October 29<sup>th</sup>, 2020 or age at death if the participant was deceased before this time as the total follow-up time. All patients with sCAA first presented at the time of their first lobar sICH, and were followed from this moment onwards.

### *Clinical data*

We used Kaplan Meier survival analysis with log-rank testing to investigate differences between males and females with CAA in age at first sICH and age at death. In the patients who suffered from at least one sICH we used Kaplan Meier survival analysis with log-rank testing to investigate differences between males and females in the survival time after the first sICH and in time between the first and the second sICH. Patients who died immediately after their first sICH (defined as death <3 weeks after 1<sup>st</sup> sICH) were included but censored in this analysis. All Kaplan Meier survival analysis were truncated when <10% of participants remained at risk. In D-CAA and sCAA patients who suffered from at least one sICH, we used Poisson regression analysis corrected for follow-up time after the first sICH to calculate adjusted rate ratios (aRR) with 95% confidence intervals (95%CI) for the difference in total number of sICH between males and females. All analyses were performed for sCAA and D-CAA separately, no comparisons were done between the groups.



### *MRI data*

We used descriptive statistics to calculate the proportion of symptomatic and presymptomatic males and females with D-CAA with CAA related MRI markers (CMB, lobar macrobleeds, cSS, CSO-EPVS, WMH) and constructed explorative scatter plots to demonstrate the association of the markers with age at time of MRI scan. Because the number of D-CAA mutation carriers in the different age categories was limited we refrained from performing formal statistical tests.

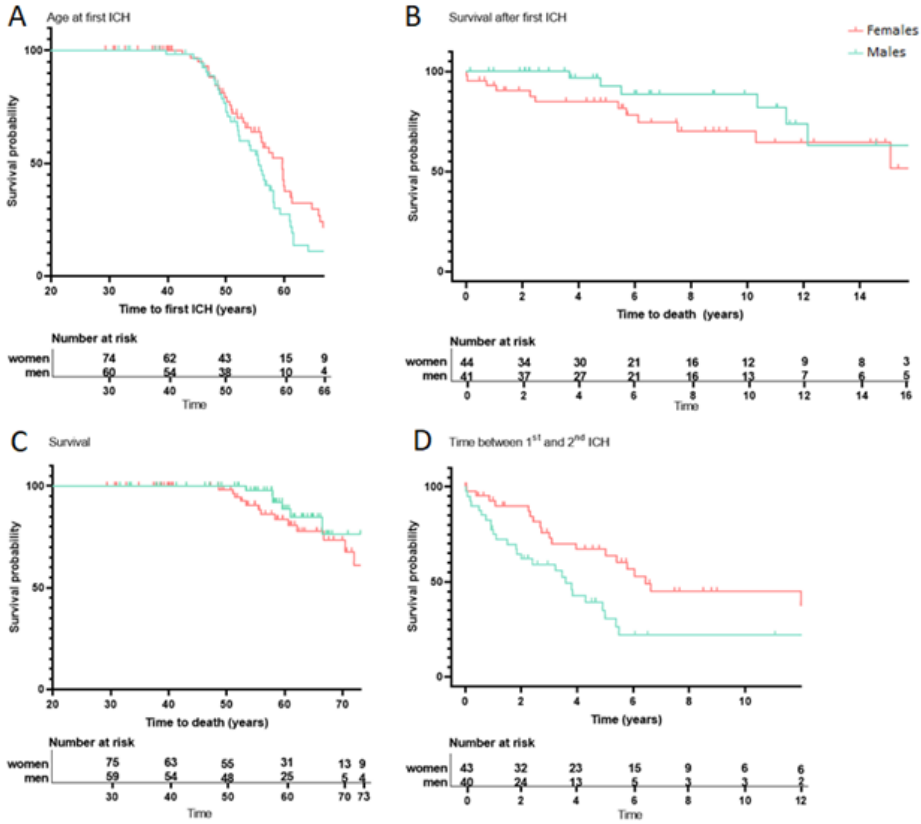
In patients with sCAA we used binomial regression with an exponential link corrected for age at time of MRI, APOE status, presence of hypertension and history of smoking to calculate adjusted odds ratios (aOR) with 95%CI for sex differences in presence of lobar CMB, presence of cSS, presence of >20 CSO-EPVS, presence of >40 CSO-EPVS, and presence of deep and periventricular WMH on MRI performed <3 months after sICH. In patients with sCAA we used Poisson regression analysis corrected for age at time of MRI, APOE status, presence of hypertension and history of smoking to calculate aRR with 95% CI for sex differences in number of lobar CMB and CAA-CSVD score, scored on MRI performed <3 months after sICH.

## Results

### *D-CAA*

We included 136 patients with D-CAA (mean age 57 years, 56% female). Eighty-seven (64%) of the 136 patients had a history of sICH (70% of the males and 59% of the females). Males with D-CAA had their first sICH at a median age of 54 years (range 40-72) and females with D-CAA at a median age of 56 years (range 42-78), ( $p=0.13$ , Figure 1A). During follow-up (mean follow-up time 58 years [range 29-87]), 7 (12%) of the males and 14 (18%) of the females with D-CAA died. All deaths except two were caused by CAA-related ICH, these two participants were excluded from the survival analysis. Survival after the first sICH was comparable between males and females with D-CAA: median of 8 years (range 4-12) for males and 5.4 years (range 0-19) for females ( $p=0.12$ , Figure 1B), as was overall survival: median survival of 59 years (range 53-67) for males and 56 years (range 49-72) for females ( $p=0.27$ , Figure 1C). Males with D-CAA had a higher number of recurrent sICH compared with females (median number of sICH 2 [range 1-9] for males and 1 [range 1-5] for females, aRR 1.5, 95% CI 1.1-1.9,  $p=0.01$ ). Median time between the first and second sICH was 1.8 years for males with D-CAA and 3.1 years for females ( $p=0.02$ , Figure 1D).

Figure 1: *slCH* onset, time between first and second *slCH* and survival in males and females with D-CAA.



1A: Difference in age at first *slCH* between males and females with D-CAA. N=60 male, 75 female (1 patient excluded from analysis as age at first *slCH* was unknown). P=0.13 (Log Rank). 1B: Difference in survival after first *slCH* between males and females with D-CAA and at least one *slCH*. N=41 mals, 43 female (2 patients excluded as death was not CAA related). P=0.12 (Log Rank). Including the patients whose death was not CAA-related did not significantly change the outcome (p=0.14). 1C: Difference in age at death between males and females with D-CAA. N= 59 male, 75 female (2 patients excluded as death was not CAA-related). P=0.27 (Log Rank). Including the patients whose death was not CAA-related did not significantly change the outcome (p=0.34). 1D: Difference in time between first and second *slCH* between males and females with D-CAA. N=40 male, 43 female (3 patients excluded because age at 2<sup>nd</sup> *slCH* was unknown, patients who died within 3 weeks of first *slCH* (n=2) included in analysis but censored). P=0.02 (Log Rank).

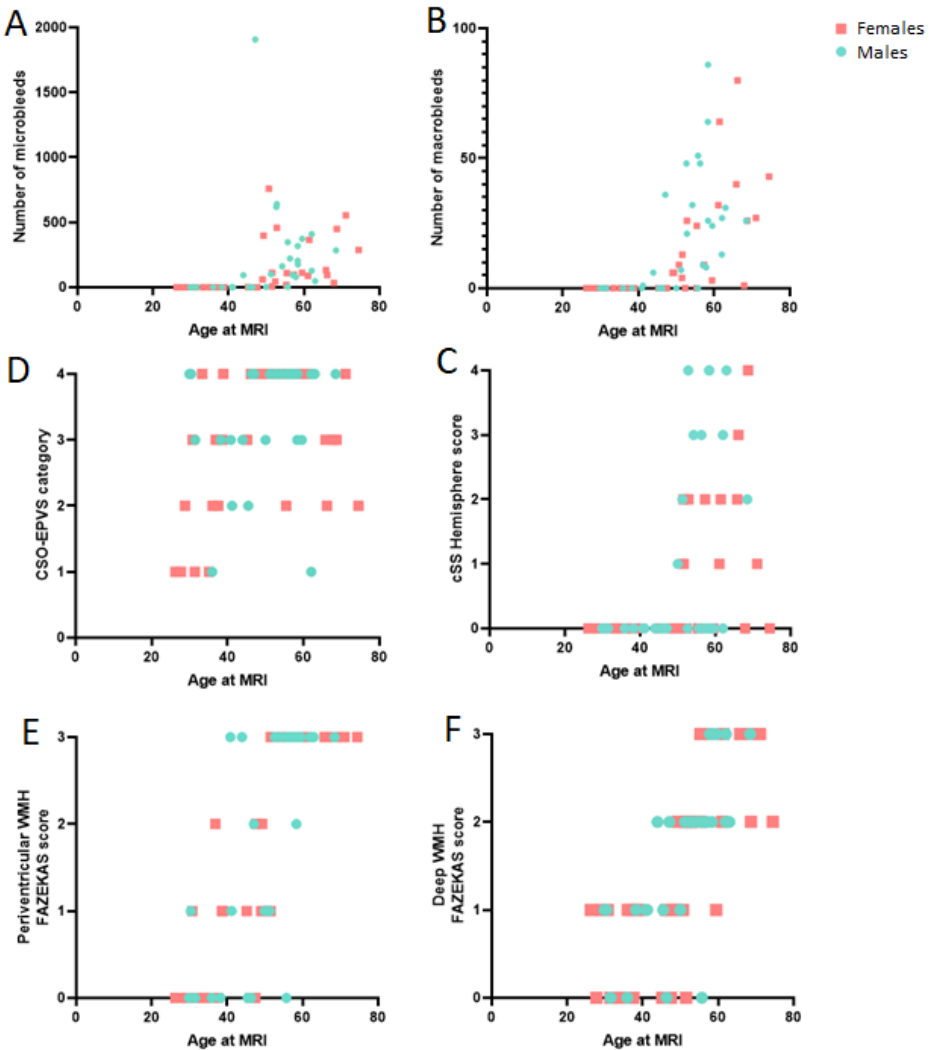
Table 1: MRI markers in males and females with D-CAA.

	No history of sICH		Positive history of sICH	
	Females with D-CAA (n=21)	Males with D-CAA (n=13)	Females with D-CAA (n=14)	Males with D-CAA (n=16)
<b>Demographics</b>				
Age at MRI (mean, range in years)	42 (26-74)	43 (30-69)	60 (49-71)	57 (47-63)
Race / Ethnicity (n, %)				
White	21 (100)	13 (100)	14 (100)	16 (100)
<b>Medical history (at time of MRI)</b>				
Hypertension (n, %)	2 (10)	4 (31)	7 (50)	1 (6)
Hypercholesterolemia (n, %)	2 (10)	1 (8)	6 (43)	5 (31)
Diabetes (n, %)	0	0	2 (14)	0
Atrial fibrillation (n, %)	1 (5)	1 (8)	2 (14)	0
Prior TIA/ Ischemic stroke (n, %)	1 (5)	0	2 (14)	2 (13)
<b>MRI markers</b>				
Macrobleeds (n, %)	3 (14)	3 (23)	14 (100)	16 (100)
Count (median, range)	0 (0-43)	0 (0-26)	19.5 (1-80)	29 (7-86)
Microbleeds (n, %)	6 (29)	4 (31)	14 (100)	16 (100)
Count (median, range)	0 (0-397)	0 (0-284)	112.5 (5-759)	213 (48-1906)
cSS (n, %)	0 (0)	2 (15)	10 (71)	8 (50)
Focal (n, %)	0 (0)	2 (15)	8 (57)	2 (13)
Disseminated (n, %)	0 (0)	0 (0)	2 (14)	6 (38)
Hemisphere score (median, range)	0 (0-0)	0 (0-2)	1.5 (0-4)	1 (0-4)
>20 CSO-EPVS (n, %)	12 (57)	10 (77)	13 (93)	15 (94)
>40 CSO-EPVS (n, %)	8 (38)	5 (39)	10 (71)	13 (81)
Periventricular WMH (n, %)	11 (52)	6 (46)	14 (100)	16 (100)
Periventricular WMH score (median, range)	1 (0-3)	0 (0-3)	3 (1-3)	3 (1-3)
Deep WMH (n, %)	14 (67)	9 (69)	13 (93)	13 (100)
Deep WMH score (median, range)	1 (0-3)	1 (0-3)	2 (0-3)	2 (2-3)
CAA-CSVD score (median, range)	1 (0-4)	1 (0-5)	5 (3-6)	4.5 (3-6)

sICH: symptomatic intracerebral hemorrhage, D-CAA: Dutch type Cerebral Amyloid Angiopathy, TIA: Transient ischemic attack, MRI: Magnetic resonance imaging, cSS: cortical superficial siderosis, CSO-EPVS: Enlarged perivascular spaces in the centrum semiovale, WMH: White matter hyperintensities, CAA-CSVD score: MRI brain burden of CAA related small vessel disease score

Sixty-four (mean age 50 years, 56% female) of the 136 patients with D-CAA participated in the prospective AURORA study. Frequencies of the different MRI markers are shown in Table 1 and Figure 2. Occurrence of CSO-EPVS and WMH on MRI were the earliest CAA related MRI markers in both pre-symptomatic males and females with D-CAA (Table 1, Figure 2, supplemental figure 2). The proportion of the different MRI markers in our exploratory analyses seemed to be comparable between males and females with D-CAA over age (Figure 2), although some males had a relatively high number of macrobleeds and cSS at a relative early age (Figure 2B and 2C).

Figure 2: MRI markers in males and females with D-CAA.



## sCAA

We included 370 participants with sCAA (mean age at sICH 76 years, 51% female); 151 were diagnosed with probable CAA (89 female and 62 male) and 219 with possible CAA (106 female and 113 male) based on the modified Boston criteria. All had a history of sICH and were followed from the moment of their first ICH onwards: average follow-up time was 71.2 months (inter-quartile range 44.7-88.6).



**Table 2:** Characteristics of males and females with sCAA.

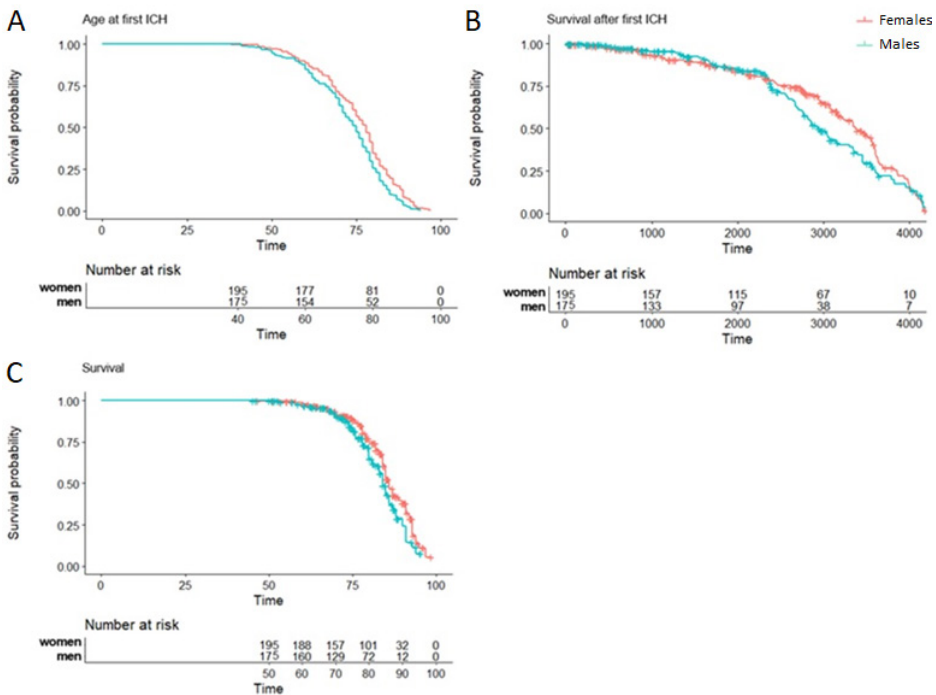
	Females with sCAA (n= 195)	Males with sCAA (n=175)
<i>Demographics</i>		
Age at first sICH (mean, SD in years)	75.4 (11.5)	72.64 (11.0)
Race /Ethnicity (n, %)		
White	170 (87.2)	155 (88.6)
Black	13 (6.7)	8 (4.6)
Other	12 (6.2)	12 (6.9)
Probable CAA (%)	89 (46)	62 (35)
<i>Medical history</i>		
Hypertension (n, %)	132 (67.7)	129 (73.7)
Diabetes (n, %)	30 (15.4)	35 (20.0)
Coronary artery disease (n, %)	24 (12.3)	35 (20.0)
Atrial fibrillation (n, %)	22 (11.3)	36 (20.6)
Prior sICH /Macrobleed	13 (6.7)	12 (6.9)
Prior TIA /Ischemic stroke (n, %)	13 (6.7)	21 (12.0)

sCAA: *sporadic cerebral amyloid angiopathy*, sICH: *symptomatic intracerebral hemorrhage*, TIA: *transient ischemic attack*.

Males with sCAA had their first sICH at a median age of 75 years (range 41-95) and females with sCAA at a median age of 78 years (range 38-97), log-rank test  $p=0.003$  (figure 3A). During follow-up, 79 (45%) of the males and 94 (48%) of the females with sCAA died. Males with sCAA died at a median age of 80 years (range 53-94) and females with sCAA at a median age of 83 years (range 47-97), log-rank test  $p=0.03$  (Figure 3C). Overall survival after the first sICH between males and females, however was comparable ( $p=0.23$ ) (Figure 3B). In total there were

91 sICH recurrent events during follow-up, 48 in males and 43 in females with sCAA. There was no difference in ICH recurrence between males and females ( $p=0.23$ ). Frequencies of the different MRI markers are shown in Table 3. Males had significantly more often lobar microbleeds compared to females ( $p=0.006$ ), and a higher median lobar microbleed count (median count of 1 in males vs 0 in females,  $p=0.02$ ). The frequencies of the other MRI markers as well as the CAA-CSVD scores were comparable between males and females with sCAA.

Figure 3. sICH onset and survival in males and females with sCAA.



3A: Difference in age at first sICH between males and females with sCAA. Time in years.  $P=0.003$  (Log Rank). 3B: Difference in survival after first sICH between males and females with sCAA. Time in days.  $P=0.23$  (Log Rank). 3C: Difference in overall survival between males and females with sCAA. Time in years.  $P=0.03$  (Log Rank).

**Table 3:** MRI markers in males and females with sCAA.

	Females with sCAA (n= 195)	Males with sCAA (n=175)	P
Lobar macrobleeds (n, %)	195 (100)	175 (100)	-
Count (median, range)	1 (1-1)	1 (1-1)	-
Lobar microbleeds (n, %)	84 (43.1)	101 (57.7)	<b>0.006*</b>
Count (median, range)	0 (0-278)	1 (0-289)	<b>0.022*</b>
cSS (n, %)	40 (20.5)	44 (25.1)	0.288
Focal (n, %)	24 (12.3)	35 (20.0)	-
Disseminated (n, %)	16 (8.2)	9 (5.1)	-
Multifocality score (median, range)	0 (0-4)	0 (0-4)	-
>20 CSO-EPVS (n, %)	80 (41.0)	75 (42.9)	0.990
>40 CSO-EPVS (n, %)	17 (8.7)	16 (9.1)	0.990
Periventricular WMH (n, %)	195 (100.0)	172 (98.3)	0.812
Periventricular WMH score (median, range)	2 (0-3)	2 (0-3)	-
Deep WMH (n, %)	181 (92.3)	160 (91.4)	0.883
Deep WMH score (median, range)	2 (0-3)	1 (0-3)	-
CAA-CSVD score (median, range)	1 (1-6)	1 (1-6)	0.732

sCAA: sporadic cerebral amyloid angiopathy, cSS: cortical superficial siderosis, CSO-EPVS: Enlarged perivascular spaces in the centrum semiovale, WMH: white matter hyperintensities, CAA-CSVD score: MRI brain burden of CAA related small vessel disease score.

\*Statistically significant.

## Discussion

We found that males with D-CAA had a higher number of recurrent sICH and a shorter time between the first and second sICH compared to females. In patients with sCAA, males had their first sICH at an earlier age than females, and males had more microbleeds on MRI compared with females. Overall, males with CAA seemed to have an earlier disease onset (sCAA) and a more hemorrhagic disease course (D-CAA and sCAA) compared with females. Survival after first sICH was comparable between sexes for both D-CAA and sCAA.

The biological sex differences in CAA increase our understanding of CAA pathophysiology and have implications for clinic and research. With our results patients can be better informed about the influence of sex differences on the disease





course. Also, the suggestion of a more hemorrhagic phenotype in males with CAA might be a factor to consider in decisions regarding (re)start of anticoagulation. The mechanisms behind the sex-differences could be possible future targets for prevention and treatment of CAA. A possible driving force behind the differences could be the influence of (female) sex specific factors such as (epi)genetic factors and sex-hormones. Previous studies in Alzheimer's disease have shown that estrogen (depletion) possibly influences disease course, including the accumulation of the amyloid- $\beta$  peptide in brain tissue, and that the hormone might protect against Alzheimer's disease.<sup>13, 14, 21, 22</sup> Due to the sex-differences found in this current study, which seem protective for females, one could hypothesize that the protective effect of estrogen also plays a role in vascular amyloid accumulation, although this has not yet been investigated. This could especially be the case in D-CAA, where women can be affected at a relatively young pre-menopausal age. However, also in sCAA it can be hypothesized that estrogen plays a protective role. Previous research has shown that inflammation is abundantly present in brain tissue of patients with CAA, and is related to microbleed development in CAA-mouse models.<sup>23-25</sup> Estrogen has anti-inflammatory effects, which might be a second possible pathway for the influence of estrogen on CAA disease course and CAA related ICH.<sup>26-28</sup> Another explanation for the sex differences in our study could be residual confounding by differences in comorbidity or (exposure to) unidentified (epi) genetic or environmental vascular risk factors. Future studies are necessary to determine whether there is a link between female sex (hormones) and CAA, for instance by investigating the effect of pregnancy and menopause (onset) and use of hormone supplementation on CAA and to investigate sex differences in histopathology, CAA animal models or organ-on chip- models.<sup>29</sup>

A previous study from the nineties found higher overall mortality in females with D-CAA compared with males.<sup>8</sup> This finding could not be reproduced in our study although median survival in females with D-CAA was shorter than in men. However, because this difference was not statistically significant it is most likely that this finding is due to chance. Alternatively, the lack of significance could be caused by the relatively small sample size and point towards a true difference in survival. It is not likely that the difference in survival was the cause of the found difference in sICH recurrence, as we adjusted for follow-up time in the analysis. Hypothetical explanations for a possible difference in survival are a more detrimental ICH course in females or a higher risk of misdiagnosis with consequent delays of acute stroke treatment, as has been recently shown to be the case in ischemic stroke.<sup>30</sup> Our current study included less participants than the previous study and the overall mortality was lower. It is, therefore, possible that we did not have enough power to



detect differences in mortality. It is also possible that due to improvement of overall clinical care and post-ICH rehabilitation compared to the nineties previously found differences in survival might have decreased. Lastly, our D-CAA database only includes patients who presented at the (outpatient) clinic, and is therefore prone to 'immortal time bias'. Patients need to be alive to be able to present themselves at the hospital and those who die due to their first sICH at home can therefore not be included. Future studies are necessary to further investigate sex-differences in survival and ICH recurrence, and the possible underlying mechanisms.

CSO-EPVS and WMH were the earliest MRI markers in both males and females with D-CAA. Macroleads and microbleeds only manifest themselves after the age of 40, comparable to what was found in previous studies regarding MRI markers in presymptomatic and symptomatic D-CAA.<sup>31, 32</sup> We found a remarkable variability in the proportions of CAA-related MRI markers within patients of the same age category (see Figure 2). This suggests that other genetic and/or life-style factors might influence CAA development in D-CAA. APOE status was not available for our D-CAA participants, however an earlier study did not identify an effect of APOE status on MRI markers (white matter hyperintensities) in D-CAA.<sup>33</sup>

Our study has limitations. Firstly, the follow-up data of D-CAA patients were in part retrospectively collected and therefore some events could have been missed. This may have also caused a selection bias, as patients who did not report at the LUMC could not be included in the study. The radiological data of both the sCAA and D-CAA populations were derived from a prospective study, in which all participants underwent the same MRI protocol. However, the number of participants with D-CAA in the different age groups was small and included relatively healthy patients, again possibly causing selection bias. Therefore we refrained from performing statistical analyses on the MRI markers in this group, and consider that part of our study explorative. Another limitation of this study is the inclusion of both possible and probable sCAA patients. Both groups were included to cover early and advanced stages of CAA and to be able to generate enough power to enable statistical comparisons. However, the addition of patients with possible CAA could have diluted our findings because of possible misclassification. Lastly, we chose in this first explorative study to only include sCAA patients with a history of ICH. sCAA has a broad spectrum of symptoms, and there are patients with sCAA who never suffer from an ICH. We chose to investigate those with ICH to enable better comparison to the D-CAA population, who are known to all eventually suffer from ICH. Future research is needed to investigate sex-differences in non-hemorrhagic symptoms such as dementia and cognitive impairment in CAA.

The strength of our study lies in the investigation of sex differences in both a unique hereditary and a sporadic cohort of CAA patients. In contrast to sCAA for which only a possible or probable diagnosis can be made during life, D-CAA can be diagnosed with absolute certainty by genetic testing.<sup>34</sup> Furthermore, as D-CAA occurs at a relatively young age, patients have far less age-related small vessel disease. Therefore, D-CAA is considered to be a relatively 'pure' form of CAA in which the early and presymptomatic stages of CAA can be investigated. We chose in this study to not combine or compare the results between sCAA and D-CAA cohorts directly, as the baseline characteristics of these cohorts are too different for a direct comparison: the cohorts differ not only in age and age-related cardiovascular risk factors, but also in race/ethnicity (see table 1 and 2), and MRI field strength and protocol. That, despite these differences, results of this study point into the same direction for both D-CAA and sCAA strengthens our findings.

To conclude, our study suggests that sex influences on the disease course of patients with D-CAA and sCAA may be present. Our results seem to show that male sex is possibly associated with an earlier onset in sCAA, and a more hemorrhagic disease course in sCAA and D-CAA. Future prospective studies are necessary to confirm these findings and determine which sex related factors could be important for CAA progression, and could therefore be a possible target for CAA prevention and treatment.

## Supplemental Material

### *Supplemental methods*

#### *Participants*

In this study we investigate the effect of biological sex on CAA. We do not investigate the effect of gender. All participants are cis-gender individuals who did not go through medical or social transitioning.

The participants with D-CAA were derived from a retrospective D-CAA database that included all consecutive D-CAA mutation carriers who visited the Leiden University Medical Center (LUMC) neurology (outpatient) clinic between January 2012 and November 2020. D-CAA was diagnosed 1) by genetic testing for the causal mutation on codon 693 in the APP gene or 2) when patients had a history of  $\geq 1$  lobar sICH with features of CAA according to the modified Boston criteria on MRI and  $\geq 1$  first-degree relative diagnosed with D-CAA. Both pre-symptomatic and symptomatic (defined as a history of sICH) D-CAA mutation carriers were included. Symptomatic ICH was defined as acute onset of symptoms which could be attributed to an ICH visible on radiological imaging. The database contains information on demographics, clinical characteristics and disease outcome (age at first sICH, sICH recurrence and survival). We obtained date and cause of death by reviewing clinical charts and, if necessary, consulting the general practitioner. A subset of the mutation carriers included in the D-CAA database also participate in the ongoing prospective D-CAA natural history study (called the AURORA study). Participants for this study were recruited via the (outpatient) clinic of the LUMC. This follow-up study started in 2018 and aims to investigate CAA disease course and to identify new CAA related biomarkers in (pre)symptomatic D-CAA. All AURORA participants underwent yearly MRI and were interviewed in-person on demographics, medical history and clinical symptoms. MRIs were performed between 2018-2020 and scored by a single observer with  $>5$  years of experience in the field (EAK).

The participants with sCAA were derived from the Massachusetts General Hospital (MGH) sCAA database that contains prospectively collected data from a longitudinal cohort of sCAA patients who visited the MGH in Boston between January 1994 and December 2012. For this study we only included patients with possible or probable sCAA according to the modified Boston criteria who had a history of lobar sICH; we excluded participants with inflammatory CAA. Information on demographics, medical history and clinical symptoms was obtained through in-person interviews. Follow-

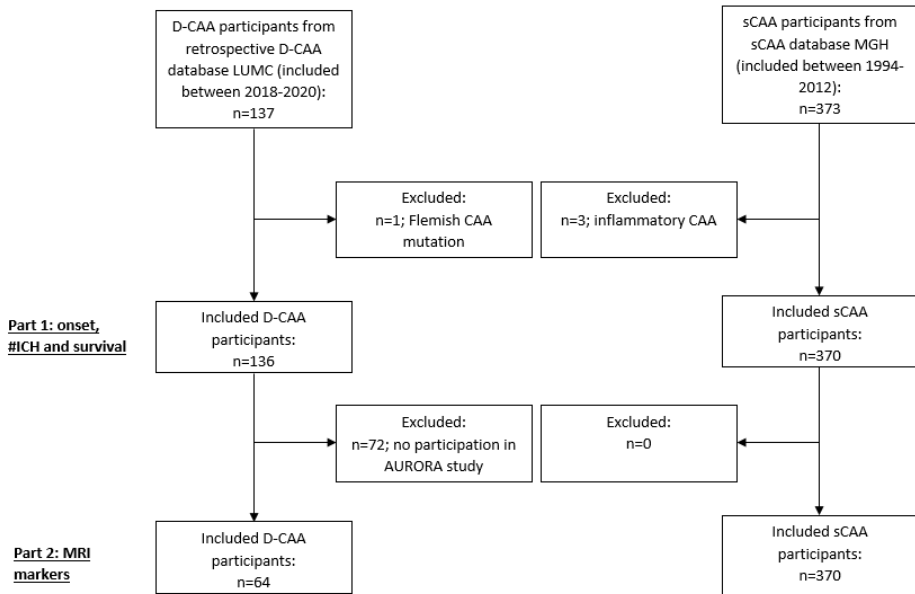


up data were collected for those patients who consented to a longitudinal follow-up via telephone calls at 3 months after enrollment and every 6 months thereafter. Patients were followed until the moment of data collection for this paper, which was August 2021. Patients underwent MRI scans performed within 3 months after lobar sICH. MRIs were performed between 1994-2012, scored by multiple experienced observers and checked by a single observer with >10 years of experience in the field (AV). See supplemental figure 1 for a flow-chart detailing inclusion of the participants with D-CAA and sCAA. This manuscript is reported against the STROBE guidelines.

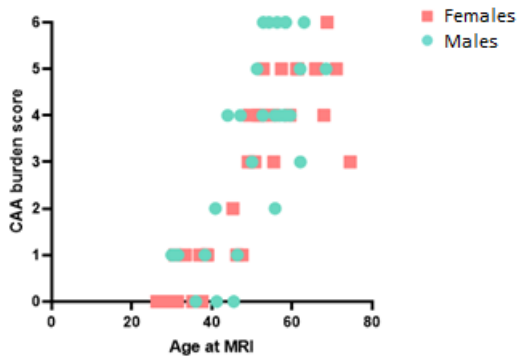
### *MRI*

In the patients with D-CAA who participated in the prospective AURORA study, MRI scans of the brain were performed on a whole body human 3 Tesla (3T) Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands). The data were acquired using a standard 32-channel head coil. The following sequences were performed: Three-dimensional T1 weighted images, T2 weighted images, three dimensional Fluid Attenuated Inversion Recovery (FLAIR) images, and susceptibility weighted images (SWI). The sCAA patients who were included in the MGH cohort were scanned with a 1.5T GE Sigma MRI scanner (GE Healthcare, Chicago, IL, USA). The following sequences were performed: a whole brain axial T2-weighted, T2\*-weighted gradient-recalled echo or SWI, FLAIR images and T1-weighted sequences.

Supplemental figure 1: Flowchart



Supplemental figure 2: CAA-CSVD burden on MRI in D-CAA.



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