Published in final edited form as:

Acta Neuropathol. 2016 August ; 132(2): 225–234. doi:10.1007/s00401-016-1580-y.

Impact of sex and *APOE4* on cerebral amyloid angiopathy in Alzheimer's disease

Mitsuru Shinohara¹, Melissa E. Murray¹, Ryan D. Frank², Motoko Shinohara¹, Michael DeTure¹, Yu Yamazaki¹, Masaya Tachibana¹, Yuka Atagi¹, Mary D. Davis¹, Chia-Chen Liu¹, Na Zhao¹, Meghan M. Painter¹, Ronald C. Petersen³, John D. Fryer¹, Julia E. Crook², Dennis W. Dickson¹, Guojun Bu^{1,*}, and Takahisa Kanekiyo^{1,*}

¹Department of Neuroscience, Mayo Clinic, Jacksonville, FL 32224, USA

²Department of Health Sciences Research, Mayo Clinic, Jacksonville, FL 32224, USA

³Department of Neurology, Mayo Clinic, Rochester, MN 55905, USA

Abstract

Cerebral amyloid angiopathy (CAA) often coexists with Alzheimer's disease (AD). *APOE4* is a strong genetic risk factor for both AD and CAA. Sex-dependent differences have been shown in AD as well as in cerebrovascular diseases. Therefore, we examined the effects of *APOE4*, sex, and pathological components on CAA in AD subjects. A total of 428 autopsied brain samples from pathologically-confirmed AD cases were analyzed. CAA severity was histologically scored in inferior parietal, middle frontal, motor, superior temporal and visual cortexes. In addition, subgroups with severe CAA (n=60) or without CAA (n=39) were subjected to biochemical analysis of amyloid- β (A β) and apolipoprotein E (apoE) by ELISA in the temporal cortex. After adjusting for age, Braak neurofibrillary tangle stage and Thal amyloid phase, we found that overall CAA scores were higher in males than females. Furthermore, carrying one or more *APOE4* alleles was associated with higher overall CAA scores. Biochemical analysis revealed that the levels of detergent soluble and insoluble A β 40, and insoluble apoE were significantly elevated in individuals with severe CAA or *APOE4*. The ratio of A β 40/A β 42 in insoluble fractions was also increased in the presence of CAA or *APOE4*, although it was negatively associated with male sex. Levels of insoluble A β 40 were positively associated with those of insoluble apoE, which were strongly influenced by CAA status. Pertaining to insoluble A β 42, the levels of apoE correlated regardless of CAA status. Our results indicate that sex and *APOE* genotypes differentially influence the presence and severity of CAA in AD, likely by affecting interaction and aggregation of A β 40 and apoE.

*Correspondence to Takahisa Kanekiyo, Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville FL 32224, Phone 904-953-2483, kanekiyo.takahisa@mayo.edu or Guojun Bu, Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville FL 32224, Phone 904-786-3419, bu.guojun@mayo.edu.

RCP: Consultant to Roche, Inc., Merck, Inc., Genentech, Inc., Biogen, Inc., Eli Lilly and Co.

Author Contributions

M.S., D.W.D., G.B. and T.K. conceived the study. M.S., M.E.M., M.S., M.D., Y.A., M.D.D., M.T., Y.Y., C.L., N.Z., D.W.D., G.B. and T.K. collected data, and M.S., M.E.M., R.D.F., J.E.C., M.S., J.D.F., M.M.P., R.C.P., D.W.D., G.B. and T.K. analyzed data. M.S., G.B. and T.K. wrote the first draft. All authors contributed to writing the final manuscript.

Conflicts of Interest: Nothing to report.

Keywords

Amyloid- β ; Alzheimer's disease; *APOE*; cerebral amyloid angiopathy; sex

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia in the elderly [2]. Epidemiologically, the prevalence of AD and other dementias is higher in females than males in the aged population. At 65 years old, the future risk of developing AD for females is 17.2%, compared with 9.1% chance for males [2]. While senile plaques, due to brain amyloid- β (A β) deposition in the parenchyma, and neurofibrillary tangles are the two major histological hallmarks for AD [41], the vast majority (>80%) of autopsy-confirmed AD cases also exhibit at least a mild degree of cerebral amyloid angiopathy (CAA) [12]. In CAA, A β deposits, predominantly in vascular smooth muscle layers, are present in leptomeningeal, cortical arteries and capillaries comprising the blood-brain barrier [3, 37, 38, 48]. Importantly, CAA is a major cause of intracerebral hemorrhage [16, 18, 27, 49] and is responsible for ~20% of cerebral hemorrhage cases in the elderly [12]. In addition, severe CAA often associates with microangiopathies (e.g. microaneurysm formation, fibrinoid necrosis) [28] and cerebral microinfarcts [44]. Although various hypotheses have been proposed to explain the increased prevalence of AD in females, whether sex-dependent differences exist in CAA remains unclear.

Genome-wide association studies have confirmed that the ϵ 4 allele of the *APOE* gene encoding apolipoprotein E (apoE) is the strongest genetic risk factor for AD [19, 25]. Meta-analysis has also shown association of *APOE4* with severe CAA [36]. Furthermore, *APOE4* is associated with increased risk of age-related cognitive decline as well as vascular cognitive impairment during normal aging [26]. Among the three human apoE isoforms, apoE4 is not only inhibitory to brain A β clearance, but is also less efficient in supporting synapses and cerebrovascular functions [8, 22]. Importantly, while *APOE4* carriers, in general, have a higher risk of conversion to mild cognitive impairment (MCI) or AD among cognitively normal individuals, the *APOE4* effect is considerably stronger in females compared to males [1].

In this study, we investigated how *APOE* genotype and sex influence the risk of CAA in pathologically-confirmed AD cases using a large sample cohort of postmortem brain tissue (n=428). Additionally, the levels of CAA-related molecules were biochemically assessed by ELISA in the temporal cortex of a subset of cases with severe CAA (n=60) or without CAA (n=39). Our results demonstrate that in AD, male sex and *APOE4* are significantly associated with CAA severity. CAA incidence, sex and *APOE4* influence brain levels of A β and apoE, and their association in AD. Our findings provide novel insights into the pathogenesis of CAA in AD.

Materials and Methods

Human autopsied brain samples

The Mayo Clinic brain bank for neurodegenerative disorders at Jacksonville was queried for neuropathologically-confirmed AD cases with available frozen tissue, known *APOE* genotype, and absence of the following; TDP-43 proteinopathies (hippocampal sclerosis, frontotemporal lobar degeneration, amyotrophic lateral sclerosis), primary tauopathies (progressive supranuclear palsy, corticobasal degeneration), argyrophilic grain disease, and α -synucleinopathies (Lewy body disease and multiple systems atrophy). The presence of cerebrovascular lesions was not considered as an exclusion criteria. A total of 428 AD cases were identified for inclusion in this study. Experimental procedures were conducted in strict accordance with protocol approved by the Mayo Clinic Institutional Review Board. All cases previously underwent standardized neuropathological assessments, as previously described [31]. Thioflavin S fluorescent microscopy was used to assign a Braak neurofibrillary tangle stage, Thal amyloid phase and CAA score (Table 1). *APOE* genotype distributions were as follows: $\epsilon 2/\epsilon 3$ (n=12), $\epsilon 2/\epsilon 4$ (n=9), $\epsilon 3/\epsilon 3$ (n=159), $\epsilon 3/\epsilon 4$ (n=188) and $\epsilon 4/\epsilon 4$ (n=60) (supplemental Figure 1).

CAA assessment

CAA severity was scored in the inferior parietal cortex, middle frontal cortex, motor cortex, superior temporal cortex and visual cortex using a semi-quantitative method through thioflavin-S staining as follows; 0 = no amyloid positive vessels; 0.5 = scattered amyloid deposition only in leptomeninges; 1 = scattered amyloid deposition in both leptomeningeal and cortical vessels; 2 = strong circumferential amyloid deposition in multiple cortical and leptomeningeal vessels; 3 = widespread strong amyloid deposition in leptomeningeal and cortical vessels; 4 = more severe CAA than score 3 (Fig. 1).

Sample preparation

Temporal cortex tissues were dissected from AD subjects with severe CAA (n=60) or without CAA (n=39), and kept frozen until extraction. Brain lysates were prepared by a three-step extraction method based upon differential solubility in detergents (Triton X-100) and the chaotropic agent guanidine hydrochloride (GuHCl) as previously described [42, 43]. In brief, after removal of meninges and blood vessels, 100–200 mg of frozen brain tissue were homogenized in 10–15 volumes (w/v) of ice-cold TBS-containing protease inhibitor cocktail (Roche Diagnostics) by Polytron homogenizer (KINEMATICA). After centrifugation at $100,000 \times g$ for 60 min at 4°C, the supernatant was aliquoted and stored at –80°C (referred to as TBS-soluble fraction or TBS). The residual pellet was re-homogenized in TBS plus 1% Triton X-100 with protease inhibitor cocktail, incubated with mild agitation for 1 h at 4°C and centrifuged as described above. The resulting supernatant was aliquoted and stored at –80°C (referred to as TX-soluble fraction or TX). The residual pellet was re-homogenized in 5 M GuHCl (pH 7.6), and incubated with mild agitation for 12–16 h at 22°C. After centrifugation as above, the resultant supernatant (referred to as the insoluble or GuHCl fraction) was diluted with nine volumes of TBS, aliquoted and stored at –80°C.

Quantification of CAA-related molecules

Levels of A β 40, A β 42 and apoE were determined by ELISA as previously described [42, 43]. Colorimetric quantification was performed on a Synergy HT plate reader (BioTek) using horseradish peroxidase (HRP)-linked streptavidin (Vector) and 3,3',5,5'-tetramethylbenzidine substrate (Sigma). All measured values by ELISA were normalized against total protein measured by BCA assay.

Data analysis

Data were descriptively summarized using median and interquartile ranges for continuous variables and frequencies and percentages for categorical variables. The overall CAA score for the primary analysis was defined as the mean of the region specific scores. Associations of sex and *APOE4* with CAA score were examined using linear regression models with adjustment for age, Braak stage, and Thal amyloid phase. Associations between CAA, sex, and *APOE4* with log-transformed A β and apoE levels were examined using multivariate logistic regression. The parameter estimates were exponentiated to obtain estimates of relative levels in one group compared to another. Age, Braak stage and Thal amyloid phase were adjusted for all models. All statistical tests were two-tailed. Analyses were primarily performed using SAS statistical software (SAS Institute Inc, Cary, NC).

Results

APOE genotype, sex and CAA severity in AD

Of the 428 pathologically-confirmed AD cases, we found that 374 AD subjects (88.4%) had CAA pathology in at least one of the five cortical regions analyzed. Visual cortex had the highest frequency of CAA with a score of 2 or more (36.7%), followed by the middle frontal cortex (35.0%), motor cortex (28.0%), inferior parietal cortex (27.8%) and superior temporal cortex (8.9%) (Fig. 2 and supplemental Table 1). There was evidence that males and those with one or more *APOE4* alleles had higher overall CAA scores, with or without adjusting for age, Braak stage and Thal amyloid phase (Table 2). In particular, male sex showed higher CAA scores than females in every cortical region (supplemental Table 2). The associations between *APOE4* and average CAA score were statistically significant only in females (n=246), but not in males (n=182), although there was no evidence of an interaction between *APOE4* and sex (p=0.84) (Fig. 3 and Table 2). Increasing *APOE4* allele number was associated with higher CAA score regardless of sex (Table 2 and supplemental Figure 1).

Biochemical analysis of A β and apoE in CAA

To further investigate the effects of *APOE4* and sex on CAA, levels of A β and apoE in temporal cortex from AD cases (males n=49; females n=50) without CAA (n=39), or with severe CAA (average CAA score >2; n=60) were subjected to biochemical analysis (Table 3). Multiple regression analyses, including variables for CAA, sex and *APOE4* status, showed statistically significant evidence that the levels of A β 40, but not A β 42, in TBS, TX and GuHCl fractions were higher in the presence of CAA or *APOE4* (Fig. 4 and Table 4). Accordingly, in those with *APOE4*, the ratios of A β 40/A β 42 in all fractions were significantly higher in cases with CAA. A β 40/A β 42 ratios in TBS and GuHCl fractions were

higher by an estimated 1.86 ($p=0.003$) and 3.62 fold ($p<0.001$), respectively. Interestingly, male sex was associated with lower A β 40/A β 42 ratios in the TBS and GuHCl fractions (Fig. 4 and Table 4). ApoE levels in GuHCl fraction were elevated in cases with CAA or *APOE4* (Table 4). There was no compelling evidence of interactions in CAA, sex and *APOE4* status among the different parameters (supplemental Table 3). When Thal amyloid phase was used as a variable instead of CAA status, neither the amounts of A β 40 and A β 42, nor the ratios of A β 40/A β 42 in any fraction were associated with Thal amyloid phase and sex in our AD cohort (data not shown).

Next, potential association between the levels of A β and apoE was assessed by linear regression models; apoE levels were positively correlated with both A β 40 ($R^2=0.100$, $p=0.001$) and A β 42 levels ($R^2=0.377$, $p<0.001$) in GuHCl fractions, but not in TBS (A β 40: $R^2=0.008$, $p=0.376$; A β 42: $R^2=0.001$, $p=0.709$) and TX (A β 40: $R^2=0.005$, $p=0.495$; A β 42: $R^2=0.011$, $p=0.301$) fractions. A β 42 levels were also positively correlated with A β 40 levels in TBS ($R^2=0.290$, $p<0.001$) and GuHCl fractions ($R^2=0.357$, $p<0.001$), but not in TX ($R^2=0.003$, $p=0.569$) fractions. ANCOVA demonstrated a strong interaction effect of CAA on insoluble apoE levels and insoluble A β 40 levels ($p<0.001$) (Fig. 5a), while the apoE levels correlated with insoluble A β 42 levels regardless of CAA status (Fig. 5b).

Discussion

CAA causes cerebrovascular lesions including intracerebral hemorrhage, cerebral ischemia and leukoencephalopathy. In the elderly, CAA pathology is often accompanied by psychiatric symptoms, such as personality change and depression [16, 18]. Currently, CAA is pathologically diagnosed by deposition and accumulation of amyloid peptides, mostly A β , in cerebral vessels [3, 37, 38, 48]. Recent findings have shown that A β in the brain interstitial fluid is eliminated through the lymphatic drainage pathway flowing from capillaries to cerebral arteries along the basement membranes of cerebrovasculature [4, 30]. Thus, the failure of this drainage pathway might contribute to CAA pathogenesis [20, 50]. The deposition of A β in vascular walls likely leads to their damage and subsequent degeneration, which compromises the integrity of the neurovascular units. In this study, we investigated CAA in a large cohort of postmortem AD brain samples. We unequivocally demonstrated a sex-dependent difference in CAA among those with AD, with men exhibiting higher CAA scores than women. Importantly, this correlation persisted even after adjusting for age, Braak stage, Thal amyloid phase and *APOE* genotype. Recently, an Oxford Project to Investigate Memory and Ageing (OPTIMA) study also reported higher CAA scores in men compared with women in AD ($n=154$), although it was not significant ($p=0.064$), potentially due to a relatively small sample size [13]. Sexual dimorphism has been observed not only in AD risk, but also in the pathologic indices [5, 10]. Among clinically diagnosed AD patients, more severe AD pathology including neuritic plaques, diffuse plaques, and neurofibrillary tangles was detected in postmortem brains from female cases than those from male cases [5]. In addition, women had more extensive senile plaque depositions throughout the brain compared to men at each early Braak stage I - III [10]. These results suggest that sex impacts pathogenic pathways for A β deposition as senile plaques in brain parenchyma and as CAA in cerebrovasculature. Interestingly, a sex-dependent difference has also been observed in the development of intracranial

atherosclerosis, where men are more susceptible than women between 30-60 years of age [35]. Stroke incidence is also higher in men than in women among those aged 45 to 84; however, the sex-dependent effect reverses in the oldest group (aged 85-94) [34]. Importantly, functions of the cerebral vasculature are affected by sex steroid hormones. For example, estrogen protects endothelial cells through diverse pathways by ameliorating mitochondrial functions and reducing reactive oxygen species [23]. Furthermore, chronic estrogen exposure has been shown to decrease cerebral vascular tone and increase cerebral blood flow, while testosterone has the opposite effects [23]. Thus, men may be at increased risk of chronic cerebrovascular damage compared with women due to a lack of estrogen-mediated protection. We propose that this damage may increase the risk of having severe CAA in AD.

Furthermore, accumulating evidence has revealed the strong contribution of *APOE4* to CAA development [13, 36, 39, 40]. Consistent with these data, our results also demonstrate that severity of CAA in AD is higher in those with *APOE4* in an allelic dose-dependent manner. *APOE4* has been shown to increase the risk of cognitive decline in healthy older women, but not men [1]. The elevated risk for the conversion of MCI to AD in *APOE4* carriers is also higher in women than men [1]. However, the difference between men and women in their association of *APOE4* with CAA was not evident in our cohort likely because of insufficient sample size for detecting a sex-dependent interaction. Further studies are desired to examine if there is a sex-dependent association of *APOE4* with AD and CAA pathogenesis. Although *APOE2* is protective against AD development, it correlates with an increased risk for CAA-related hemorrhage [7, 33]. However, the potential association of *APOE2* with CAA risk is somewhat controversial [32, 36] likely because the high risk associated with *APOE4* [36] and the low frequency of *APOE2* genotype making assessment relatively difficult. Our current study also did not detect a statistically significant effect of *APOE* $\epsilon 2/\epsilon 3$ genotype on average CAA score compared to *APOE* $\epsilon 3/\epsilon 3$ (supplemental Figure 1). Nonetheless, a better understanding on the contribution of *APOE2* to CAA risk and CAA-related cerebrovascular pathology is critical for addressing the roles of sex and apoE isoforms in these pathogenic conditions.

Whereas A β 42 is the major species that accumulates in senile plaques [29], we found that both soluble and insoluble levels of A β 40, but not A β 42, were increased in the temporal cortex from AD patients with severe CAA compared to those without CAA (Fig. 4), which is consistent with previous reports [17, 45]. Furthermore, the ratio of A β 40/A β 42 was elevated in AD brains with severe CAA. Since A β 40 is diffusible compared to A β 42, more A β 40 is predicted to traverse through cerebrovasculature than A β 42. Thus, increased levels of parenchymal A β 40 might promote its diffusion into perivascular regions, resulting in CAA formation. On the other hand, the ratio of A β 40/A β 42 was negatively associated with male sex despite the fact that CAA was exacerbated in male AD patients. Thus, increasing A β 40 levels and the A β 40/A β 42 ratio are not the only factors facilitating CAA formation during AD pathogenesis. In contrast, it is possible that cerebrovascular damage through CAA and/or CAA progression, *per se*, predominantly interferes with clearance of A β 40, which causes A β 40 accumulation in the brain parenchyma. The outcome of a current phase II clinical trial in probable CAA patients will assess the therapeutic efficacy of an A β 40-targeted immunotherapy using Ponezumab (PF-04360365) [24] ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier

number: NCT01821118), and will hopefully shed light on the pathogenic relationship between CAA and A β 40.

Additionally, we found that insoluble apoE levels were elevated in CAA cases, which were positively correlated with insoluble A β levels. Of note, there was a strong association between apoE levels and A β 40 levels for those with severe CAA, but not for those without CAA. The same pattern was not apparent for A β 42, with a similar degree of association between apoE levels and A β 40 levels for those with and without CAA. These results indicate that apoE may co-deposit with A β 40 in CAA, which could contribute to the pathogenic process. In AD cases without CAA, the interaction between apoE and A β 40 might not be as prevalent. It is also possible that the clearance pathways for A β 40 are more efficient in the absence of CAA, thereby lowering its chance to encounter apoE. Further studies are needed to determine if apoE promotes the aggregation of A β 40 or whether apoE is merely captured by A β 40 aggregates during CAA development.

APOE4 has been shown to be strongly associated with increased A β depositions in both senile plaques and CAA [40]. Given that apoE4 accelerates A β aggregation and suppresses A β clearance as compared to other apoE isoforms [22], the exacerbated effects of *APOE4* on CAA are also predicted to be mediated by A β . In a transgenic amyloid AD model with apoE4-targeted replacement (TR) background in which mice express human apoE4, the brain ratio of A β 40/A β 42 is elevated in soluble fractions prior to A β deposition compared to brain fractions from apoE3-TR mice, leading to exacerbated CAA formation in aged mice [15]. Since we found that *APOE4* is correlated with an increase in both detergent-soluble and -insoluble A β 40 levels, apoE4 may directly influence the metabolism of A β 40 by altering its clearance and/or brain distribution. In addition, *APOE4* is significantly associated with increased insoluble apoE levels. Thus, the binding properties of apoE4 may also be involved in the mechanism underlying the risk of CAA in *APOE4* carriers. On the other hand, *APOE4* may also contribute to CAA development through A β -independent pathways. For example, apoE4 negatively affects brain homeostasis by influencing neuronal functions, lipid/glucose metabolism, cerebrovascular system and neuroinflammation compared to apoE3 [8, 21]. In particular, reduced cerebral blood flow has been described in elderly *APOE4* carriers relative to non-carriers [14, 47]. Furthermore, apoE4-TR mice exhibit dysregulated blood-brain barrier integrity and reduced brain microcirculation compared to apoE2-TR and apoE3-TR mice [6]. Importantly, the detection of regional cerebral hypoperfusion through neuroimaging techniques can identify individuals at risk for AD since cerebral hypoperfusion precedes hypometabolism, cognitive decline and neurodegeneration in AD [11]. Thus, apoE4-related cerebrovascular dysfunction may trigger the pathogenesis of both AD and CAA. Since the cerebrovascular system critically mediates brain A β clearance [51], CAA-mediated disturbances may facilitate further A β deposition.

In summary, using a large cohort (n=428) of AD patients, our current study has shown that the majority (88.4%) of AD cases has concurrent CAA, where *APOE4* and sex significantly influence its severity. One limitation of our study is that capillary CAA was not specifically addressed. Because AD cases with capillary CAA are likely associated with *APOE4* to a greater extent than those lacking capillary CAA [46], future studies should be designed by addressing CAA types as well as its severity. CAA data was obtained from our databased

reports, which may have suffered from ‘evaluation drift’ over the years. The CAA data, however, was scored by a single neuropathologist (DWD) on the same microscope limiting variability to some extent. Our data further demonstrate that severe CAA in AD is more frequent in men than women, regardless of *APOE4* genotype. In addition, A β 40 levels and A β 40/A β 42 ratio in temporal cortex are elevated in CAA cases and *APOE4* carriers. Intriguingly, the association between apoE levels and A β 40 and A β 42 levels are differently influenced by CAA status. Despite increasing evidence that shows sex-dependent differences in AD [9], the interaction between *APOE* and sex has received limited attention in studies of CAA and cerebrovascular diseases. Future studies that focus on sex, apoE and the cerebrovascular system may discover new genetic or environmental factors for CAA that would aid in better understanding of the mechanisms underlying pathogenesis of AD and CAA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH grants RF1AG051504, R01AG027924, R01AG035355, R01AG046205, and P01 NS074969 (to G.B.), P50 AG016574 (to G.B. and R.C.P.), U01AG006786 and R01AG034676 (to R.C.P.), and R01NS094137 (to J.D.F), grants from Florida Department of Health Ed and Ethel Moore Alzheimer’s Disease Research Program (to G.B. [5AZ08] and M.E.M [6AZ01]), and a Scientist Development Grant from the American Heart Association (to T.K.). The authors would like to acknowledge the continuous commitment and teamwork offered by Amanda M. Liesinger, Linda G. Rousseau, Virginia R. Phillips, and Monica Castanedes-Casey for their dedicated efforts to Mayo Clinic Brain Bank.

References

1. Altmann A, Tian L, Henderson VW, Greicius MD. Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann Neurol*. 2014; 75:563–573. DOI: 10.1002/ana.24135 [PubMed: 24623176]
2. Alzheimer’s Association. 2015 Alzheimer’s Disease Facts and Figures. *Alzheimer’s & Dementia*. 2015; 11:332–384.
3. Attems J. Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms. *Acta Neuropathol*. 2005; 110:345–359. DOI: 10.1007/s00401-005-1074-9 [PubMed: 16170565]
4. Bakker EN, Bacsakai BJ, Arbel-Ornath M, Aldea R, Bedussi B, Morris AW, Weller RO, Carare RO. Lymphatic Clearance of the Brain: Perivascular, Paravascular and Significance for Neurodegenerative Diseases. *Cell Mol Neurobiol*. 2016; 10.1007/s10571-015-0273-8 [pii]. doi: 10.1007/s10571-015-0273-8
5. Barnes LL, Wilson RS, Bienias JL, Schneider JA, Evans DA, Bennett DA. Sex differences in the clinical manifestations of Alzheimer disease pathology. *Arch Gen Psychiatry*. 2005; 62:685–691. Doi 62/6/685 [pii]. DOI: 10.1001/archpsyc.62.6.685 [PubMed: 15939846]
6. Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, Holtzman DM, Betsholtz C, Armulik A, Sallstrom J, et al. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature*. 2012; 485:512–516. DOI: 10.1038/nature11087 [PubMed: 22622580]
7. Biffi A, Sonni A, Anderson CD, Kissela B, Jagiella JM, Schmidt H, Jimenez-Conde J, Hansen BM, Fernandez-Cadenas I, Cortellini L, et al. Variants at APOE influence risk of deep and lobar intracerebral hemorrhage. *Ann Neurol*. 2010; 68:934–943. DOI: 10.1002/ana.22134 [PubMed: 21061402]

8. Bu G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat Rev Neurosci.* 2009; 10:333–344. nrn2620 [pii]. DOI: 10.1038/nrn2620 [PubMed: 19339974]
9. Carter CL, Resnick EM, Mallampalli M, Kalbarczyk A. Sex and gender differences in Alzheimer's disease: recommendations for future research. *J Womens Health (Larchmt).* 2012; 21:1018–1023. DOI: 10.1089/jwh.2012.3789 [PubMed: 22917473]
10. Corder EH, Ghebremedhin E, Taylor MG, Thal DR, Ohm TG, Braak H. The biphasic relationship between regional brain senile plaque and neurofibrillary tangle distributions: modification by age, sex, and APOE polymorphism. *Ann N Y Acad Sci.* 2004; 1019:24–28. 1019/1/24 [pii]. DOI: 10.1196/annals.1297.005 [PubMed: 15246987]
11. de la Torre JC. Alzheimer disease as a vascular disorder: nosological evidence. *Stroke.* 2002; 33:1152–1162. [PubMed: 11935076]
12. Ellis RJ, Olichney JM, Thal LJ, Mirra SS, Morris JC, Beekly D, Heyman A. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. *Neurology.* 1996; 46:1592–1596. [PubMed: 8649554]
13. Esiri M, Chance S, Joachim C, Warden D, Smallwood A, Sloan C, Christie S, Wilcock G, Smith AD. Cerebral amyloid angiopathy, subcortical white matter disease and dementia: literature review and study in OPTIMA. *Brain Pathol.* 2015; 25:51–62. DOI: 10.1111/bpa.12221 [PubMed: 25521177]
14. Filippini N, Ebmeier KP, MacIntosh BJ, Trachtenberg AJ, Frisoni GB, Wilcock GK, Beckmann CF, Smith SM, Matthews PM, Mackay CE. Differential effects of the APOE genotype on brain function across the lifespan. *Neuroimage.* 2011; 54:602–610. S1053-8119(10)01076-1 [pii]. DOI: 10.1016/j.neuroimage.2010.08.009 [PubMed: 20705142]
15. Fryer JD, Simmons K, Parsadanian M, Bales KR, Paul SM, Sullivan PM, Holtzman DM. Human apolipoprotein E4 alters the amyloid-beta 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model. *J Neurosci.* 2005; 25:2803–2810. Doi 25/11/2803 [pii]. DOI: 10.1523/JNEUROSCI.5170-04.2005 [PubMed: 15772340]
16. Gahr M, Nowak DA, Connemann BJ, Schonfeldt-Lecuona C. Cerebral Amyloid Angiopathy--a disease with implications for neurology and psychiatry. *Brain Res.* 2013; 1519:19–30. S0006-8993(13)00634-3 [pii]. DOI: 10.1016/j.brainres.2013.04.052 [PubMed: 23651976]
17. Gravina SA, Ho L, Eckman CB, Long KE, Otvos L Jr. Younkin LH, Suzuki N, Younkin SG. Amyloid beta protein (A beta) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43). *J Biol Chem.* 1995; 270:7013–7016. [PubMed: 7706234]
18. Greenberg SM, Al-Shahi Salman R, Biessels GJ, van Buchem M, Cordonnier C, Lee JM, Montaner J, Schneider JA, Smith EE, Vernooij M, et al. Outcome markers for clinical trials in cerebral amyloid angiopathy. *Lancet Neurol.* 2014; 13:419–428. S1474-4422(14)70003-1 [pii]. DOI: 10.1016/S1474-4422(14)70003-1 [PubMed: 24581702]
19. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet.* 2009; 41:1088–1093. Doi ng.440 [pii]. DOI: 10.1038/ng.440 [PubMed: 19734902]
20. Herzig MC, Van Nostrand WE, Jucker M. Mechanism of cerebral beta-amyloid angiopathy: murine and cellular models. *Brain Pathol.* 2006; 16:40–54. [PubMed: 16612981]
21. Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. *Cell.* 2012; 148:1204–1222. S0092-8674(12)00278-4 [pii]. DOI: 10.1016/j.cell.2012.02.040 [PubMed: 22424230]
22. Kanekiyo T, Xu H, Bu G. ApoE and Abeta in Alzheimer's disease: accidental encounters or partners? *Neuron.* 2014; 81:740–754. S0896-6273(14)00099-3 [pii]. DOI: 10.1016/j.neuron.2014.01.045 [PubMed: 24559670]
23. Krause DN, Duckles SP, Pelligrino DA. Influence of sex steroid hormones on cerebrovascular function. *J Appl Physiol (1985).* 2006; 101:1252–1261. Doi 01095.2005 [pii]. DOI: 10.1152/jappphysiol.01095.2005 [PubMed: 16794020]
24. La Porte SL, Bollini SS, Lanz TA, Abdiche YN, Rusnak AS, Ho WH, Kobayashi D, Harrabi O, Pappas D, Mina EW, et al. Structural basis of C-terminal beta-amyloid peptide binding by the

- antibody ponesumab for the treatment of Alzheimer's disease. *J Mol Biol.* 2012; 421:525–536. S0022-2836(11)01292-7 [pii]. DOI: 10.1016/j.jmb.2011.11.047 [PubMed: 22197375]
25. Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet.* 2009; 41:1094–1099. Doi ng.439 [pii]. DOI: 10.1038/ng.439 [PubMed: 19734903]
 26. Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol.* 2013; 9:106–118. nrneurol.2012.263 [pii]. DOI: 10.1038/nrneurol.2012.263 [PubMed: 23296339]
 27. Maia LF, Mackenzie IR, Feldman HH. Clinical phenotypes of Cerebral Amyloid Angiopathy. *J Neurol Sci.* 2007; 257:23–30. Doi S0022-510X(07)00073-1 [pii]. DOI: 10.1016/j.jns.2007.01.054 [PubMed: 17341423]
 28. Mandybur TI. Cerebral amyloid angiopathy: the vascular pathology and complications. *J Neuropathol Exp Neurol.* 1986; 45:79–90. [PubMed: 3941328]
 29. Miller DL, Papayannopoulos IA, Styles J, Bobin SA, Lin YY, Biemann K, Iqbal K. Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. *Arch Biochem Biophys.* 1993; 301:41–52. Doi S0003-9861(83)71112-4 [pii]. DOI: 10.1006/abbi.1993.1112 [PubMed: 8442665]
 30. Morris AW, Sharp MM, Albargothy NJ, Fernandes R, Hawkes CA, Verma A, Weller RO, Carare RO. Vascular basement membranes as pathways for the passage of fluid into and out of the brain. *Acta Neuropathol.* 2016; 10.1007/s00401-016-1555-z [pii]. doi: 10.1007/s00401-016-1555-z
 31. Murray ME, Graff-Radford NR, Ross OA, Petersen RC, Duara R, Dickson DW. Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. *The Lancet Neurology.* 2011; 10:785–796. DOI: 10.1016/S1474-4422(11)70156-9 [PubMed: 21802369]
 32. Nelson PT, Pious NM, Jicha GA, Wilcock DM, Fardo DW, Estus S, Rebeck GW. APOE-epsilon2 and APOE-epsilon4 correlate with increased amyloid accumulation in cerebral vasculature. *J Neuropathol Exp Neurol.* 2013; 72:708–715. DOI: 10.1097/NEN.0b013e31829a25b9 [PubMed: 23771217]
 33. Nicoll JA, Burnett C, Love S, Graham DI, Dewar D, Ironside JW, Stewart J, Vinters HV. High frequency of apolipoprotein E epsilon 2 allele in hemorrhage due to cerebral amyloid angiopathy. *Ann Neurol.* 1997; 41:716–721. DOI: 10.1002/ana.410410607 [PubMed: 9189032]
 34. Petrea RE, Beiser AS, Seshadri S, Kelly-Hayes M, Kase CS, Wolf PA. Gender differences in stroke incidence and poststroke disability in the Framingham heart study. *Stroke.* 2009; 40:1032–1037. STROKEAHA.108.542894 [pii]. DOI: 10.1161/STROKEAHA.108.542894 [PubMed: 19211484]
 35. Pu Y, Liu L, Wang Y, Zou X, Pan Y, Soo Y, Leung T, Zhao X, Wong KS. Geographic and sex difference in the distribution of intracranial atherosclerosis in China. *Stroke.* 2013; 44:2109–2114. STROKEAHA.113.001522 [pii]. DOI: 10.1161/STROKEAHA.113.001522 [PubMed: 23760212]
 36. Rannikmae K, Kalaria RN, Greenberg SM, Chui HC, Schmitt FA, Samarasekera N, Al-Shahi Salman R, Sudlow CL. APOE associations with severe CAA-associated vasculopathic changes: collaborative meta-analysis. *J Neurol Neurosurg Psychiatry.* 2014; 85:300–305. jnnp-2013-306485 [pii]. DOI: 10.1136/jnnp-2013-306485 [PubMed: 24163429]
 37. Rensink AA, de Waal RM, Kremer B, Verbeek MM. Pathogenesis of cerebral amyloid angiopathy. *Brain Res Brain Res Rev.* 2003; 43:207–223. [PubMed: 14572915]
 38. Revesz T, Ghiso J, Lashley T, Plant G, Rostagno A, Frangione B, Holton JL. Cerebral amyloid angiopathies: a pathologic, biochemical, and genetic view. *J Neuropathol Exp Neurol.* 2003; 62:885–898. [PubMed: 14533778]
 39. Ringman JM, Sachs MC, Zhou Y, Monsell SE, Saver JL, Vinters HV. Clinical predictors of severe cerebral amyloid angiopathy and influence of APOE genotype in persons with pathologically verified Alzheimer disease. *JAMA Neurol.* 2014; 71:878–883. 1867706 [pii]. DOI: 10.1001/jamaneurol.2014.681 [PubMed: 24797962]
 40. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD. Increased amyloid beta-peptide deposition in cerebral cortex as a

- consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A*. 1993; 90:9649–9653. [PubMed: 8415756]
41. Selkoe DJ. Deciphering the genesis and fate of amyloid beta-protein yields novel therapies for Alzheimer disease. *J Clin Invest*. 2002; 110:1375–1381. DOI: 10.1172/JCI16783 [PubMed: 12438432]
 42. Shinohara M, Fujioka S, Murray ME, Wojtas A, Baker M, Rovelet-Lecrux A, Rademakers R, Das P, Parisi JE, Graff-Radford NR, et al. Regional distribution of synaptic markers and APP correlate with distinct clinicopathological features in sporadic and familial Alzheimer's disease. *Brain*. 2014; 137:1533–1549. awu046 [pii]. DOI: 10.1093/brain/awu046 [PubMed: 24625695]
 43. Shinohara M, Petersen RC, Dickson DW, Bu G. Brain regional correlation of amyloid-beta with synapses and apolipoprotein E in non-demented individuals: potential mechanisms underlying regional vulnerability to amyloid-beta accumulation. *Acta Neuropathol*. 2013; 125:535–547. DOI: 10.1007/s00401-013-1086-9 [PubMed: 23371365]
 44. Soontornniyomkij V, Lynch MD, Mermash S, Pomakian J, Badkoobehi H, Clare R, Vinters HV. Cerebral microinfarcts associated with severe cerebral beta-amyloid angiopathy. *Brain Pathol*. 2010; 20:459–467. BPA322 [pii]. DOI: 10.1111/j.1750-3639.2009.00322.x [PubMed: 19725828]
 45. Suzuki N, Iwatsubo T, Odaka A, Ishibashi Y, Kitada C, Ihara Y. High tissue content of soluble beta 1-40 is linked to cerebral amyloid angiopathy. *Am J Pathol*. 1994; 145:452–460. [PubMed: 8053502]
 46. Thal DR, Papassotiropoulos A, Saido TC, Griffin WS, Mrak RE, Kolsch H, Del Tredici K, Attems J, Ghebremedhin E. Capillary cerebral amyloid angiopathy identifies a distinct APOE epsilon4-associated subtype of sporadic Alzheimer's disease. *Acta Neuropathol*. 2010; 120:169–183. DOI: 10.1007/s00401-010-0707-9 [PubMed: 20535486]
 47. Thambisetty M, Beason-Held L, An Y, Kraut MA, Resnick SM. APOE epsilon4 genotype and longitudinal changes in cerebral blood flow in normal aging. *Arch Neurol*. 2010; 67:93–98. 67/1/93 [pii]. DOI: 10.1001/archneurol.2009.913 [PubMed: 20065135]
 48. Vinters HV. Cerebral amyloid angiopathy. A critical review. *Stroke*. 1987; 18:311–324. [PubMed: 3551211]
 49. Vinters HV, Gilbert JJ. Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. *Stroke*. 1983; 14:924–928. [PubMed: 6658996]
 50. Weller RO, Subash M, Preston SD, Mazanti I, Carare RO. Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. *Brain Pathol*. 2008; 18:253–266. BPA133 [pii]. DOI: 10.1111/j.1750-3639.2008.00133.x [PubMed: 18363936]
 51. Zlokovic BV. Cerebrovascular effects of apolipoprotein e: implications for Alzheimer disease. *JAMA Neurol*. 2013; 70:440–444. DOI: 10.1001/jamaneurol.2013.2152 [PubMed: 23400708]

Figure 1. Morphologies of CAA with different scores in AD brains

Cerebral amyloid angiopathy (CAA) was evaluated using thioflavin-S fluorescent microscopy. (a) Cases with no CAA were given a score of 0. Cases found to have CAA only in leptomeninges were given a score of 0.5. (b) Scattered amyloid deposition in both leptomeningeal and cortical vessels was given a score of 1. (c) Strong, circumferential amyloid deposition in some, but not all vessels was given a score of 2. (d) Widespread, strong amyloid deposition in leptomeningeal and cortical vessels was given a score of 3. (e) Cases with more severe CAA than score 3 were given a score of 4. (a'-e') Higher magnification views from each image are shown. Scale bar= 200 μ m.

Figure 2. CAA severity in different cortical regions

The distribution of CAA scores in inferior parietal cortex, middle frontal cortex, motor cortex, superior temporal cortex and visual cortex are shown as they relate to sex and *APOE4* status.

Figure 3. Sex- and *APOE4*-dependent differences in CAA severity

The overall averaged CAA scores from the five cortical regions were plotted as they relate to sex and *APOE4* status. Horizontal lines are medians and boxes are interquartile ranges (IQRs).

Figure 4. CAA-, Sex- and APOE4-dependent differences in Aβ40 levels and Aβ40/Aβ42 ratios
The levels of Aβ40 in TBS (a), TX (b) and GuHCl (c) fractions and Aβ40/Aβ42 ratio in GuHCl fraction (d) from temporal cortex of AD cases with or without CAA were plotted as they relate to sex and APOE4 status. Horizontal lines are medians and boxes are interquartile ranges (IQRs).

Figure 5. Associations between the levels of apoE and Aβ as a function of CAA

The regression plots for insoluble levels of apoE vs. Aβ40 (a) and apoE vs. Aβ42 (b) were shown.

Table 1

Subject characteristics of AD brain samples

	<i>APOE4</i> non-carrier		<i>APOE4</i> carrier	
	Female (N=103)	Male (N=68)	Female (N=143)	Male (N=114)
Age	81 (75, 88)	80 (73, 85)	83 (78, 88)	79 (73, 83)
Braak Stage				
<5	12 (12)	8 (12)	11 (8)	10 (9)
5-5.5	39 (38)	27 (40)	39 (27)	45 (39)
6	52 (50)	33 (49)	93 (65)	59 (52)
Thal Stage				
Missing	1	0	2	2
<4	11 (11)	6 (9)	8 (6)	11 (10)
4	8 (8)	7 (10)	11 (8)	10 (9)
5	83 (81)	55 (81)	122 (87)	91 (81)

Median and interquartile range (IQR) shown for age; number (%) shown for Braak and Thal stages.

Table 2Association of Sex and *APOE4* with CAA Score

Covariate	Subgroup	Sex and <i>APOE4</i> in model ^c		Additionally adjusting for age, Braak stage and Thal stage ^c	
		Effect (95% CI)	p-value ^d	Effect (95% CI)	p-value ^d
Male	All ^a	0.25 (0.10, 0.40)	<.001	0.27 (0.12, 0.42)	<.001
	<i>APOE4</i> ⁻ ^b	0.27 (0.03, 0.51)	0.027	0.29 (0.05, 0.53)	0.017
	<i>APOE4</i> ⁺ ^b	0.24 (0.05, 0.43)	0.014	0.26 (0.06, 0.46)	0.010
<i>APOE4</i> ⁺	All ^a	0.23 (0.08, 0.38)	0.003	0.22 (0.06, 0.37)	0.005
	Females ^b	0.24 (0.04, 0.44)	0.017	0.23 (0.03, 0.43)	0.024
	Males ^b	0.21 (-0.02, 0.45)	0.074	0.20 (-0.03, 0.43)	0.095
<i>APOE4</i> allelic dose	All ^a	0.30 (0.20, 0.41)	<.001	0.30 (0.19, 0.40)	<.001
	Females ^b	0.30 (0.16, 0.44)	<.001	0.29 (0.15, 0.43)	<.001
	Males ^b	0.30 (0.15, 0.46)	<.001	0.31 (0.15, 0.46)	<.001

^aResults from models with no interaction terms.^bResults from models with interaction of *APOE4* and sex.^cAge is included as a continuous variable, Braak stage as dichotomous (6 vs. <6), and Thal as dichotomous (5 vs. <5).^dWald p-value.

Table 3Levels of A β and apoE in temporal cortex from AD subjects with or without CAA

	Without CAA (N=39)	With CAA (N=60)
Sex; Male	17 (44)	32 (53)
<i>APOE4+</i>	21 (54)	42 (70)
Age; years	80 (76, 85)	80 (75, 85)
Braak Stage		
<5	4 (10)	3 (5)
5-5.5	14 (36)	24 (40)
6	21 (54)	33 (55)
Thal phase		
<4	2 (5)	4 (7)
4	3 (8)	7 (12)
5	34 (87)	49 (82)
A β 40 (ng/mg protein)		
TBS	0.26 (0.03)	1.43 (0.19)
TX	0.25 (0.03)	1.15 (0.14)
GuHCl	35.1 (9.8)	708.0 (108.4)
A β 42 (ng/mg protein)		
TBS	0.46 (0.11)	0.58 (0.07)
TX	5.16 (0.55)	3.92 (0.27)
GuHCl	687.6 (57.3)	839.3 (65.7)
A β 40/A β 42		
TBS	2.65 (1.01)	3.89 (0.83)
TX	0.09 (0.03)	0.35 (0.05)
GuHCl	0.06 (0.02)	0.74 (0.09)
ApoE (ng/mg protein)		
TBS	1.27 (0.08)	1.37 (0.07)
TX	1.59 (0.08)	1.74 (0.11)
GuHCl	381.4 (78.2)	585.3 (59.0)

Median and interquartile range (IQR) shown for age; number (%) shown for gender, *APOE4+*, Braak stage and, Thal phase; Mean (SEM) shown for A β and apoE levels.

Table 4Associations of CAA, sex, and *APOE4* with levels of A β and apoE in temporal cortex

	TBS		TX		GuHCl	
	Relative Level ^a (95% CI)	p-value ^b	Relative Level ^a (95% CI)	p-value ^b	Relative Level ^a (95% CI)	p-value ^b
A β 40						
CAA+	3.50 (2.45, 5.00)	<.001	3.64 (2.37, 5.59)	<.001	17.55 (10.17, 30.29)	<.001
Sex; Male	0.71 (0.50, 1.00)	0.053	0.77 (0.51, 1.16)	0.213	0.68 (0.40, 1.17)	0.161
<i>APOE4</i> +	1.71 (1.20, 2.46)	0.003	1.61 (1.06, 2.47)	0.027	3.99 (2.30, 6.92)	<.001
A β 42						
CAA+	0.88 (0.67, 1.17)	0.377	1.74 (0.99, 3.07)	0.053	1.22 (0.98, 1.51)	0.077
Sex; Male	1.10 (0.83, 1.45)	0.513	0.99 (0.57, 1.73)	0.978	1.15 (0.93, 1.42)	0.208
<i>APOE4</i> +	0.92 (0.69, 1.22)	0.575	1.26 (0.72, 2.22)	0.423	1.10 (0.88, 1.37)	0.387
A β 40/A β 42						
CAA+	3.97 (2.67, 5.92)	<.001	2.30 (1.43, 3.69)	<.001	14.43 (8.74, 23.83)	<.001
Sex; Male	0.64 (0.43, 0.96)	0.029	0.91 (0.57, 1.44)	0.676	0.59 (0.36, 0.97)	0.038
<i>APOE4</i> +	1.86 (1.24, 2.78)	0.003	1.13 (0.70, 1.81)	0.624	3.62 (2.18, 6.01)	<.001
ApoE						
CAA+	1.08 (0.94, 1.25)	0.275	1.07 (0.92, 1.24)	0.409	1.69 (1.15, 2.48)	0.007
Sex; Male	1.09 (0.95, 1.26)	0.231	1.15 (0.99, 1.34)	0.068	0.74 (0.51, 1.08)	0.119
<i>APOE4</i> +	0.80 (0.69, 0.93)	0.003	0.96 (0.83, 1.13)	0.649	1.69 (1.15, 2.49)	0.008

^aAnalyses use logarithm of biochemical measures as response variable in linear regression analyses including variables CAA group (severe versus none), sex (male vs. female) and *APOE4* (presence vs. absence) and also adjusting for age; estimated effects are exponentiated to provide effect expressed as a relative level or fold change.

^bWald p-value.