



## The APOE4 allele shows opposite sex bias in microbleeds and Alzheimer's disease of humans and mice



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### ABSTRACT

The apolipoprotein APOE4 allele confers greater risk of Alzheimer's disease (AD) for women than men, in conjunction with greater clinical deficits per unit of AD neuropathology (plaques, tangles). Cerebral microbleeds, which contribute to cognitive dysfunctions during AD, also show APOE4 excess, but sex-APOE allele interactions are not described. We report that elderly men diagnosed for mild cognitive impairment and AD showed a higher risk of cerebral cortex microbleeds with APOE4 allele dose effect in 2 clinical cohorts (ADNI and KIDS). Sex-APOE interactions were further analyzed in EFAD mice carrying human APOE alleles and familial AD genes (5XFAD<sup>+/+</sup>/human APOE<sup>+/+</sup>). At 7 months, E4FAD mice had cerebral cortex microbleeds with female excess, in contrast to humans. Cerebral amyloid angiopathy, plaques, and soluble A $\beta$  also showed female excess. Both the cerebral microbleeds and cerebral amyloid angiopathy increased in proportion to individual A $\beta$  load. In humans, the opposite sex bias of APOE4 allele for microbleeds versus the plaques and tangles is the first example of organ-specific, sex-linked APOE allele effects, and further shows AD as a uniquely human condition.

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### 1. Introduction

The APOE4 allele is the strongest heritable risk factor for sporadic Alzheimer's disease (AD), with sex-bias of >50% for women (Altmann et al., 2014; Farrer et al., 1997; Payami et al., 1996). The neuropathological load showed parallel sex and apolipoprotein E (APOE) differences in benchmark postmortem studies of AD brains, with female excess of senile plaques and of neurofibrillary tangles (NFT) (Barnes et al., 2005; Corder et al., 2004). Moreover, women

incurred 5-fold greater cognitive deficits per unit of brain  $\beta$ -amyloid ( $A\beta$ ) than men (Barnes et al., 2005). Other studies also show female excess vulnerability in brain aging, with 1%–1.5% faster brain atrophy in magnetic resonance imaging (MRI) studies of probable AD (Hua et al., 2010), and higher tau in cerebrospinal fluid of prodromal AD (mild cognitive impairment [MCI]) (Altmann et al., 2014), but also in cognitively healthy elderly (Damoiseaux et al., 2012). Correspondingly, women *APOE4* carriers had excess of  $A\beta$  plaque and NFT (Barnes et al., 2005; Corder et al., 2004) and more hippocampal atrophy in prodromal AD (Fleisher et al., 2005). Mouse transgenic models of AD with familial AD mutations (FAD) have shown consistent female excess of brain  $A\beta$  and cognitive deficits (Carroll et al., 2010; Dubal et al., 2012; Sturchler-Pierrat and Staufenbiel, 2000; Vest and Pike, 2013).

In contrast to brain  $A\beta$  and NFT, cerebral microbleeds (microhemorrhages) typically show male 50% excess, for example, in the Karolinska Imaging Dementia Study (KIDS) for AD (Shams et al., 2015) and the Amsterdam Dementia Cohort (Benedictus et al., 2015). Microbleeds, together with cerebral amyloid angiopathy (CAA) (Shams et al., 2015), are of emerging importance as a contributing factor to preclinical cognitive decline and as an additional clinical burden in AD (Meier et al., 2014; Reijmer et al., 2015). In cognitively normal elderly, the presence of cerebral cortex microbleeds was associated with lower resting-state cerebral blood flow and subtle cognitive deficits (Gregg et al., 2015). *APOE4* is also associated with cerebral microbleeds (Rannikmae et al., 2013; Yates et al., 2014) and CAA in leptomeningeal and cerebral cortex vessels (Rannikmae et al., 2013; Schmechel et al., 1993). Because microbleeds are associated with CAA (Premkumar et al., 1996; Yates et al., 2014), it is cogent to evaluate their association with sex. Some postmortem studies show a slight female excess of CAA (Lee and Stemmermann, 1978; Masuda et al., 1988) that was not seen in other samples (Allen et al., 2014; Gilbert and Vinters, 1983; Love et al., 2003). FAD mice develop CAA (Fryer et al., 2005) but sex differences and interactions of FAD with human *APOE* transgenes are not reported.

AD may have uniquely evolved in humans, because no FAD rodent or aging primate has clinical grade AD-like cognitive impairment with major neuron loss (Finch and Austad, 2015). Although aging great apes develop CAA (Gearing et al., 1994) and incur sporadic strokes (Jean et al., 2012; Rosen et al., 2008), no aging wild-type rodent has shown CAA or stroke (Sullivan et al., 2008). Wildtype rodents are famously resistant to diet-induced atherosclerosis, unless made hypercholesterolemic by lipoprotein gene knockout, for example, *APOE-KO* mice on high cholesterol diets developed aortic atheromas and intimal-media thickening (Smith et al., 2010). However, spontaneous cerebrovascular ischemic lesions are unknown in lab rodents even with induced hypercholesterolemia. Thus, 4 major pathologic changes associated with human brain aging and AD have not been reported for aging wildtype rodents: brain  $A\beta$ -amyloid deposits, major neurodegeneration or neuron loss, CAA, and cerebrovascular ischemic lesions. One clue may be in *APOE* differences by species.

Humans have a unique multiallele *APOE* system, whereas the primates and rodents examined so far have a single *APOE* isoform which resembles *APOE4* in some amino acid positions (Fullerton et al., 2000; McIntosh et al., 2012). Notably during aging, *APOE*-targeted replacement (TR) mice transgenic for human *APOE* by TR (knock-in) developed gross lobar hemorrhages by 18 months (41% of *APOE4-TR*; 8% of *APOE3-TR*) (Sullivan et al., 2008). Moreover, these hemorrhages were associated with classic CAA. The vascular thioflavin-positive fibrillar amyloid on wildtype background was unexpected because the endogenous murine  $A\beta$  has lower aggregability than human  $A\beta$  (Boyd-Kimball et al., 2004). The greater prevalence of CAA in *APOE4*- versus *E3-TR* is consistent with

findings from mice transgenic for the combination of FAD genes together human *APOE*, in which *APOE4* increased CAA and plaques above *APOE3* (Fryer et al., 2005). These studies did not examine sex differences.

To extend the correlations of  $A\beta$  with CAA for sex-*APOE* allele interactions in FAD mice and in human AD (see the previous paragraphs), we used the EFAD mice, which are 5XFAD<sup>+/+</sup>/human *APOE*<sup>+/+</sup> (*E3FAD*, *E4FAD*) (for derivation, see Youmans et al., 2012). Previous publications demonstrated that by immunochemistry, male EFAD mice accumulate significant extracellular  $A\beta$  in the subiculum and then deep layers of the frontal cortex from 2 to 6 months of age, with *E4FAD* > *E3FAD* (Youmans et al., 2012). In addition, soluble levels of  $A\beta_{42}$  and oligomeric  $A\beta$  ( $oA\beta$ ) in both the hippocampus and cortex are higher in *E4FAD* compared to *E3FAD* mice (Youmans et al., 2012). The early deposition of  $A\beta$  in EFAD mice gives an attractive model to study quantitative relationships between CAA and the  $A\beta$  load at young ages before changes in sex steroids. Cerebral cortex was analyzed at age 7 months for sex-*APOE* interactions in microbleeds and CAA, and for correlations with plaque  $A\beta$ . We also assayed oligomeric  $A\beta$ , a driver of neurodegeneration in AD (Klein et al., 2001; Mucke and Selkoe, 2012), which has not been reported for associations with sex or *APOE* in mouse models. Novel sex-*APOE* allele interactions were found in microvascular pathologies of brain aging that differ from AD, with species differences that may arise during sexual differentiation.

## 2. Material and methods

### 2.1. Subjects

Informed written consent was obtained from all participants at each site (Fig. 1, Tables 1–4).

#### 2.1.1. Alzheimer's Disease Neuroimaging Initiative (USA and Canada)

The Alzheimer's disease neuroimaging initiative (ADNI) database ([www.adni-info.org](http://www.adni-info.org)) represents a longitudinal study of MRI and PET, combined with biological markers and clinical assessment of MCI and early AD. More than 1500 community-dwelling subjects, aged 48–91 years, were recruited at 50 sites across the US and Canada. Microbleeds were assessed by MRI (<http://adni.loni.usc.edu/methods/documents/mri-protocols>) as hypointense lesions within the brain parenchyma <10 mm dia on the MRI sequence: GRE/T2\*; MRI field strength: 1.5 T/3.0 T (Greenberg et al., 2009; Wardlaw et al., 2013). Excluding *APOE2*, the sample was 658 subjects (166 cognitively normal, 402 with MCI, and 90 with early AD).

#### 2.1.2. Karolinska Imaging dementia study (KIDS) cohort (Sweden)

The KIDS database represents a cross-sectional cohort of consecutive patients undergoing memory investigation with an accompanying MRI scan for hemosiderin at Karolinska University Hospital, Stockholm, Sweden ( $n = 1572$ , 2006–2012, age 36–88 years). Informed consent: if the patient was too confused, consent was obtained from a legal guardian. Ethics approval was obtained from the regional ethics board, Stockholm. After excluding scans of insufficient quality and *APOE2*, the analysis cohort consisted of 448 subjects: 152 AD, 152 MCI, and 144 controls which were a group of patients with subjective cognitive impairment (not clinical grade) (Caracciolo et al., 2012). Diagnoses were set according to International Classification of Diseases, Tenth Revision by senior geriatricians at the memory clinic in multidisciplinary meetings that considered all diagnostic analyses. MRI scans were made on 1.5/3.0 T scanners with full protocols with axial hemosiderin sequences T2\* and/or SWI. Microbleeds were characterized by the microbleed anatomical rating scale

and identified as hypointense lesions <10 mm diameter (Gregoire et al., 2009; Wardlaw et al., 2013).

## 2.2. Animals

Procedures were approved by the USC Institutional Animal Care and Use Committee. EFAD mice are 5XFAD<sup>+/-</sup>/human APOE<sup>+/+</sup> (E3FAD, E4FAD) (for derivation, see Youmans et al., 2012). Breeding trios for EFAD mice were generously provided by Mary Jo LaDu (University of Illinois at Chicago). Mice were examined at age 6–7 months, when the female bias in behavioral deficits emerges in the NSE-APOE mouse (Raber et al., 1998). The C57BL/6Nj mice were used as the background mice strain. Two different cohorts of mice were used: one (4–5 mice/group) for microbleeds (Fig. 2) and one for CAA (6–7 mice/group) (Fig. 3) analysis. Both were analyzed for A $\beta$  load (Figs. 4 and 5).

## 2.3. Tissues

After euthanization by isoflurane anesthesia, mice were perfused transcardially with phosphate-buffered saline (PBS). Brains were hemisected and fixed in 4% paraformaldehyde/24 hours, immersed in sequential sucrose (10–20–30%, 24 hours each) and Optimal Cutting Temperature compound (OCT, Sakura, Torrance, CA), and frozen on dry ice. The other hemisphere was dissected and stored at –80 °C. All assays used observer-blinded protocols.

## 2.4. Immunohistochemistry

After sagittal sectioning 0.5–2 mm from midline (25  $\mu$ m), sections were stored at –80 °C. A $\beta$  amyloid was immunostained with 4G8 (residues 17–24 at N-terminal of APP; Covance, Princeton, NJ) (Rasool et al., 2013). Sections were immersed in 70% formic acid/5 minutes. Endogenous peroxidases were blocked by 3% H<sub>2</sub>O<sub>2</sub> and 10% methanol in Tris-buffered saline, 30 min/22 °C. Sections were permeabilized in 0.1% Triton X-100/15 minutes, blocked by 30 minutes incubation in Tris-buffered saline with 2% BSA (bovine serum albumin) and 0.1% Triton, followed by primary and then biotinylated antimouse secondary antibodies (1:250), and stained by ABC peroxidase and DAB (3,3'-diaminobenzidine) substrate (Vector, Burlingame, CA). For plaque quantification bright field microscopic images were converted to 8-bit grayscale, thresholded to highlight plaques and to diminish background signal with visual inspection to confirm each object as a plaque. The entire cortex section was evaluated for total plaque number and percentage of area covered (A $\beta$  load) by the “analyze particles” function of NIH ImageJ software.

Fibrillar A $\beta$  was stained by 0.1% thioflavin-S (Youmans et al., 2012). CAA was assessed using a primary antibody directed against A $\beta$  (Invitrogen, Grand Island, NY) with a standard avidin: biotinylated enzyme complex immunoperoxidase method and ABC Elite and diaminobenzidine kits (Vector Laboratories, CA), see the previous paragraphs. Nonoverlapping images of cerebral cortex (6–9 per immunostained section) were collected from 7–8 horizontal sections (40  $\mu$ m) per brain with an average of 65 images. Images were captured with an Olympus DP73 digital camera paired with cellSens software and a 10 $\times$  objective to include the entire medial-lateral span of the cortex in 1 image. NIH ImageJ 1.48 software was used to define the region of interest (ROI) within each image, which included all cortical layers. The white matter at the edge of the cortex was excluded and served as the medial boundary. Gray scale images were thresholded for discrimination of immunopositive and negative A $\beta$  zones. Total A $\beta$  load represents the percentage of pixels within the cerebrocortical ROI that showed

positive A $\beta$  immunoreactivity. To determine CAA load, A $\beta$ -immunoreactive vessels were identified morphologically. Identified vessels were analyzed only if they were visually distinct from cells with diameter >10  $\mu$ m and length of  $\geq$ 20  $\mu$ m. The entirety of each immunoreactive vessel within a section was outlined to generate a CAA ROI that was contained within the larger cerebrocortical ROI. The CAA load was calculated as the number of immunoreactive pixels within the CAA ROI and expressed as % the total pixels in the cerebrocortical ROI. Numbers of A $\beta$  immunoreactive vessels within each section were determined. CAA load from each section was averaged per brain.

Microbleeds were assayed by Prussian blue staining for hemosiderin (Sullivan et al., 2008), with counterstaining by nuclear fast red (Sigma-Aldrich). Hemosiderin deposits (puncta) were individually analyzed for number and area.

## 2.5. Enzyme-linked immunosorbent assay

A $\beta$  peptides were assayed in brain supernates (Jayaraman et al., 2012). Cerebral half-cortexes were homogenized in DEA buffer (0.2% diethylamine, 50 mm NaCl; 1 mL/200 mg tissue) with complete protease inhibitor cocktail (Sigma). After centrifugation (20,800 g  $\times$  30 minutes), supernatants were neutralized with Tris-HCl, pH 6.2 (“Tris extract”). Pellets were resuspended in 1% SDS-PBS (sodium dodecyl sulfate in phosphate buffered saline) and centrifuged (supernatants, “SDS extract”). Oligomeric A $\beta$  was assayed in Tris extracts by oligospecific MOAB-2 enzyme-linked immunosorbent assay (monoclonal antibody; Youmans et al., 2012) (Biosensis, Thebarton, Australia). A $\beta$ -38, -40, -42 fragments were assayed by Peptide Panel 1 (4G8) Kit V-PLEX (Meso Scale Discovery, Rockville, MD).

## 2.6. Statistical analyses

### 2.6.1. Mouse

Two-way analysis of variance and post hoc *t* test by GraphPad Prism version 5; *Human*: STATA 13 (StataCorp, College Station, TX) and SPSS 22.0. APOE allele association with number of microbleeds was analyzed by Kruskal-Wallis test. All analyses were observer blinded.

## 3. Results

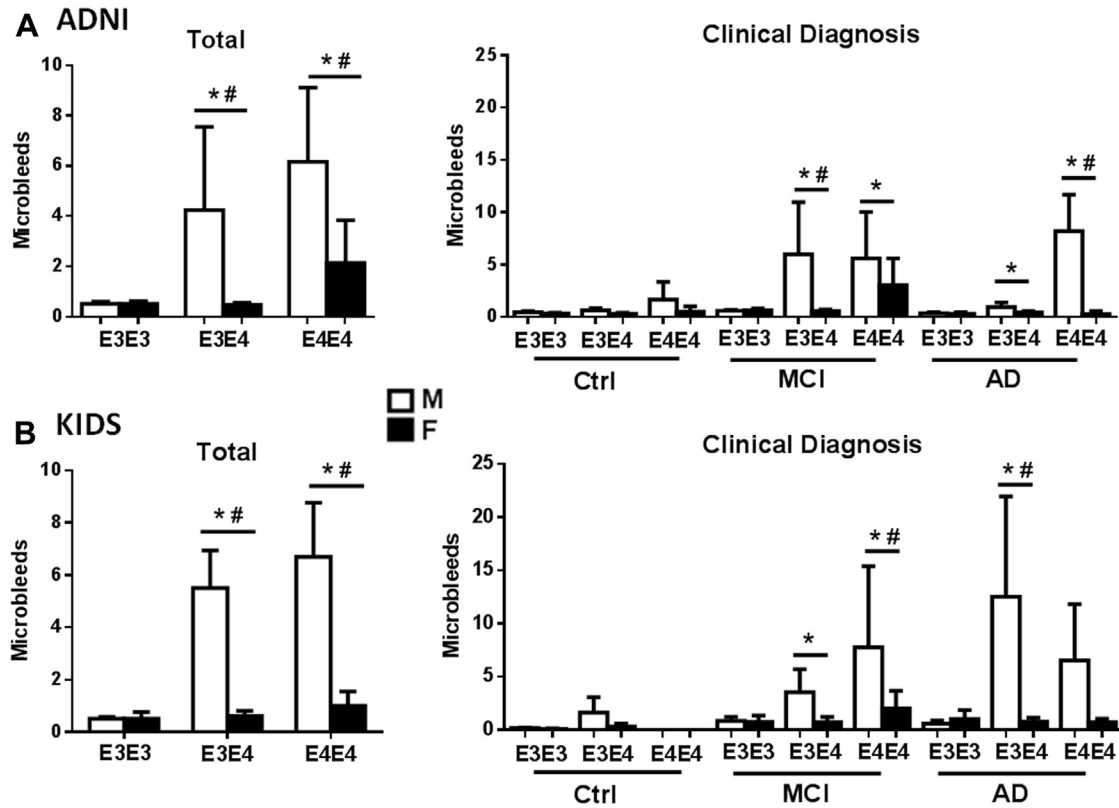
### 3.1. Microbleeds show opposite sex bias in human and mice

#### 3.1.1. Humans

Because sex-APOE interactions for human microbleeds have not been reported, we interrogated 2 clinical MRI studies of well-characterized elderly for microbleeds: the ADNI Study, a longitudinal MRI study of a large North American cohort, and the Karolinska Imaging Dementia Study (KIDS), a cross-sectional cohort study (Section 2.1; Tables 1 and 2). In both ADNI and KIDS, APOE3 homozygotes did not differ by sex in microbleeds. Both ADNI and KIDS male subjects carrying APOE4 had more microbleeds; for both sexes, APOE4 homozygotes had more microbleeds (Fig. 1A and B, left panels; Tables 3 and 4). Only men showed an APOE4 allele interaction with the microbleed burden (Table 4). After stratification by clinical diagnosis, only the MCI and AD subjects had significant male-APOE4 excess of microbleeds (Fig. 1A and B, right panels; Tables 3 and 4).

#### 3.1.2. Mice

In EFAD mice, cerebral cortex microbleeds had the opposite sex bias from humans. By Prussian blue histochemistry, females had 2-fold more microbleeds, with 5-fold greater total hemosiderin



**Fig. 1.** Human male *APOE4* carriers have more cerebral microbleeds. MRI analysis of ADNI and KIDS clinical cohorts showed male excess of microbleeds, increasing with *APOE4* allele dose. *APOE3* did not differ by sex in microbleed numbers. In both studies, a minority of subjects had microbleeds: ADNI, 35%; KIDS, 19%. Data are shown as total sample (left panel) and by diagnostic group (right) for mean number of microbleeds  $\pm$  95% CI. Tables 1–4 show statistical findings and subject numbers by diagnostic group and *APOE* allele. Both studies used a negative binomial regression model adjusted for age and a diagnostic group specified for each study. (A) ADNI, the Alzheimer Disease Neuroimaging Initiative, representing the USA and Canada. Total subjects (N = 658); clinical diagnosis: Ctrl, cognitively normal; MCI, mild cognitive impairment; and clinical AD. MRI sequence: GRE-T2\*; MRI field strength, 3T. (B) KIDS, Karolinska Imaging Dementia Study. Total subjects (N = 448); clinical diagnosis: Ctrl, subjective cognitive impairment (not clinical grade); MCI, and clinical AD; same MRI as ADNI. \**APOE* allele dose effect; #*APOE* allele-male sex interaction (see Tables 1–4 for details). Abbreviations: AD, Alzheimer Disease; *APOE*, apolipoprotein E; Ctrl, control group; F, female; M, male; MCI, mild cognitive impairment; MRI, magnetic resonance imaging.

(Fig. 2A–C). Only female EFAD mice showed *APOE4* allele effects for total hemosiderin (Fig. 2C).

We also analyzed for sex effects that may be independent of FAD genes. The EFAD non-carrier mice (human 5XFAD<sup>-/-</sup>/*APOE*<sup>+/+</sup>) without FAD transgenes in these groups also had a 2-fold female excess of microbleeds. However, their 20-fold lower levels did not

show significant *APOE* allele effects (Supplementary Fig. 1). Seven-month-old C57BL/6 wildtype mice from an independent sample also had cerebral microbleeds in both sexes at very low levels and of small size; these low levels did not allow resolution of possible sex differences (not shown).

### 3.2. EFAD associations of *A $\beta$* levels with microbleeds and CAA

Because microbleeds are associated with CAA in humans (Premkumar et al., 1996; Yates et al., 2014) and in mice (Zipfel et al., 2009), and because *A $\beta$*  peptide levels drove leptomeningeal CAA in mice above a threshold *A $\beta$*  level (Han et al., 2008; Zipfel et al., 2009), we evaluated effects of *APOE* alleles and sex on these relationships in EFAD mice.

**Table 1**  
Statistical analysis of human cohorts: demography

Parameters	ADNI (%)	KIDS (%)
Patients	658	448
Age, median, IQR		
Total	48–91, median 73 (IQR: 67–78)	36–88, median 62 (IQR: 57–66)
Control	56–90, median 74 (IQR: 70–79)	36–79, median 57 (IQR: 53–63)
MCI	48–89, median 72 (IQR: 66–77)	40–81, median 63 (IQR: 57–67)
AD	56–91, median 76 (IQR: 70–80)	50–88, median 64 (IQR: 60–72)
Male	363 (53)	183 (41)
Patients with microbleeds	228 (35)	88 (19)
<i>APOE</i> 3/3	335 (51)	206 (46)
<i>APOE</i> 3/4	249 (38)	174 (39)
<i>APOE</i> 4/4	74 (11)	68 (15)
Control	166 (25)	144 (32)
MCI	402 (61)	152 (34)
AD	90 (14)	152 (34)

Key: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; IQR, interquartile range; KIDS, Karolinska Imaging Dementia Study; MCI, mild cognitive impairment.

**Table 2**  
Statistical analysis of human cohorts: clinical diagnosis

<i>APOE</i> allele	ADNI			KIDS		
	E3/E3	E3/E4	E4/E4	E3/E3	E3/E4	E4/E4
Control (M/F)	58/51	22/30	3/2	26/47	21/36	2/4
MCI (M/F)	106/95	91/63	29/18	27/23	22/41	10/29
AD (M/F)	10/15	24/19	15/7	38/38	29/25	8/15

Key: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE*, apolipoprotein E; F, female; M, male; MCI, mild cognitive impairment.

**Table 3**  
Statistical analysis of human cohorts: sex differences

Male excess	ADNI		KIDS	
	Coeff	p-value	Coeff	p-value
Total	0.61	0.003	1.7	<0.001
Control			1.73	<0.001
MCI			1.12	<0.001
AD	0.9	0.07	2.02	<0.001

Nonsignificant values are not shown.

Key: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; Coeff, coefficient of regression analysis; MCI, mild cognitive impairment.

### 3.2.1. Cerebral amyloid angiopathy

In cerebral cortex, A $\beta$  immunoreactivity exhibited a patchy pattern within morphologically identified vessels (Fig. 3A). Thioflavin S staining for fibrillar amyloid showed a similar pattern characteristic of CAA (Fig. 3A). Female EFAD mice had more A $\beta$  positive vessels, with additive effects of APOE4 (Fig. 3B). The A $\beta$  load per vessel was higher in E4FAD, with no significant sex effect (Fig. 3C).

### 3.2.2. Plaque A $\beta$

In cerebral cortex, female EFAD mice had 2-fold more A $\beta$  as plaque deposits than males for both APOE alleles (Fig. 4A–C). Female sex and the APOE4 allele had additive effects for plaque number (Fig. 4B) and for A $\beta$  load (total immunoreactive area) (Fig. 4C). Plaque size differed 5-fold by sex, with no additive APOE allele effect (modal frequency size in  $\mu\text{m}^2$ : 127, male E3FAD; 146, male E4; 1020, female E3; 1320, female E4).

### 3.2.3. Soluble A $\beta$ peptides

Oligomeric A $\beta$ 42 was higher in Tris extracts of cerebral cortex (no detergents) for E4FAD mice (Fig. 4D), with effects of APOE4 but not sex. For total soluble A $\beta$  in Tris extracts, females of both APOE alleles had higher levels of A $\beta$ 38, -40, and -42, with the greatest female excess for A $\beta$ 40 by >7-fold (Fig. 4D). Sequential extraction of the initial Tris-extracted pellet with SDS-PBS also showed the highest A $\beta$  levels in female E4FAD (Fig. 4F). The A $\beta$ 42:40 ratios did not differ by sex or APOE allele (Fig. 4 legend).

### 3.2.4. Correlations of cerebrovascular pathology with A $\beta$

Most hemosiderin puncta (67%) resided within A $\beta$  immunopositive deposits (Fig. 5A), which represent a minority of all A $\beta$  plaques (15%). Some hemosiderin deposits (33%) were not embedded within immunodetectable A $\beta$ , which we designate as “naked microbleeds.”

**Table 4**  
Statistical analysis of human cohorts: APOE allele interactions

APOE allele	ADNI				KIDS			
	E3/E4		E4/E4		E3/E4		E4/E4	
	Coeff	p-value	Coeff	p-value	Coeff	p-value	Coeff	p-value
APOE allele dose*								
Total	1.4	0.003	2.4	<0.001	0.6	<0.001	0.8	<0.001
Control					2.26	<0.001		
MCI	1.7	<0.001	2.2	<0.001			1.2	<0.001
AD	1.2	0.04	3.1	<0.001	0.69	0.004		
APOE allele-male sex interaction#								
Total	1.6	<0.001	1.6	0.007	0.6	<0.001	0.8	0.02
Control					1.8	0.06		
MCI	1.9	0.001					1.2	0.04
AD			3.0	0.02	1.6	<0.001	0.7	<0.08

Nonsignificant values are not shown.

Key: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE, apolipoprotein E; Coeff, coefficient of regression analysis; MCI, mild cognitive impairment; \*APOE allele dose, Fig. 1; #APOE allele-male sex interaction, Fig. 1.

Only female EFAD mice showed a significant correlation of microbleed numbers with total A $\beta$  load in immunohistochemically defined plaques (Fig. 5B). The number of microbleeds was chosen for this correlation analysis in parallel with the human MRI studies (Fig. 1). Since the effect of sex was stronger than APOE alleles, data were combined for both APOE alleles. Besides immunohistochemically detected A $\beta$ , other A $\beta$  fractions also showed correlation with microbleeds, again restricted to females but with lower statistical strength, in rank order: A $\beta$  plaque number > PBS extracted A $\beta$ 42 > PBS extracted A $\beta$ 40 > oligomeric A $\beta$ 42 (Fig. 5 legend). A threshold level of A $\beta$  plaque load for a linear correlation with microbleeds was determined iteratively to be significant at  $\geq 1.70$  A $\beta$  units (Fig. 5 legend). The CAA load also strongly correlated with A $\beta$  plaque load for both APOE alleles, again only in females (Fig. 5C). Because different cohorts of mice were analyzed for microbleeds or CAA with A $\beta$ , it was not possible to examine correlations of CAA and microbleeds in this study.

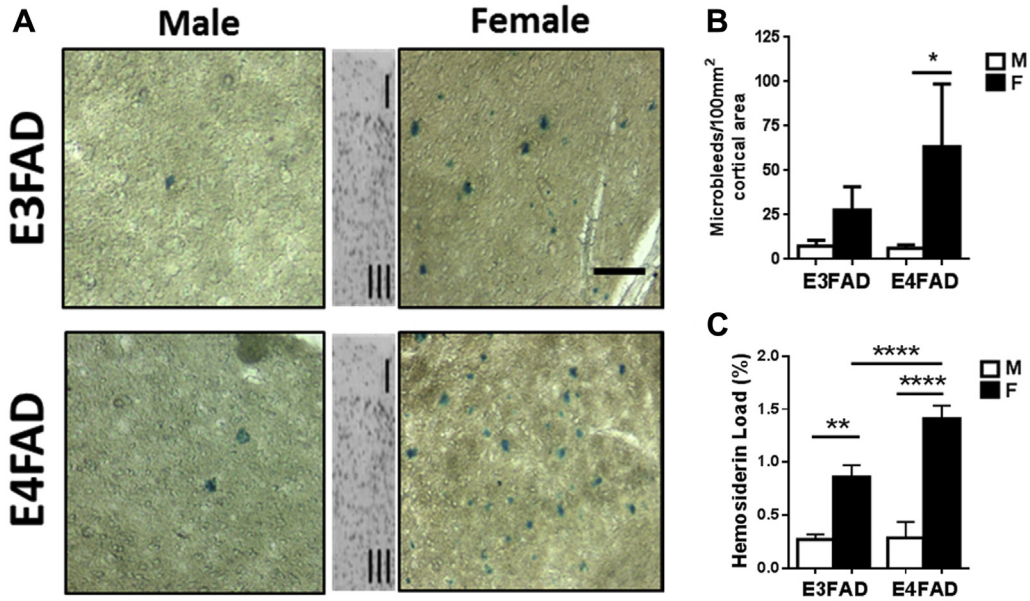
## 4. Discussion

We report novel sex-APOE allele interactions for cerebral microbleeds and for brain A $\beta$  amyloid levels with species differences. Microbleeds were analyzed from 2 clinical MRI imaging studies from different national populations (ADNI, U.S and Canada; KIDS, Sweden). In both ADNI and KIDS, elderly men with clinical grade symptoms of MCI or AD had 2-fold more microbleeds than women, with APOE4 allele effects. The control groups had much fewer microbleeds in both ADNI and KIDS and did not show sex or APOE allele associations. The male bias for microbleeds confirms prior findings (Benedictus et al., 2015; Shams et al., 2015). This sex bias is opposite to the >50% higher risk of AD in women carrying APOE4 (Altmann et al., 2014; Corder et al., 2004).

In contrast to these human findings, EFAD mice showed female APOE4 excess of microbleeds with correspondingly higher CAA and A $\beta$  levels. The strong correlation of CAA and microbleeds with the levels of A $\beta$  in EFAD female mice does not appear to generalize to human AD. Although APOE4 carriers have excess A $\beta$  (Altmann et al., 2014; Corder et al., 2004; Damoiseaux et al., 2012; Reznick et al., 2015), women have fewer microbleeds than men (Benedictus et al., 2015; Shams et al., 2015 this study). Thus, APOE4 has sex specificity for different causes of cognitive decline, with greater cerebrovascular contributions in men than in women. The neurobiological basis for these sex-APOE allele interactions and the species differences is unknown.

The excess of microbleeds in APOE4 men suggests its role in the human male excess of stroke (Go et al., 2013) and of vascular disease generally (Beltrán-Sanchez et al., 2015). Population-based studies (Ding et al., 2015; Jeerakathil et al., 2014; Romero et al., 2012; Sveinbjornsdottir et al., 2008) and memory clinic-based cohorts (Shams et al., 2015) also showed male bias of microbleeds but did not assess sex-APOE4 interactions. The differing sex bias between mice and humans for microbleeds could reflect the influence of hypertension interacting with CAA. Hypertension is more prevalent in men (Piper et al., 2014), whereas hypertension during aging is not reported for most mouse genotypes. Future studies could consider ethnic differences of sex-APOE allele interactions in cerebrovascular pathology, for example, Asians generally have a lower prevalence of CAA than Caucasians (Chen et al., 2010).

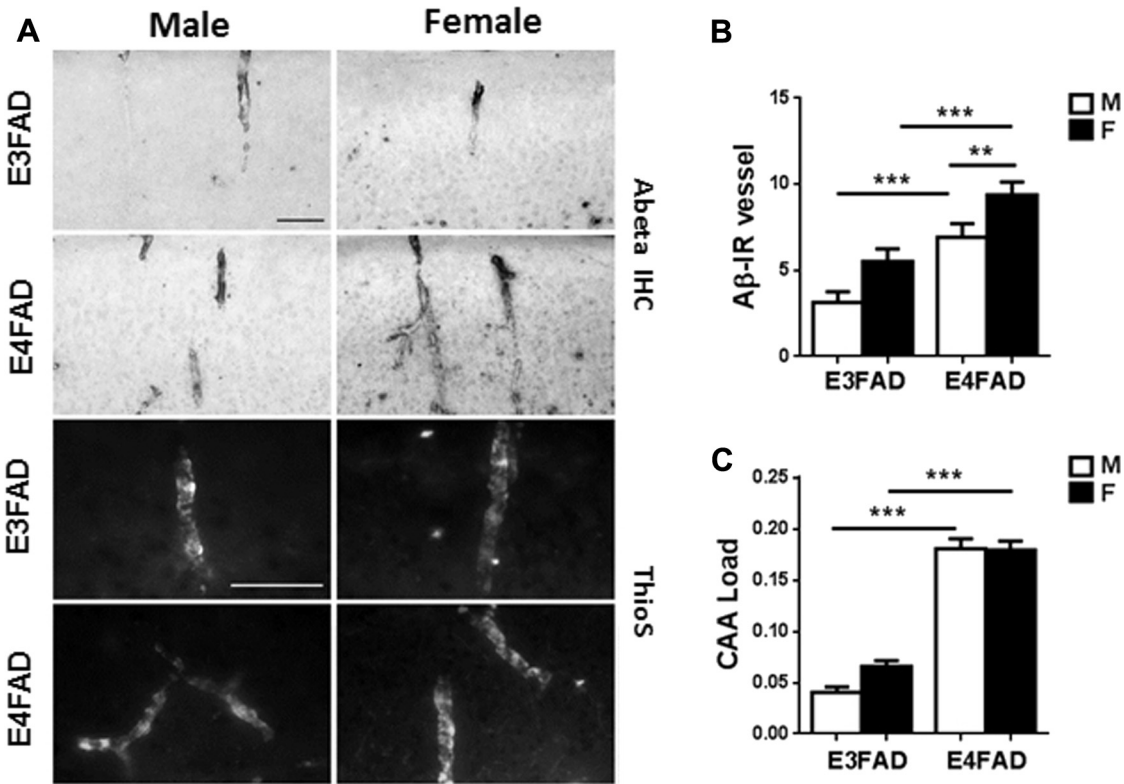
Cerebral atrophy also shows sex differences, but with fewer sex-APOE interactions. In ADNI, older women incurred 1% faster cerebral cortex atrophy than men, with greatest effect in MCI and in proportion to APOE4 allele dose (Hua et al., 2010), but with no sex-APOE4 interaction (Xue Hua, personal communication). Similarly, a study of healthy elderly did not find sex-APOE effects on gray



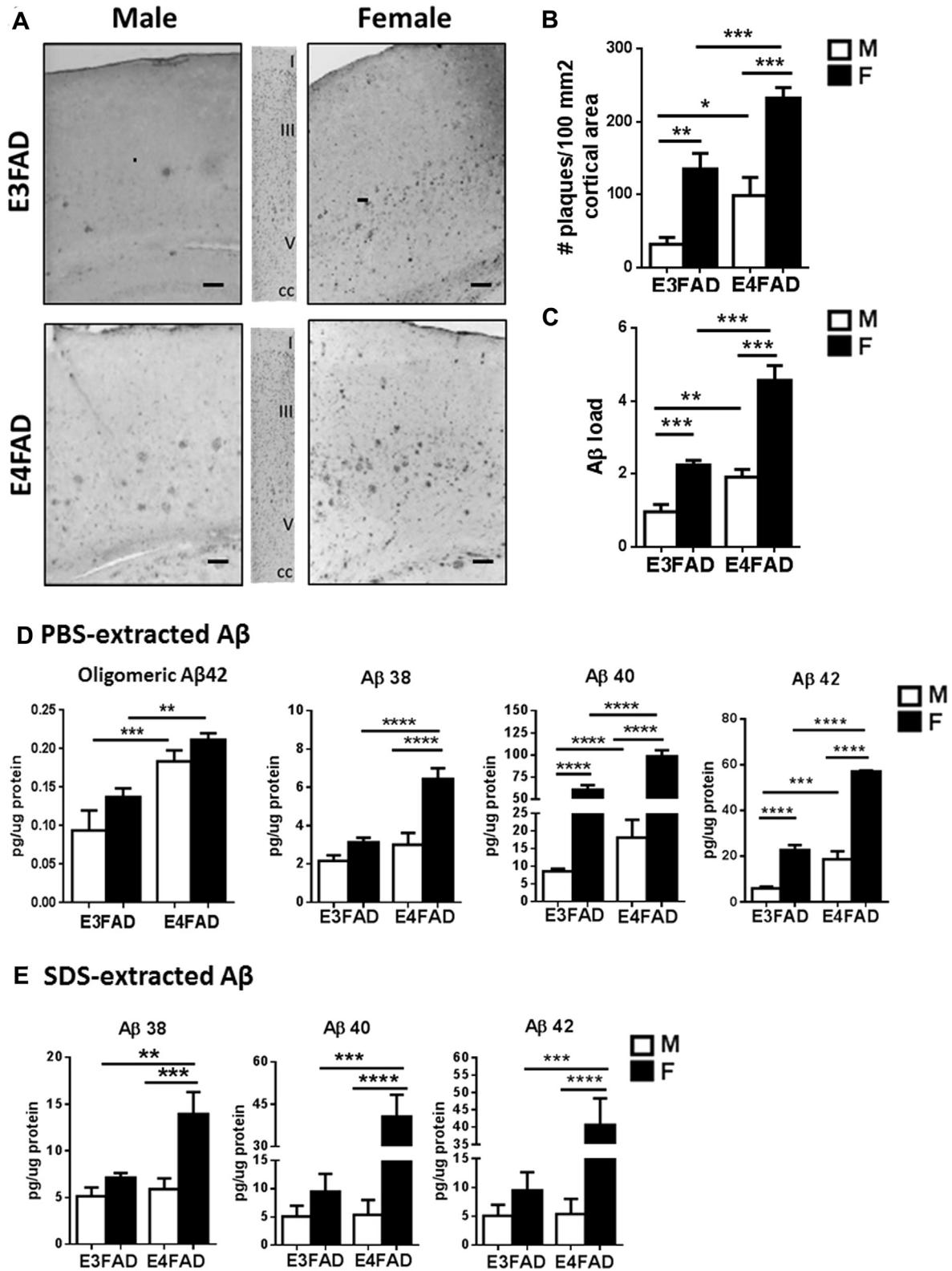
**Fig. 2.** Microbleeds in cerebral cortex of EFAD mice: female excess is additive with *APOE4*. (A) Microbleeds visualized as extravasated hemosiderin by Prussian blue histochemistry in cerebral cortex sagittal sections (0.5–2 mm lateral from midline; counterstained by nuclear fast red; scale bar 50  $\mu$ m). The inset shows cortical layers I and III. (B) Number of microbleeds per 100 mm<sup>2</sup> of cortical area for EFAD mice. Female excess was observed in both EFAD mice. (C) Hemosiderin load (total Prussian blue area, %) showed female excess and additivity with *APOE4* in EFAD. \**p* < 0.05; \*\**p* < 0.01; \*\*\*\**p* < 0.001. Male microbleeds did not differ by *APOE* allele. Abbreviations: *APOE*, apolipoprotein E; F, female; M, male. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

matter volume, despite strong sex-*APOE* interactions in functional brain connectivity (Damoiseaux et al., 2012). These sex differences also imply mechanisms of neuronal atrophy that are independent of focal vascular pathology.

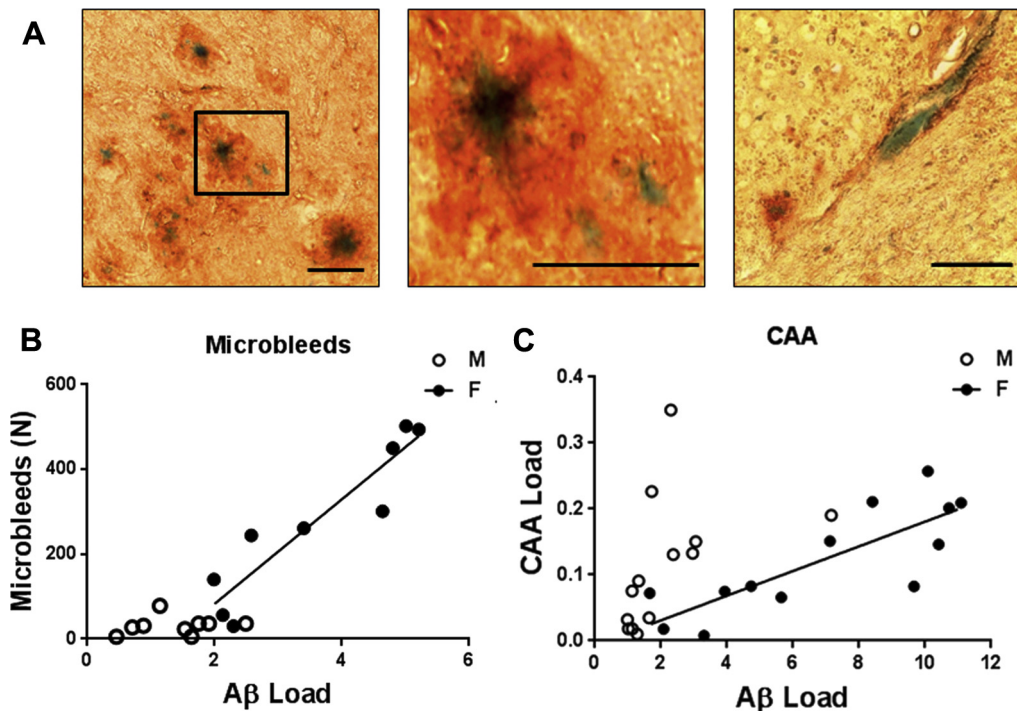
The mouse models showed new cerebrovascular features. Two transgenic *APOE* models, EFAD and FAD non-carriers with human *APOE* developed cortical microbleeds with female bias in 100% of the mice by 7 months. In contrast, only a minority of elderly human



**Fig. 3.** Cerebrovascular amyloid in EFAD mice shows female excess. (A) Cortical vessels immunopositive for  $A\beta$  (upper panels) and for thioflavin-S (lower panels) in EFAD mice. Scale bars: 50  $\mu$ m. (B) The number of  $A\beta$ -immunopositive ( $A\beta$ -IR) vessels was higher in females, with additive effects of *APOE4*. (C) CAA load of  $A\beta$  within vessels was higher in *E4FAD* mice but did not differ by sex. Mean  $\pm$  SEM, *N* = 6–8/group, \*\**p* < 0.01; \*\*\**p* < 0.005. Abbreviations:  $A\beta$ , amyloid beta; CAA, cerebral amyloid angiopathy; F, female; M, male; SEM, standard error mean.



**Fig. 4.** Levels of plaque and soluble Aβ show female excess in EFAD mice. (A) Cerebral cortex sagittal sections (0.5–2 mm lateral from midline) were immunostained for Aβ (4G8 antibody); middle panels, Cresyl violet histochemistry showing cortical layers; scale bar, 100 μm. (B) Number of plaques per 100 mm<sup>2</sup> of cortical area (C) Aβ load, total plaque Aβ immunostaining. The subiculum had similar female and APOE4 excess Aβ plaque (not shown). The sex-APOE interaction was significant for Aβ load ( $p = 0.02$ ). (D and E) Soluble Aβ peptides in cerebral cortex. Oligomeric Aβ42 in PBS extract (oligospecific ELISA) differed by APOE allele, but not by sex (MOAB-1 ELISA). Total Aβ peptides (multiplex ELISA) showed female excess, with additive effects for APOE4 for Aβ38, Aβ40, and Aβ42. PBS extracts had consistently 2-fold more Aβ40 than Aβ42 (Aβ40:42 ratio male: E3, 1.5 ± 0.1, E4, 1.7 ± 0.5; female: E3, 2.6 ± 0.4, E4, 1.6 ± 0.1), whereas the ratio was closer to 1 in SDS extracts (male: E3, 1.5 ± 0.1, E4, 1.7 ± 0.5; female: E3, 2.6 ± 0.4, E4, 1.6 ± 0.1). Sex-APOE allele interactions were significant for total soluble Aβ levels in both the PBS (Aβ 38,  $p < 0.01$ ; Aβ 40,  $p < 0.01$ ; Aβ 42,  $p = 0.001$ ) and SDS extractions (Aβ 40,  $p < 0.005$ ; Aβ 42  $p < 0.01$ ). Mean ± SEM, N = 6–8/group. \* $p < 0.05$ ; \*\* $p < 0.01$ , \*\*\* $p < 0.005$ , \*\*\*\* $p < 0.001$ . Abbreviations: APOE, apolipoprotein E; ELISA, enzyme-linked immunosorbent assay; F, female; M, male; PBS, phosphate-buffered saline; SEM, standard error mean.



**Fig. 5.** A $\beta$  total load correlated with microbleeds and CAA only in EFAD females. (A) Cerebral cortex histochemistry costaining for A $\beta$  (4G8 antibody, brown) and microbleeds (Prussian Blue, hem siderin); these images show the diverse morphology of hemosiderin cores within A $\beta$  deposits, scale bars, 50  $\mu$ m. Middle panel shows particular from left panel (black box). (B) The number of microbleeds correlated most strongly with total A $\beta$  plaque load for female EFAD mice, but not males, in rank order of total plaque A $\beta$  load (Male,  $r^2 = 0.09$ ,  $p = 0.3$ ; Female,  $r^2 = 0.89$ ,  $p < 0.001$ ) >PBS-extracted A $\beta$ 42 (Male,  $r^2 = 0.002$ ,  $p = 0.9$ ; Female,  $r^2 = 0.64$ ,  $p < 0.01$ ) and PBS extracted A $\beta$ 40 (Male,  $r^2 = 0.002$ ,  $p = 0.9$ ; Female,  $r^2 = 0.47$ ,  $p < 0.05$ ), >oligomeric A $\beta$ 42 (Male,  $r^2 = 0.003$ ,  $p = 0.9$ ; Female,  $r^2 = 0.27$ ,  $p = 0.15$ ). The threshold level of total A $\beta$  for linear correlation with microbleed numbers was determined iteratively: A $\beta \geq 1.70$ ,  $t = 8.6294$ ,  $df = 10$ ,  $p = 6.03e-06$ ; A $\beta < 1.70$ ,  $t = 0.4536$ ,  $df = 5$ ,  $p = 0.6692$ . (C) CAA load correlated with total A $\beta$  load in females, but not males (Male,  $r^2 = 0.22$ ,  $p = 0.1$ ; Female,  $r^2 = 0.75$ ,  $p < 0.001$ ). CAA load represents 1.8% of the total A $\beta$  load in females and 5.3% in males. Removal of one male outlier in the A $\beta$  plaque load yields a significant correlation ( $p = 0.05$ ); nonetheless, the resulting  $r^2$  value for males (0.34) would still be less than the females (0.75). Abbreviations: A $\beta$ , amyloid beta; CAA, cerebral amyloid angiopathy; F, female; M, male; PBS, phosphate-buffered saline.

patients showed microbleeds with male bias (35% ADNI; 18.5% KIDS; Fig. 1 legend). Female EFAD mice showed strong correlations of microbleeds with the total A $\beta$  load above an A $\beta$  threshold level (Fig. 5). These A $\beta$ -microbleed correlations extend those for single FAD gene transgenic mice with endogenous murine APOE, in which vascular A $\beta$  levels in leptomeningeal arteries correlated with impaired vasodilation (sex unspecified) (Han et al., 2008; Zipfel et al., 2009). These authors hypothesized that increasing microvascular A $\beta$  levels above a threshold can drive a pathological progression, from impaired dilation to microaneurysms and white matter lesions (Dumas et al., 2012; Peca et al., 2013). Future human in vivo imaging studies could evaluate possible A $\beta$  thresholds for microbleeds.

Intriguingly, wildtype C57BL/6 mice, the background “wildtype” strain, also developed microbleeds within the lower range of APOE-TR, confirming Liu et al. (2014). With human transgenic A $\beta$  at high levels, the EFAD mice showed a 20-fold increase of microbleeds. Because a subgroup of 18 months and older APOE-TR mice developed gross cerebral hemorrhages in association with thioflavin-positive CAA (Sullivan et al., 2008), we conclude that the endogenous murine A $\beta$  is permissive for cerebrovascular pathology in the presence of human APOE. (The FAD non-carriers with human APOE in the present studies are not identical with the original APOE-TR of Sullivan et al., 1997 because of their derivation via backcrossing with the 5XFAD which is on a different background strain). In the present FAD non-carriers with human APOE mice aged 7 months, we did not detect amyloid by 4G8 immunostaining or thioflavin histochemistry (not shown). Analysis of a full range of ages in FAD carriers and non-carriers with human APOE mice may

reveal transitional stages from microbleeds to gross hemorrhages and the onset age of sex-APOE allele interactions. The rapid development of A $\beta$  deposits and CAA in EFAD mice by 3–7 months gives a model for A $\beta$ -driven changes, distinct from neuro-inflammatory changes that arise during middle-age in healthy mice.

Most microbleeds in EFAD mice were colocalized within A $\beta$  deposits, also seen in another FAD model by dye visualization of blood leaks (Tanifum et al., 2014). The correlations of microbleed numbers and of the CAA load with human A $\beta$  suggest that increased A $\beta$  levels drive cerebrovascular degeneration with blood-brain barrier leakage. Moreover, extravasated blood can induce A $\beta$  deposition. We observed that one-third of microbleeds did not colocalize with immunohistochemically defined A $\beta$  deposits, which we designate as “naked microbleeds.” Because trace bleeds from a fine needle can induce A $\beta$  deposits in FAD mice (Chuang et al., 2012), we hypothesize that naked microbleeds may initiate local A $\beta$  plaque genesis. Thus, the progressive leakiness of the blood-brain barrier in normal human aging (Montagne et al., 2015) which is increased by APOE4 (Bell et al., 2012) could contribute to preclinical A $\beta$ .

CAA is incompletely documented for sex-APOE interactions. This gap may reflect different operational definitions for sporadic CAA, as a pathological entity, a clinical syndrome (e.g., symptomatic intracerebral hemorrhage, cognitive impairment), or as a neuro-imaging phenotype of small vessel disease markers. APOE4 associations with CAA as reported (Berg et al., 1998; Nelson et al., 2013; Olichney et al., 2000; Premkumar et al., 1996; Walker et al., 2000; Yip et al., 2005), however, were not replicated by others (Love



et al., 2003; Xu et al., 2003). In EFAD mice, CAA showed independent effects of sex and *APOE4*. The stronger correlation of total cortical A $\beta$  and CAA loads in females more than males of both *APOE* genotypes was paralleled by correlations of microbleeds with total A $\beta$  (Fig. 5). The sex-specific correlation of both microbleeds and CAA with total A $\beta$  load implies causal links to the female excess A $\beta$  in human AD and in FAD mice. We note several caveats. By identifying vessels based on morphological appearance, we may have missed smaller A $\beta$ -containing vessels, thus underestimating total CAA. We also note a potential issue in interpreting findings from ADNI and KIDS groups of postmenopausal women with much lower sex steroid levels than EFAD mice at age 7 months before major decreases of sex steroids. However, young E3FAD and E4FAD female mice showed divergent A $\beta$  responses to estrogen (Kunzler et al., 2014).

Could developmental variations of sex steroids alter the penetrance of *APOE4* in AD risk? In FAD mice, blood levels of sex steroids influence neurodegenerative changes (Carroll et al., 2010; Rosario et al., 2012). Besides these “activating” effects, sex steroids have “organizational” effects on brain development (Arnold, 2014; Gorski, 1979) that extend to A $\beta$  deposits. As shown by the Pike lab, the masculinization of neonatal female FAD mice by testosterone decreased adult brain A $\beta$ ; conversely, demasculinization of male neonates with flutamide increased adult A $\beta$  (Carroll et al., 2010). Early sex differences include the greater vulnerability of neonatal male mouse neurons to hypoxia in primary culture (Fairbanks et al., 2013). In human congenital adrenal hyperplasia syndrome, fetal exposure to excess androgens alters sex-linked behaviors (Hines et al., 2015) and thus might alter sex differences in CAA and AD. Cerebral arteries may also be sensitive to organizational effects of sex steroids, for example, aneurysms, which are male biased in humans and mice, can be induced in female mice by neonatal androgenization (Zhang et al., 2012). While sex differences in response to stroke in adult mice are sensitive to sex steroid levels independently of the sex chromosome complement (Manwani et al., 2014), sex differences in cerebral vascular biology are largely unexplored.

Sex-*APOE4* interactions may be relevant to AD as a human-specific disease (Finch and Austad, 2015) because only humans have *APOE* isoforms with differing lipid binding characteristics (Fullerton et al., 2000; McIntosh et al., 2012). The *APOE4* allele was present in earlier *Homo* at least 600,000 years ago, while *APOE3* emerged about 225,000 years ago (Fullerton et al., 2000). The persistence of *APOE4* in modern populations, despite its later costs to lifespan and brain aging, may be due to benefits to young ages. *APOE4* women have higher luteal phase blood progesterone (Jasienska et al., 2015), which may enhance fertility. Moreover, *APOE4* may be adaptive in immunity by higher cytokine responses (Finch and Morgan, 2007) and in pathogen resistance (Azevedo et al., 2014; Finch and Martin, 2015; Rougeron et al., 2013).

Species differences in CAA and microbleeds may involve both APP and *APOE*. Mice and humans differ in A $\beta$  sequence at 3 sites, which decrease aggregation and cytotoxicity (Boyd-Kimball et al., 2004; De Strooper et al., 1995). Because brain amyloid fibrils are unknown in aging wildtype mice, their presence in *APOE*-TR mice by 18 months (Sullivan et al., 2008) implies unknown interactions of human *APOE* with endogenous murine APP, possibly through allele-specific affinity binding to APP that modulates A $\beta$  production (Theendakara et al., 2013).

## 5. Conclusion

For both humans and mice, *APOE4* interacted with sex differences of microbleeds and A $\beta$  levels. Comparison of human brain aging with mouse models reveals major gaps in our understanding

of sex differences across species: aging men incur several-fold more microbleeds than women, opposite to male EFAD mice which had several-fold fewer. For CAA, the EFAD mice showed modest female excess in prevalence of A $\beta$ -positive vessels, agreeing with some postmortem human studies. Because the mouse sex differences in brain A $\beta$  and in aortic aneurysms are sensitive to organizational effects of steroids, we suggest that outcomes of cerebrovascular aging may also be shaped by sex steroids during development. This could be evaluated with postmortem brain studies of women exposed to congenital adrenal hyperplasia who show partial masculinization of behavior. Meanwhile, we need further analysis of sex-*APOE* interactions for CAA. The NIH directive of May 2014 to include both sexes in preclinical studies should soon expand the small data base on sex differences in brain aging. The greater risk of male *APOE4* carriers for microbleeds may be a target for genotype-informed therapy to reduce this contribution to cognitive decline.

## Disclosure statement

The authors have no conflicts of interest to disclose.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2015.10.010>.

## References

- Allen, N., Robinson, A.C., Snowden, J., Davidson, Y.S., Mann, D.M., 2014. Patterns of cerebral amyloid angiopathy define histopathological phenotypes in Alzheimer's disease. *Neuropathol. Appl. Neurobiol.* 40, 136–148.
- Altmann, A., Tian, L., Henderson, V.W., Greicius, M.D., Alzheimer's Disease Neuroimaging Initiative Investigators, 2014. Sex modifies the *APOE*-related risk of developing Alzheimer disease. *Ann. Neurol.* 75, 563–573.
- Arnold, A.P., 2014. Conceptual frameworks and mouse models for studying sex differences in physiology and disease: why compensation changes the game. *Exp. Neurol.* 259, 2–9.

- Azevedo, O.G., Bolick, D.T., Roche, J.K., Pinkerton, R.F., Lima, A.A., Vitek, M.P., Warren, C.A., Oriá, R.B., Guerrant, R.L., 2014. Apolipoprotein E plays a key role against cryptosporidial infection in transgenic undernourished mice. *PLoS One* 9, e89562.
- Barnes, L.L., Wilson, R.S., Bienias, J.L., Schneider, J.A., Evans, D.A., Bennett, D.A., 2005. Sex differences in the clinical manifestations of Alzheimer disease pathology. *Arch. Gen. Psychiatry* 62, 685–691.
- Bell, R.D., Winkler, E.A., Singh, I., Sagare, A.P., Deane, R., Wu, Z., Holtzman, D.M., et al., 2012. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* 485, 512–516.
- Beltrán-Sánchez, H., Finch, C.E., Crimmins, E.M., 2015. The 20<sup>th</sup> Century surge of excess adult male mortality. *Proc. Natl. Acad. Sci. U. S. A.* 112, 8993–8998.
- Benedictus, M.R., Prins, N.D., Goos, J.D., Scheltens, P., Barkhof, F., van der Flier, W.M., 2015. Microbleeds, Mortality, and Stroke in Alzheimer Disease: The MISTRAL Study. *JAMA Neurol.* 72, 539–545.
- Berg, L., McKeel Jr., D.W., Miller, J.P., Storandt, M., Rubin, E.H., Morris, J.C., Baty, J., Coats, M., Norton, J., Goate, A.M., Price, J.L., Gearing, M., Mirra, S.S., Saunders, A.M., 1998. Clinicopathologic studies in cognitively healthy aging and Alzheimer's disease: relation of histologic markers to dementia severity, age, sex, and apolipoprotein E genotype. *Arch. Neurol.* 55, 326–335.
- Boyd-Kimball, D., Sultana, R., Mohammad-Abdul, H., Butterfield, D.A., 2004. Rodent Abeta(1–42) exhibits oxidative stress properties similar to those of human Abeta(1–42): implications for proposed mechanisms of toxicity. *J. Alzheimers Dis.* 16, 515–525.
- Caracciolo, B., Gatz, M., Xu, W., Pedersen, N.L., Fratiglioni, L., 2012. Differential distribution of subjective and objective cognitive impairment in the population: a nation-wide twin-study. *J. Alzheimers Dis.* 29, 393–403.
- Carroll, J.C., Rosario, E.R., Kreimer, S., Villamagna, A., Gentzsch, E., Stanczyk, F.Z., Pike, C.J., 2010. Sex differences in beta-amyloid accumulation in 3xTg-AD mice: role of neonatal sex steroid hormone exposure. *Brain Res.* 1366, 233–245.
- Chen, Y.W., Lee, M.J., Smith, E.E., 2010. Cerebral amyloid angiopathy in East and West. *Int. J. Stroke* 5, 403–411.
- Chuang, J.Y., Lee, C.W., Shih, Y.H., Yang, T., Yu, L., Kuo, Y.M., 2012. Interactions between amyloid-beta and hemoglobin: implications for amyloid plaque formation in Alzheimer's disease. *PLoS One* 7, e33120.
- Corder, E.H., Ghebremedhin, E., Taylor, M.G., Thal, D.R., Ohm, T.G., Braak, H., 2004. The biphasic relationship between regional brain senile plaque and neurofibrillary tangle distributions: modification by age, sex, and APOE polymorphism. *Ann. N. Y. Acad. Sci.* 1019, 24–28.
- Damoiseaux, J.S., Seeley, W.W., Zhou, J., Shiner, W.R., Coppola, G., Karydas, A., Rosen, H.J., Miller, B.L., Kramer, J.H., Greicius, M.D., Alzheimer's Disease Neuroimaging Initiative, 2012. Alzheimer's Disease Neuroimaging, Gender modulates the APOE epsilon4 effect in healthy older adults: convergent evidence from functional brain connectivity 516 and spinal fluid tau levels. *J. Neurosci.* 32, 8254–8262.
- De Strooper, B., Simons, M., Multhaup, G., Van Leuven, F., Beyreuther, K., Dotti, C.G., 1995. Production of intracellular amyloid-containing fragments in hippocampal neurons expressing human amyloid precursor protein and protection against amyloidogenesis by subtle amino acid substitutions in the rodent sequence. *EMBO J.* 14, 4932–4938.
- Ding, J., Sigurdsson, S., Garcia, M., Phillips, C.L., Eiriksdottir, G., Gudnason, V., van Buchem, M.A., Launer, L.J., 2015. Risk Factors Associated With Incident Cerebral Microbleeds According to Location in Older People: The Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. *JAMA Neurol.* 72, 682–688.
- Dubal, D.B., Broestl, L., Worden, K., 2012. Sex and gonadal hormones in mouse models of Alzheimer's disease: what is relevant to the human condition? *Biol. Sex. Diff.* 3, 24.
- Dumas, A., Dierksen, G.A., Gurol, M.E., Halpin, A., Martinez-Ramirez, S., Schwab, K., Rosand, J., Viswanathan, A., Salat, D.H., Polimeni, J.R., Greenberg, S.M., 2012. Functional magnetic resonance imaging detection of vascular reactivity in cerebral amyloid angiopathy. *Ann. Neurol.* 72, 76–81.
- Fairbanks, S.L., Vest, R., Verma, S., Traystman, R.J., Herson, P.S., 2013. Sex stratified neuronal cultures to study ischemic cell death pathways. *J. Vis. Exp.* 82, e50758.
- Farrer, L.A., Cupples, L.A., Haines, J.L., Hyman, B., Kukull, W.A., Mayeux, R., Myers, R.H., Pericak-Vance, M.A., Risch, N., van Duijn, C.M., 1997. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 278, 1349–1356.
- Finch, C.E., Austad, S.N., 2015. Commentary: is Alzheimer's disease uniquely human? *Neurobiol. Aging* 36, 553–555.
- Finch, C.E., Martin, G.M., 2015. Dementias of the Alzheimer type: views through the lens of evolutionary biology suggest amyloid-driven brain aging is a trade-off with host defence. In: Alvergne, A. (Ed.), *Evolutionary Thinking in Medicine: From Research to Policy and Practice*. Ed. Springer, Germany.
- Finch, C.E., Morgan, T.E., 2007. Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: a position paper. *Curr. Alzheimer Res.* 42, 185–189.
- Fleisher, A., Grundman, M., Jack Jr., C.R., Petersen, R.C., Taylor, C., Kim, H.T., Schiller, D.H., Bagwell, V., Sencakova, D., Weiner, M.F., DeCarli, C., DeKosky, S.T., van Dyck, C.H., Thal, L.J., Alzheimer's Disease Cooperative Study, 2005. Sex, apolipoprotein E epsilon 4 status, and hippocampal volume in mild cognitive impairment. *Arch. Neurol.* 62, 953–957.
- Fryer, J.D., Simmons, K., Parsadanian, M., Bales, K.R., Paul, S.M., Sullivan, P.M., Holtzman, D.M., 2005. 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.* 25, 2803–2810.
- Fullerton, S.M., Clark, A.G., Weiss, K.M., Nickerson, D.A., Taylor, S.L., Stengård, J.H., Salomaa, V., Vartiainen, E., Perola, M., Boerwinkle, E., Sing, C.F., 2000. Apolipoprotein E variation at the sequence haplotype level: implications for the origin and maintenance of a major human polymorphism. *Am. J. Hum. Genet.* 67, 881–900.
- Gearing, M., Rebeck, G.W., Hyman, B.T., Tigges, J., Mirra, S.S., 1994. Neuropathology and apolipoprotein E profile of aged chimpanzees: implications for Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* 91, 9382–9386.
- Gilbert, J.J., Vinters, H.V., 1983. Cerebral amyloid angiopathy: incidence and complications in the aging brain. I. Cerebral hemorrhage. *Stroke* 14, 915–923.
- Go, A.S., Mozaffarian, D., Roger, V.L., Benjamin, E.J., Berry, J.D., Borden, W.B., Bravata, D.M., Dai, S., Ford, E.S., Fox, C.S., Franco, S., Fullerton, H.J., Gillespie, C., Hailpern, S.M., Heit, J.A., Howard, V.J., Huffman, M.D., Kissela, B.M., Kittner, S.J., Lackland, D.T., Lichtman, J.H., Lisabeth, L.D., Magid, D., Marcus, G.M., Marelli, A., Mather, D.B., McGuire, D.K., Mohler, E.R., Moy, C.S., Mussolino, M.E., Nichol, G., Paynter, N.P., Schreiner, P.J., Sorlie, P.D., Stein, J., Turan, T.N., Virani, S.S., Wong, N.D., Woo, D., Turner, M.B., American Heart Association Statistics Committee and Stroke Statistics Subcommittee, 2013. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. *Circulation* 127, e245.
- Gorski, R.A., 1979. The neuroendocrinology of reproduction: an overview. *Biol. Reprod.* 20, 111–127.
- Greenberg, S.M., Vernooij, M.W., Cordonnier, C., Viswanathan, A., Al-Shahi Salman, R., Warach, S., Launer, L.J., Van Buchem, M.A., Breteler, M.M., Microbleed Study Group, 2009. Cerebral microbleeds: a guide to detection and interpretation. *Lancet Neurol.* 8, 165–174.
- Gregg, N.M., Kim, A.E., Gurol, M.E., Lopez, O.L., Aizenstein, H.J., Price, J.C., Mathis, C.A., James, J.A., Snitz, B.E., Cohen, A.D., Kamboh, M.I., Minhas, D., Weissfeld, L.A., Tamburo, E.L., Klunk, W.E., 2015. Incidental Cerebral Microbleeds and Cerebral Blood Flow in Elderly Individuals. *JAMA Neurol.* 72, 1021–1028.
- Gregoire, S.M., Chaudhary, U.J., Brown, M.M., Yousry, T.A., Kallis, C., Jager, H.R., Werring, D.J., 2009. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology* 73, 1759–1766.
- Han, B.H., Zhou, M.L., Abusaleh, F., Brendza, R.P., Dietrich, H.H., Koenigsnecht-Talboo, J., Cirrito, J.R., Milner, E., Holtzman, D.M., Zipfel, G.J., 2008. Cerebrovascular dysfunction in amyloid precursor protein transgenic mice: contribution of soluble and insoluble amyloid-beta peptide, partial restoration via gamma-secretase inhibition. *J. Neurosci.* 28, 13542–13550.
- Hines, M., Constantinescu, M., Spencer, D., 2015. Early androgen exposure and human gender development. *Biol. Sex. Diff.* 6, 3.
- Hua, X., Hibar, D.P., Lee, S., Toga, A.W., Jack Jr., C.R., Weiner, M.W., Thompson, P.M., 2010. Alzheimer's Disease Neuroimaging Initiative, Sex and age differences in atrophic rates: an ADNI study with n=1368 MRI scans. *Neurobiol. Aging* 31, 1463–1480.
- Jasienska, G., Ellison, P.T., Galbarczyk, A., Jasienski, M., Kalemba-Drozd, M., Kapiszewska, M., Nenko, I., Thune, I., Ziolkiewicz, A., 2015. Apolipoprotein E (ApoE) polymorphism is related to differences in potential fertility in women: a case of antagonistic pleiotropy? *Proc. Biol. Sci.* 282, 20142395.
- Jayaraman, A., Carroll, J.C., Morgan, T.E., Lin, S., Zhao, L., Arimoto, J.M., Murphy, M.P., Beckett, T.L., Finch, C.E., Brinton, R.D., Pike, C.J., 2012. 17beta-estradiol and progesterone regulate expression of beta-amyloid clearance factors in primary neuron cultures and female rat brain. *Endocrinology* 153, 5467–5479.
- Jean, S.M., Preuss, T.M., Sharma, P., Anderson, D.C., Provenzale, J.M., Strobert, E., Ross, S.R., Stroud, F.C., 2012. Cerebrovascular accident (stroke) in captive, group-housed, female chimpanzees. *Compar. Med.* 62, 322–329.
- Jeerakathil, T., Wolf, P.A., Beiser, A., Hald, J.K., Au, R., Kase, C.S., Massaro, J.M., DeCarli, C., 2014. Cerebral microbleeds: prevalence and associations with cardiovascular risk factors in the Framingham Study. *Stroke* 35, 1831–1835.
- Klein, W.L., Krafft, G.A., Finch, C.E., 2001. Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? *Trends. Neurosci.* 24, 219–224.
- Kunzler, J., Youmans, K.L., Yu, C., Ladu, M.J., Tai, L.M., 2014. APOE modulates the effect of estrogen therapy on Aβ accumulation EFAD-Tg mice. *Neurosci. Lett.* 560, 131–136.
- Lee, S.S., Stemmermann, G.N., 1978. Congophilic angiopathy and cerebral hemorrhage. *Arch. Pathol. Lab. Med.* 102, 317–321.
- Liu, S., Grigoryan, M.M., Vasilevko, V., Sumbria, R.K., Paganini-Hill, A., Cribbs, D.H., Fisher, M.J., 2014. Comparative analysis of H&E and Prussian blue staining in a mouse model of cerebral microbleeds. *J. Histochem. Cytochem.* 62, 767–773.
- Love, S., Nicoll, J.A., Hughes, A., Wilcock, G.K., 2003. APOE and cerebral amyloid angiopathy in the elderly. *Neuroreport* 14, 1535–1536.
- Masuda, J., Tanaka, K., Ueda, K., Omae, T., 1988. Autopsy study of incidence and distribution of cerebral amyloid angiopathy in Hisayama, Japan. *Stroke* 19, 205–210.
- Manwani, B., Bentivegna, K., Benashski, S.E., Venna, V.R., Xu, Y., Arnold, A.P., McCullough, L.D., 2014. Sex differences in ischemic stroke sensitivity are influenced by gonadal hormones, not by sex chromosome complement. *J. Cereb. Blood. Flow. Metab.* 35, 221–229.
- McIntosh, A.M., Bennett, C., Dickson, D., Anestis, S.F., Watts, D.P., Webster, T.H., Fontenot, M.B., Bradley, B.J., 2012. The apolipoprotein E (APOE) gene appears functionally monomorphic in chimpanzees (Pan troglodytes). *PLoS One* 7, e47760.
- Meier, I.B., Gu, Y., Guzman, V.A., Wiegman, A.F., Schupf, N., Manly, J.J., Luchsinger, J.A., Viswanathan, A., Martinez-Ramirez, S., Greenberg, S.M.,

- Mayeux, R., Brickman, A., 2014. Lobar microbleeds are associated with a decline in executive functioning in older adults. *Cerebrovasc. Dis.* 38, 377–383.
- Montagne, A., Barnes, S.R., Sweeney, M.D., Halliday, M.R., Sagare, A.P., Zhao, Z., Toga, A.W., Jacobs, R.E., Liu, C.Y., Amezcua, L., Harrington, M.G., Chui, H.C., Law, M., Zlokovic, B.V., 2015. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* 85, 296–302.
- Mucke, L., Selkoe, D.J., 2012. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. *Cold. Spring Harb. Perspect. Med.* 2, a006338.
- Nelson, P.T., Pious, N.M., Jicha, G.A., Wilcock, D.M., Fardo, D.W., Estus, S., Rebeck, G.W., 2013. APOE-epsilon2 and APOE-epsilon4 correlate with increased amyloid accumulation in cerebral vasculature. *J. Neuropathol. Exp. Neurol.* 72, 708–715.
- Olichney, J.M., Hansen, L.A., Lee, J.H., Hofstetter, C.R., Katzman, R., Thal, L.J., 2000. Relationship between severe amyloid angiopathy, apolipoprotein E genotype, and vascular lesions in Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 903, 138–143.
- Payami, H., Zarepari, S., Montee, K.R., Sexton, G.J., Kaye, J.A., Bird, T.D., Yu, C.E., Wijsman, E.M., Heston, L.L., Litt, M., Schellenberg, G.D., 1996. Gender difference in apolipoprotein E-associated risk for familial Alzheimer disease: a possible clue to the higher incidence of Alzheimer disease in women. *Am. J. Hum. Genet.* 58, 803–811.
- Peca, S., McCreary, C.R., Donaldson, E., Kumarpillai, G., Shobha, N., Sanchez, K., Charlton, A., Steinback, C.D., Beaudin, A.E., Flück, D., Pillay, N., Fick, G.H., Poulin, M.J., Frayne, R., Goodyear, B.G., Smith, E.E., 2013. Neurovascular decoupling is associated with severity of cerebral amyloid angiopathy. *Neurology* 81, 1659–1665.
- Piper, M.A., Evans, C.V., Burda, B.U., Margolis, K.L., O'Connor, E., Smith, N., Webber, E., Perdue, L.A., Bigler, K.D., Whitlock, E.P., 2014. Screening for High Blood Pressure in Adults: A Systematic Evidence Review for the US Preventive Services Task Force. Agency for Healthcare Research and Quality (US), Rockville, MD.
- Premkumar, D.R., Cohen, D.L., Hedera, P., Friedland, R.P., Kalaria, R.N., 1996. Apolipoprotein E-epsilon4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease. *Am. J. Pathol.* 148, 2083–2095.
- Raber, J., Wong, D., Buttini, M., Orth, M., Bellosta, S., Pitas, R.E., Mahley, R.W., Mucke, L., 1998. Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: increased susceptibility of females. *Proc. Natl. Acad. Sci. U. S. A.* 95, 10914–10919.
- Rannikmae, K., Samarasekera, N., Martinez-Gonzalez, N.A., Al-Shahi Salman, R., Sudlow, C.L., 2013. Genetics of cerebral amyloid angiopathy: systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* 84, 901–908.
- Rasool, S., Martinez-Coria, H., Wu, J.W., LaFerla, F., Glabe, C.G., 2013. Systemic vaccination with anti-oligomeric monoclonal antibodies improves cognitive function by reducing Abeta deposition and tau pathology in 3xTg-AD mice. *J. Neurochem.* 126, 473–482.
- Reijmer, Y.D., Fotiadis, P., Martinez-Ramirez, S., Salat, D.H., Schultz, A., Shoamanesh, A., Ayres, A.M., Vashkevich, A., Rosas, D., Schwab, K., Leemans, A., Biessels, G.J., Rosand, J., Johnson, K.A., Viswanathan, A., Gurol, M.E., Greenberg, S.M., 2015. Structural network alterations and neurological dysfunction in cerebral amyloid angiopathy. *Brain* 138, 179–188.
- Resnick, S.M., Bilgel, M., Moghekar, A., An, Y., Cai, Q., Wang, M.C., Thambisetty, M., Prince, J.L., Zhou, Y., Soldan, A., Wong, D.F., O'Brien, R.J., Ferrucci, L., Albert, M.S., 2015. Changes in Aβ biomarkers and associations with APOE genotype in 2 longitudinal cohorts. *Neurobiol. Aging* 36, 2333–2339.
- Romero, J.R., Preis, S.R., Beiser, A.S., DeCarli, C., Lee, D.Y., Viswanathan, A., Benjamin, E.J., Fontes, J., Au, R., Pikula, A., Wang, J., Kase, C.S., Wolf, P.A., Izzari, M.C., Seshadri, S., 2012. Lipoprotein phospholipase A2 and cerebral microbleeds in the Framingham Heart Study. *Stroke* 43, 3091–3094.
- Rosario, E.R., Carroll, J., Pike, C.J., 2012. Testosterone regulation of Alzheimer-like neuropathology in male 3xTg-AD mice involves both estrogen and androgen pathways. *Brain Res.* 1359, 281–290.
- Rosen, R.F., Farberg, A.S., Gearing, M., Dooyema, J., Long, P.M., Anderson, D.C., Davis-Turak, J., Coppola, G., Geschwind, D.H., Paré, J.F., Duong, T.Q., Hopkins, W.D., Preuss, T.M., Walker, L.C., 2008. Tauopathy with paired helical filaments in an aged chimpanzee. *J. Compar. Neurol.* 509, 259–270.
- Rougeron, V., Woods, C.M., Tiedje, K.E., Bodeau-Livinec, F., Migot-Nabias, F., Deloron, P., Luty, A.J., Fowkes, F.J., Day, K.P., 2013. Epistatic Interactions between apolipoprotein E and hemoglobin S Genes in regulation of malaria parasitemia. *PLoS One* 8, e76924.
- Schmechel, D.E., Saunders, A.M., Strittmatter, W.J., Crain, B.J., Hulette, C.M., Joo, S.H., Pericak-Vance, M.A., Goldgaber, D., Roses, A.D., 1993. 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.* 90, 9649–9653.
- Shams, S., Martola, J., Granberg, T., Li, X., Shams, M., Fereshtehnejad, S.M., Cavallin, L., Aspelin, P., Kristoffersen-Wiberg, M., Wahlund, L.O., 2015. Cerebral microbleeds: different prevalence, topography, and risk factors depending on dementia diagnosis—the Karolinska Imaging Dementia Study. *Am. J. Neuroradiol.* 36, 661–666.
- Smith, D.D., Tan, X., Tawfik, O., Milne, G., Stechschulte, D.J., Dileepan, K.N., 2010. Increased aortic atherosclerotic plaque development in female apolipoprotein E-null mice is associated with elevated thromboxane A2 and decreased prostacyclin production. *J. Physiol. Pharmacol.* 61, 309–316.
- Sturchler-Pierrat, C., Staufienbiel, M., 2000. Pathogenic mechanisms of Alzheimer's disease analyzed in the APP23 transgenic mouse model. *Ann. N. Y. Acad. Sci.* 920, 134–139.
- Sullivan, P.M., Mace, B.E., Estrada, J.C., Schmechel, D.E., Alberts, M.J., 2008. Human apolipoprotein E4 targeted replacement mice show increased prevalence of intracerebral hemorrhage associated with vascular amyloid deposition. *J. Stroke Cerebrovasc. Dis.* 17, 303–311.
- Sullivan, P.M., Mezdour, H., Aratani, Y., Knouff, C., Najib, J., Reddick, R.L., Quarfordt, S.H., Maeda, N., 1997. Targeted replacement of the mouse apolipoprotein E gene with the common human APOE3 allele enhances diet-induced hypercholesterolemia and atherosclerosis. *J. Biol. Chem.* 272, 17972–17980.
- Sveinbjörnsdóttir, S., Sigurdsson, S., Aspelund, T., Kjartansson, O., Eiriksdóttir, G., Valtysdóttir, B., Lopez, O.L., van Buchem, M.A., Jonsson, P.V., Gudnason, V., Launer, J.J., 2008. Cerebral microbleeds in the population based AGES-Reykjavik study: prevalence and location. *J. Neurol. Neurosurg. Psych.* 79, 1002–1006.
- Tanifum, E.A., Starosolski, Z.A., Fowler, S.W., Jankowsky, J.L., Annapragada, A.V., 2014. Cerebral vascular leak in a mouse model of amyloid neuropathology. *J. Cereb. Blood Flow Metab.* 34, 1646–1654.
- Theendakara, V., Patent, A., Peters Libeu, C.A., Philpot, B., Flores, S., Descamps, O., Poksay, K.S., Zhang, Q., Cailing, G., Hart, M., John, V., Rao, R.V., Bredesen, D.E., 2013. Neuroprotective Sirtuin ratio reversed by ApoE4. *Proc. Natl. Acad. Sci. U. S. A.* 110, 18303–18308.
- Vest, R.S., Pike, C.J., 2013. Gender, sex steroid hormones, and Alzheimer's disease. *Horm. Behav.* 63, 301–307.
- Walker, L.C., Pahnke, J., Madauss, M., Vogelgesang, S., Pahnke, A., Herbst, E.W., Stausske, D., Walther, R., Kessler, C., Warzok, R.W., 2000. Apolipoprotein E4 promotes the early deposition of Abeta42 and then Abeta40 in the elderly. *Acta Neuropathol.* 100, 36–42.
- Wardlaw, J.M., Smith, E.E., Biessels, G.J., Cordonnier, C., Fazekas, F., Frayne, R., Lindley, R.I., O'Brien, J.T., Barkhof, F., Benavente, O.R., Black, S.E., Brayne, C., Breteler, M., Chabriat, H., Decarli, C., de Leeuw, F.E., Doubal, F., Duering, M., Fox, N.C., Greenberg, S., Hachinski, V., Kilimann, I., Mok, V., Oostenbrugge, R., Pantoni, L., Speck, O., Stephan, B.C., Teipel, S., Viswanathan, A., Werring, D., Chen, C., Smith, C., van Buchem, M., Norrving, B., Gorelick, P.B., Dichgans, M., 2013. Standards for Reporting Vascular changes on neuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol.* 12, 822–838.
- Xu, D., Yang, C., Wang, L., 2003. Cerebral amyloid angiopathy in aged Chinese: a clinico-neuropathological study. *Acta Neuropathol.* 106, 89–91.
- Yates, P.A., Desmond, P.M., Phal, P.M., Steward, C., Szoek, C., Salvado, O., Ellis, K.A., Martins, R.N., Masters, C.L., Ames, D., Villemagne, V.L., Rowe, C.C., AIBL Research Group, 2014. Incidence of cerebral microbleeds in preclinical Alzheimer disease. *Neurology* 82, 1266–1273.
- Yip, A.G., McKee, A.C., Green, R.C., Wells, J., Young, H., Cupples, L.A., Farrer, L.A., 2005. APOE, vascular pathology, and the AD brain. *Neurology* 65, 259–265.
- Youmans, K.L., Tai, L.M., Nwabuisi-Heath, E., Jungbauer, L., Kanekiyo, T., Gan, M., Kim, J., Eimer, W.A., Estus, S., Rebeck, G.W., Weeber, E.J., Bu, G., Yu, C., Ladu, M.J., 2012. APOE4-specific changes in Abeta accumulation in a new transgenic mouse model of Alzheimer disease. *J. Biol. Chem.* 287, 41774–41786.
- Zhang, X., Thatcher, S.E., Rateri, D.L., Brummer, D., Charnigo, R., Daugherty, A., Cassis, L.A., 2012. Transient exposure of neonatal female mice to testosterone abrogates the sexual dimorphism of abdominal aortic aneurysms. *Circ. Res.* 110, e73–e85.
- Zipfel, G.J., Han, H., Ford, A.L., Lee, J.M., 2009. Cerebral amyloid angiopathy: progressive disruption of the neurovascular unit. *Stroke* 40, S16–S19.