



# Risk factors and global cognitive status related to brain arteriolosclerosis in elderly individuals

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## Abstract

Risk factors and cognitive sequelae of brain arteriolosclerosis pathology are not fully understood. To address this, we used multimodal data from the National Alzheimer's Coordinating Center and Alzheimer's Disease Neuroimaging Initiative data sets. Previous studies showed evidence of distinct neurodegenerative disease outcomes and clinical-pathological correlations in the "oldest-old" compared to younger cohorts. Therefore, using the National Alzheimer's Coordinating Center data set, we analyzed clinical and neuropathological data from two groups according to ages at death: < 80 years ( $n = 1008$ ) and  $\geq 80$  years ( $n = 1382$ ). In both age groups, severe brain arteriolosclerosis was associated with worse performances on global cognition tests. Hypertension (but not diabetes) was a brain arteriolosclerosis risk factor in the younger group. In the  $\geq 80$  years age at death group, an *ABCC9* gene variant (rs704180), previously associated with aging-related hippocampal sclerosis, was also associated with brain arteriolosclerosis. A post-hoc arterial spin labeling neuroimaging experiment indicated that *ABCC9* genotype is associated with cerebral blood flow impairment; in a convenience sample from Alzheimer's Disease Neuroimaging Initiative ( $n = 15$ , homozygous individuals), non-risk genotype carriers showed higher global cerebral blood flow compared to risk genotype carriers. We conclude that brain arteriolosclerosis is associated with altered cognitive status and a novel vascular genetic risk factor.

## Keywords

Arteriosclerosis, CVD, HS-Aging, TDP-43, VCID

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## Introduction

Cerebrovascular pathologies affecting small arteries and arterioles are common findings in the aged brain<sup>1–3</sup> and are often seen in brains of persons with dementia.<sup>4–7</sup> Here, we focused on brain arteriolosclerosis (B-ASC), a subtype of cerebral small vessel pathology characterized by degenerative wall thickening of arterioles.<sup>8–10</sup> The true prevalence of B-ASC is unknown but has been observed in 39–80% of autopsied elderly individuals.<sup>11,12</sup> The pathologic changes of B-ASC include hypertrophy and/or death of vascular smooth muscle cells and extracellular deposition of elastin and collagen.<sup>1,2,13,14</sup> In the present study, we sought to better understand the risk factors and global cognitive status associated with B-ASC pathology among aged individuals.

Hypertension and diabetes are presumed risk factors for arteriolosclerosis (ASC) in the brain and other organs. Chronic hypertension is associated with ASC pathology in the brain, kidney, and other organs.<sup>15–17</sup> Hypertensive animal models have thicker cerebral arteriolar walls, larger vessel cross-sectional areas, and smaller inner arteriolar diameters compared to control animals.<sup>18,19</sup> Diabetic patients have thicker subcutaneous gluteal arteriolar walls and larger cross-sectional areas compared to controls.<sup>20,21</sup> Diabetic animal models have thicker retinal capillary walls<sup>22</sup> and renal arteriolar glomerular basement membranes<sup>23</sup> compared to controls.

In addition to clinical risk factors, recent studies suggest that a single nucleotide polymorphism (SNP) located in *ABCC9*, ATP-binding cassette sub-family C member 9, may be a genetic risk factor for B-ASC pathology in older elderly individuals. Evidence in support of the link between an *ABCC9* SNP and B-ASC pathology are as follows: (1) The rs704180 SNP located in *ABCC9* is associated with hippocampal sclerosis of aging (HS-Aging),<sup>24,25</sup> a neurodegenerative disease affecting individuals >80 years at death.<sup>8,26</sup> (2) Individuals with HS-Aging have worse B-ASC pathology compared to individuals without HS-Aging pathology.<sup>8</sup> (3) The gene product of *ABCC9* is a subunit of ATP-sensitive potassium channels found in vascular smooth muscle cells, including arterioles.<sup>27</sup> Thus, by extension, *ABCC9* gene variants may constitute a risk factor for B-ASC pathology in elderly individuals. However, this credible hypothesis has not been tested previously.

As the clinical and genetic risk factors for B-ASC are imperfectly understood, so is the cognitive impairment associated with this pathology. Studies on the global cognitive status of patients with B-ASC have included the analyses of Mini Mental State Examination (MMSE) scores,<sup>28</sup> Clinical Dementia Rating Scale (CDR) scores,<sup>29</sup> and CDR Sum of Boxes (CDRSUM) scores.<sup>30</sup> The MMSE is an assessment tool used in measuring global cognitive function,<sup>31</sup> while the CDR is a measure of a person's ability to accomplish activities of

daily living.<sup>32,33</sup> The CDRSUM score is derived by summing scores from all CDR domains.<sup>34</sup> Prior analyses of data from 334 elderly individuals did not reveal an association between B-ASC pathology and MMSE scores.<sup>28</sup> However, in an autopsy study with 52 cases, widespread B-ASC pathology in cases with Alzheimer's disease (AD) was associated with worse global CDR scores.<sup>29</sup> Similarly, in an autopsy study using 715 AD cases with CDRSUM information, researchers found that high B-ASC severity was associated with worse CDRSUM scores.<sup>30</sup> Conflicting results from these studies may be due to a number of experimental factors including small sample size (statistical power), particular cognitive domains affected by small blood vessel pathologies, frequent presence of comorbid pathologies, and parameters that vary in different parts of the human aging spectrum.

In order to gain insight into B-ASC risk factors and global cognitive status while factoring in other dementia-associated pathologies, we analyzed a subset of individuals from the National Alzheimer's Disease Coordinating Center (NACC) data set. Because there is evidence of distinct neurodegenerative outcomes and clinical-pathological correlation differences between the "younger-old" and "oldest-old" persons,<sup>35–41</sup> we analyzed groups separately according to ages at death: < 80 years and ≥80 years. The goals of the study were to determine if autopsy-verified B-ASC is associated with global cognitive status, to assess the association between vascular risk factors and B-ASC pathology, and to determine the relationship between *ABCC9* HS-Aging risk genotype and B-ASC pathology. In order to further test the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology, we studied the relationship between the *ABCC9* HS-Aging risk genotype and cerebral blood flow (CBF) (a possible in vivo manifestation of B-ASC pathology). Genetic and neuroimaging data on a sub-sample of individuals from the Alzheimer's Disease Neuroimaging Initiative (ADNI) data set were used to test this association.

## Methods

This study used data from the NACC and ADNI data sets. Patient recruitment and data collection in the NACC data set have been previously described including details related to institutional review board approval and patient consent.<sup>42–45</sup> Research using NACC data was approved by the University of Washington Human Subjects Division. Patient recruitment, neuroimaging acquisition, and data collection on individuals included in the ADNI data set have been previously described with approval by local ethics review boards at each participating site.<sup>46,47</sup>

## Study subjects

The NACC data set contains research subject information collected from 34 past and present Alzheimer's Disease Centers (ADCs) across the United States, comprising clinical, genetic, and neuropathological (autopsy) data.<sup>43</sup> More detailed information on data collection is available online ([https://www.alz.washington.edu/WEB/dataforms\\_main.html](https://www.alz.washington.edu/WEB/dataforms_main.html)). Archival data from September 2005 through December 2013 were obtained for this study.

The initial pulled archival data from the NACC data set comprised 29,483 cases. Living cases ( $n=26,686$ ) and autopsied cases with missing information which would preclude making an assessment of B-ASC status ( $n=407$ ) were excluded from the analysis. For analysis, cases were split into two age at death groups:  $<80$  years ( $n=1008$ ) and  $\geq 80$  years ( $n=1382$ ).

The ADNI data set is from a multicenter longitudinal study in the United States and Canada in which subjects with normal cognition, MCI and AD were followed with cognitive testing, neuroimaging techniques, and other biomarker.<sup>46</sup> A convenience sample ( $n=15$ ) of rs704180 homozygous (A\_A or G\_G genotype) individuals was used for CBF analysis. Neither other scans nor SNPs were assessed from ADNI.

## Cognitive and functional assessment in the NACC data set

MMSE (0–30; 30=no global impairment) and CDRSUM (0–18; 0=no global impairment) scores were used as measures of global cognitive status.<sup>31–33</sup> Scores from the most recent ADC clinical visit before each individual's death were used (median interval between final visit and death: 0.83 years).

## Clinical, neuropathologic, and genetic parameters in the NACC data set

Clinical data were obtained from each participant's final ADC clinical visit before death. During clinical research visits, medical histories were obtained from subjects, caregivers (particularly if the subject was cognitively impaired), and/or patient records. The following self-reported vascular risk factors and cerebrovascular diseases were used in the analyses: medical histories of hypertension, diabetes, hypercholesterolemia, sex, smoking, stroke, and atrial fibrillation. Responses were coded initially as unknown, absent, recent/active, or remote/inactive, and subsequently, "recent" and "remote" responses were combined into one category (i.e. history of a condition) for analytical purposes. Body mass index (BMI) values were derived from height and weight measurements. Pack years were derived from self-reported cigarette packs smoked per day and years of smoking.

Neuropathologic details from all cases included Braak staging,<sup>48</sup> CERAD neuritic plaque densities,<sup>49</sup> and other parameters as described in detail previously.<sup>44</sup> In the NACC Neuropathology Data Set Coding Guidebook version 9.1 ([https://www.alz.washington.edu/WEB/forms\\_np.html](https://www.alz.washington.edu/WEB/forms_np.html)), B-ASC was described as "hyalinosis of the media and adventitia of small parenchymal and/or leptomeningeal vessels." B-ASC pathology was diagnosed using a semi-quantitative four-tier categorization system with responses scored to indicate "none", "mild", "moderate", or "severe" B-ASC pathology. In the NACC guidebook, neuropathologists were instructed to estimate the overall severity of B-ASC pathology. No specific brain region for B-ASC pathological diagnosis was mentioned; thus, this diagnostic methodology was left to the discretion of each individual neuropathologist and/or research center.

Genetic data were obtained and analyzed as described previously.<sup>24,25</sup> Briefly, the Alzheimer's Disease Genetic Consortium (ADGC) accrued genetic data from 29 different ADCs, with multiple iterations of SNP data,<sup>50–52</sup> which were analyzed together with neuropathological and clinical data gathered through NACC.<sup>53</sup> The three alleles identified were analyzed according to ADGC SNP nomenclature and were *GRN*.rs5848 (A/G), *TMEM106B*.rs1990622 (A/G; note that other reports have used T/C for this allele: the "A" allele is analogous to "T" allele in other reports, whereas the "G" allele we report is analogous to "C" allele), and *ABCC9*.rs704180 (A/G).<sup>24,25</sup> *APOE*  $\epsilon 4$  genotype information from NACC was also used in the analysis because *APOE* alleles are known to be associated with cerebral amyloid angiopathy (CAA), which could lead to vascular wall distortions.<sup>54</sup>

## Neuroimaging and genetic parameters in ADNI data set

T<sub>1</sub>-weighted brain MRI scans were acquired using a sagittal MP-RAGE sequence following the ADNI MRI protocol.<sup>47,55</sup> Arterial spin labeling (ASL) images were obtained from ADNI data set. Data from 15 Caucasian individuals were acquired from six different American research centers using a standardized pulsed arterial spin labeling (pASL) protocol: Field Strength = 3.0 tesla; Flip angle = 90.0 degree; Manufacturer = SIEMENS; Matrix X = 320.0 pixels; Matrix Y = 320.0 pixels; Pixel Spacing X = 4.0 mm; Pixel Spacing Y = 4.0 mm; Pulse Sequence = EP; Slice Thickness = 4.0 mm; TE = 12.0 ms; TR = 3400.0 ms. Control and label images were subtracted, and quantitative CBF (mL/100 g/min units) was calculated using in-house Matlab software using the following

equation<sup>56,57</sup> and then correlated with genotyping

$$\text{CBF} = \frac{\lambda \cdot (\text{SI}_{\text{control}} - \text{SI}_{\text{label}})}{2 \cdot \alpha \cdot M_0 \cdot T_{I1} \cdot \exp(-T_{I2}/T_{1,\text{blood}})} [\text{ml}/100\text{g}/\text{min}]$$

where  $\lambda$  was the brain/blood partition coefficient in mL/g,  $\text{SI}_{\text{control}}$  and  $\text{SI}_{\text{label}}$  were the time-averaged signal intensities in the control and label images, respectively,  $T_{1,\text{blood}}$  was the longitudinal relaxation time of blood in seconds,  $\alpha$  was the labeling efficiency,  $M_0$  was the equilibrium brain tissue magnetization,  $T_{I1}$  was post-labeling delay, and  $T_{I2}$  was the label duration. SNP rs704180 in *ABCC9* and *APOE*  $\epsilon 4$  status information came from ADNI.

### Statistical analyses

Exploratory bivariate analyses and regression modeling were used to assess the association between clinical vascular risk factors and B-ASC pathology. Initially, a Chi-square test, a Mann-Whitney U test, or a Kruskal–Wallis test were used to determine possible risk factors for B-ASC severity in the two age at death groups. A Chi-square test was applied for categorical variables, whereas a Mann-Whitney U test or Kruskal–Wallis test was applied for continuous non-normally distributed variables. Subsequently, clinical variables that yielded a  $p < 0.05$  in these analyses were included as independent variables in an ordinal logistic regression to further elucidate the association between clinical variables and B-ASC pathology while controlling for confounding effects. The variables in this logistic regression model included age at death, sex, hypertension, diabetes, smoking pack years, and hypercholesterolemia.

Logistic regression modeling was used to determine the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology. Age at death, sex, hypertension, diabetes, pack years, and hypercholesterolemia were used as covariates. Mild, moderate and severe B-ASC pathologies were collapsed into one category and treated as a dependent variable.

A linear regression model was used to assess the association between B-ASC pathology with MMSE and CDRSUM scores. B-ASC pathology was treated as the main independent variable. MMSE or CDRSUM scores were treated as dependent variables while adjusting for age at death, sex, Braak neurofibrillary tangles (NFT) stage, presence of any microinfarcts, presence of neocortical Lewy bodies, and presence of HS-Aging pathology. Adjusted mean MMSE and CDRSUM scores derived from the linear regression analyses were reported for each B-ASC severity category and compared using the least squares method.

In order to assess the relationship between the *ABCC9* HS-Aging risk genotype and CBF, a possible

manifestation of B-ASC pathology, a Welch's two sample t-test was used to compare CBF measurements between individuals with the *ABCC9* HS-Aging homozygous non-risk and risk genotypes. All statistical analyses were performed using IBM SPSS Statistics 22 Properties and PC-SAS 9.34 (SAS Institute, Inc.; Cary, NC, USA).

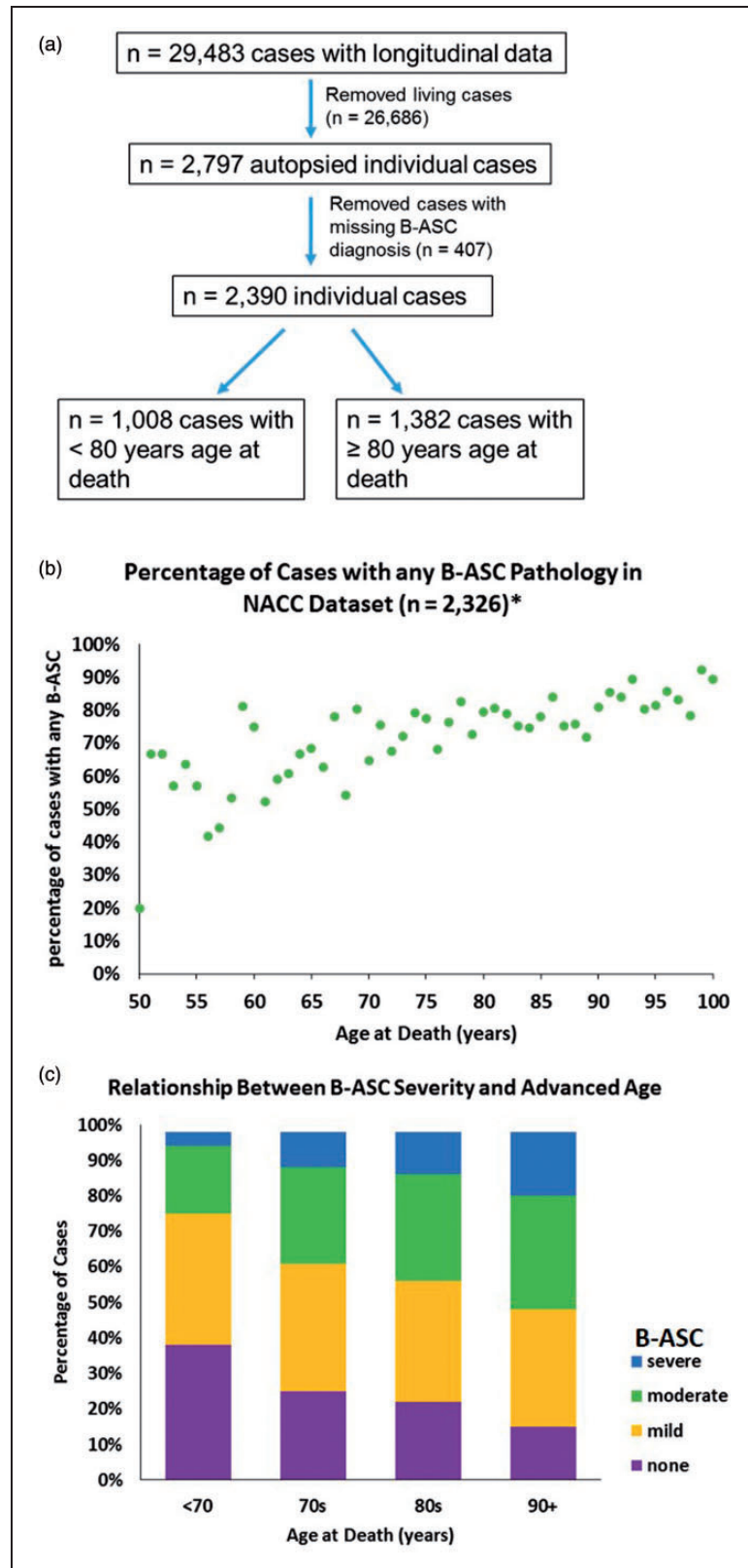
### Results

The inclusion/exclusion criteria applied to individuals used in our analyses from the NACC data set are shown in Figure 1(a). Individuals used in these analyses were predominantly (>90%) Caucasian (data not shown), and median year at death was 2010 (range: 2005–2013). In order to convey examples of blood vessel profiles representing the spectrum of B-ASC pathology, images were obtained from four human brain sections stained with hematoxylin and eosin (H&E) (Figure 2). The percentage of individuals with B-ASC pathology trended upward with increasing age at death. (Figure 1(b)). When stratifying by age at death, the percentage of individuals with moderate or severe B-ASC pathology was higher in older age at death groups ( $p < 0.0001$ ) (Figure 1(c)). These results indicate that B-ASC is a common pathology in the NACC data set, becoming more severe with increasing age at death.

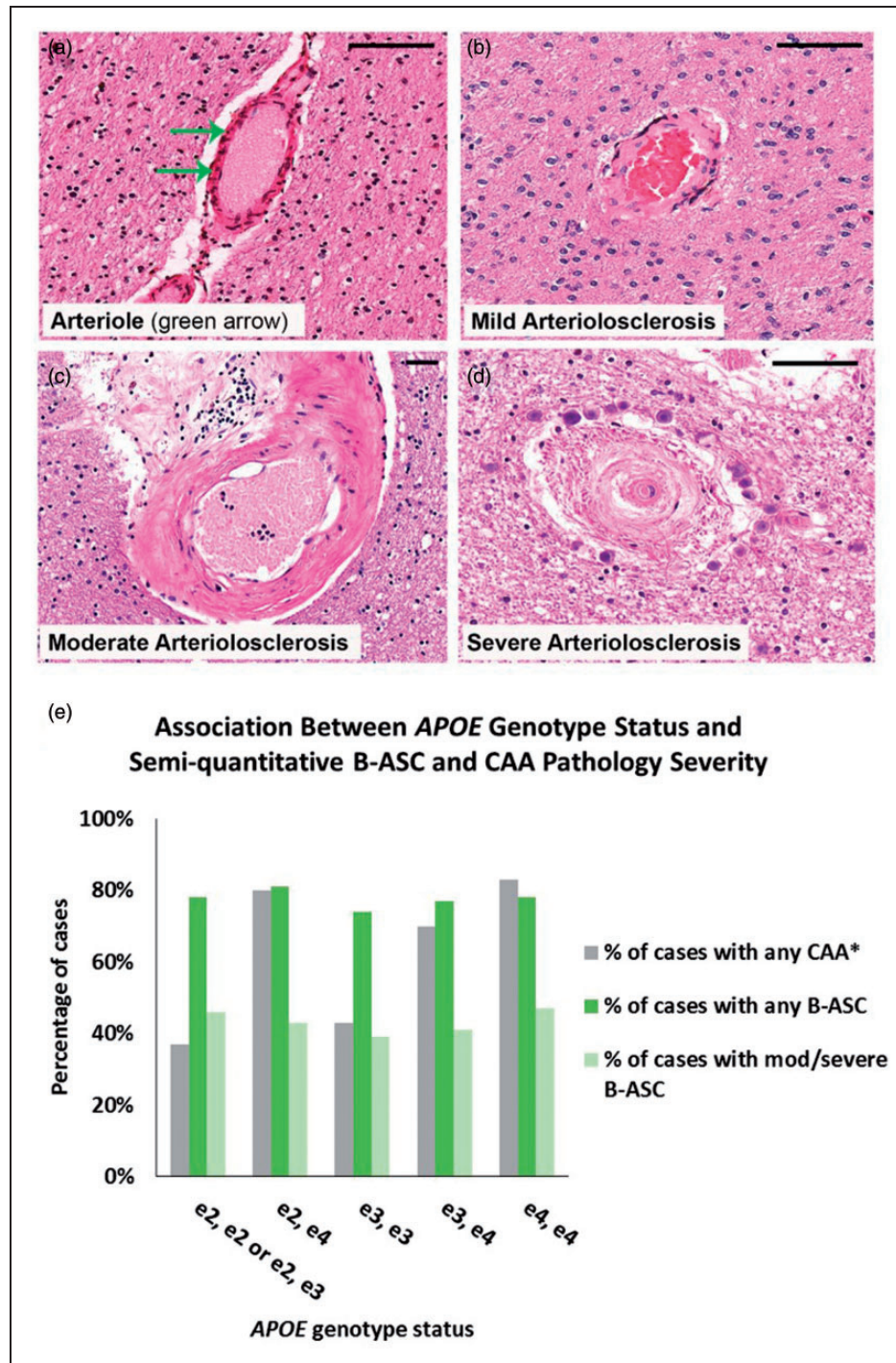
As a result of prior studies showing different outcomes and clinical-pathological correlations in the “oldest old,”<sup>35–37</sup> we analyzed the overall cohort in two separate age groups. More specifically, the cutoff of 80 years was chosen because it has been used before to help highlight neurodegenerative disease and/or neuropathologic features that differ – often quite dramatically – among the “oldest-old”.<sup>39–41,58–60</sup> Furthermore, this cutoff was close to the overall cohort mean (80.0 years) and median (82.0 years) age at death. Comparing the two age at death groups in the NACC data set, individuals in the  $\geq 80$  years age at death group were more often female, hypertensive, and less often impaired cognitively when compared to individuals in the  $< 80$  years age at death group (Table 1). In addition, individuals in the  $\geq 80$  years age at death group were less likely to show “high” levels of AD pathology, but more likely to show B-ASC and HS-Aging pathologies at autopsy, compared to individuals in the  $< 80$  years age at death group. These data, in addition to the prior precedents in the literature, confirmed that the two age groups show differing clinical risk factors, cognitive profiles, and neuropathological autopsy results.

### Global cognitive status associated with B-ASC pathology

To analyze the global cognitive status of individuals with B-ASC pathology, linear regression analyses were



**Figure 1.** Exclusion/inclusion criteria and frequency of brain arteriolosclerosis (B-ASC) pathology in autopsied cases from the National Alzheimer's Coordinating Center (NACC) data set. (a) Living cases and autopsied cases with missing information which would preclude making an assessment of B-ASC status were excluded from analysis. (b) The percentage of cases with any B-ASC pathology (mild, moderate or severe) in the NACC data set. Asterisk (\*) indicates that 64 cases with age at death < 50 years or > 100 years were not plotted. (c) A stacked bar graph showing that B-ASC severity increases with age at death, Chi-square  $p$ -value < 0.0001.



**Figure 2.** Semi-quantitative severity grading of B-ASC pathology and its non-association with APOE  $\epsilon 4$  allele in the NACC data set. (a, b, c, d) Photomicrographs of hematoxylin- and eosin-stained blood vessels. (a) What we assume to be a normal arteriole (green arrow). (b) mild B-ASC with relatively mild thickening of vessel wall. (c) moderate B-ASC severity grade 2 with increased thickening of vessel wall. (d) severe B-ASC with prominent thickening of vessel wall and partly occluded vessel lumen. Scale bars = 100  $\mu\text{m}$ . (e) The association between APOE  $\epsilon 4$  genotypes and any degree of CAA (mild, moderate, severe) combining both age at death groups: \*Chi-square  $p$ -value < 0.0001 among 1883 individuals for whom APOE genotype data and CAA diagnosis were available. Those with  $\epsilon 2/\epsilon 3$  and  $\epsilon 2/\epsilon 2$  genotypes were combined into one category. There were no statistically significant associations that could be determined between B-ASC severity and APOE genotype.

B-ASC: brain arteriolosclerosis; CAA: cerebral amyloid angiopathy.

**Table 1.** Age at death group comparison on clinical, cognitive, neuropathologic, and genetic variables.

	<80 years age of death group <i>n</i> = 1008	≥80 years age of death group <i>n</i> = 1382	Missing cases, <i>n</i>	<i>p</i> <sup>a</sup>
<b>Demographic variables</b>				
Age of death, median (25th, 75th quartile)	70.0 (64.0, 76.0)	88.00 (84.0, 92.0)		<0.0001 <sup>b,c</sup>
Gender (%)				<0.0001 <sup>b,c</sup>
Male	61.7	48.3		
Female	38.3	51.7		
<b>Clinical variables</b>				
Hypertension (%) <sup>d</sup>	46.1	64.6	8	<0.0001 <sup>b,c</sup>
Diabetes (%) <sup>d</sup>	9.6	13.2	2	0.008 <sup>c</sup>
Hypercholesterolemia (%) <sup>d</sup>	47.7	49.8	28	0.315
Pack years median (25th, 75th quartile) <sup>d</sup>	0.0 (0.0, 13.1)	0.0 (0.0, 15.0)	181	0.496
BMI median (25th, 75th quartile) <sup>e</sup>	25.4 (22.8, 28.5)	24.9 (22.2, 27.6)	980	0.004 <sup>c</sup>
<b>Cognitive variables</b>				
MMSE median (25th, 75th quartile)	16.0 (6.0, 24.0)	21.0 (14.0, 27.0)	697	<0.0001 <sup>b,c</sup>
CDRSUM median (25th, 75th quartile)	13.0 (7.0, 18.0)	9.0 (2.0, 15.0)		<0.0001 <sup>b,c</sup>
<b>Neuropathological variables</b>				
AD pathology “High” <sup>f</sup>	47.3	42.5	36	<0.0001 <sup>b,c</sup>
HS-Aging (%) <sup>e</sup>	7.1	14.2	113	<0.0001 <sup>b,c</sup>
B-ASC (%)				<0.0001 <sup>a,b,c</sup>
None	31.3	19.9		
Mild	37.0	33.9		
Moderate	23.8	31		
Severe	7.8	15.2		
<b>Genetic variables</b>				
ABCC9.rs704180 genotype A_A (%)	23.8	25.8	1465	0.508
APOE ε4 allele (%)	48.1	39.9	467	<0.0001 <sup>b,c</sup>

In comparing the two age at death groups, *p*-values for age at death, pack years, and BMI are from Mann-Whitney U analyses. *p*-Values for gender, hypertension, diabetes, hypercholesterolemia, B-ASC, ABCC9 HS-Aging risk genotype (rs704180.A\_A), and APOE ε4 allele are determined from Chi-square tests. Percentages were recorded after excluding missing cases for each variable. AD: Alzheimer’s disease; B-ASC: brain arteriolosclerosis; BMI: body mass index; CDRSUM: clinical dementia rating sum of boxes; HS-Aging: hippocampal sclerosis of aging; MMSE: mini mental state examination. <sup>a</sup>Group comparisons exclude cases with missing data. <sup>b</sup>*p*-Value <0.05. <sup>c</sup>Significant *p*-value after Bonferroni correction for multiple comparisons. <sup>a,b,c,d,e,f</sup>Self-reported vascular risk factors. <sup>e</sup>Derived variables from NACC variables. <sup>f</sup>NIA/Reagan Institute Criteria, 1997.

performed using B-ASC pathology as a predictor, other dementia-inducing pathologies as covariates, and MMSE or CDRSUM scores as outcome variables. After adjusting for age at death, cortical microinfarcts, AD pathology (Braak NFT stage and CERAD neuritic plaque rating), neocortical Lewy bodies, and HS-Aging pathologies, B-ASC was a significant pathological predictor in the MMSE model using cases from the <80 years age at death group and in the CDRSUM model using cases from both age groups (Supplementary Tables 3 and 4).

In the < 80 age at death group, the adjusted MMSE and CDRSUM group means for cases with severe B-ASC were worse compared to that of cases with none, mild, or moderate B-ASC pathology (Table 2). In the ≥ 80 age at death group, the adjusted MMSE group mean score for cases with severe B-ASC was worse compared to that of cases with none or mild B-ASC pathology. The adjusted CDRSUM group mean score for cases with severe B-ASC pathology was worse compared to that of cases with none, mild, or moderate B-ASC pathology

**Table 2.** Adjusted final MMSE and CDRSUM Group means associated with brain arteriolosclerosis (B-ASC) pathology.<sup>a</sup>

B-ASC	Age of death < 80 years		Age of death ≥ 80 years	
	MMSE	CDRSUM	MMSE	CDRSUM
None	17.0 ± 0.7	10.5 ± 0.4	21.3 ± 0.6	6.8 ± 0.4
Mild	16.8 ± 0.6	10.8 ± 0.3	21.2 ± 0.4	6.8 ± 0.3
Moderate	17.1 ± 0.8	10.3 ± 0.4	20.4 ± 0.5	<b>7.5 ± 0.3<sup>b</sup></b>
Severe	<b>13.1 ± 1.3<sup>c</sup></b>	<b>12.7 ± 0.7<sup>c</sup></b>	<b>19.5 ± 0.7<sup>d</sup></b>	<b>9.0 ± 0.4<sup>c</sup></b>

CDRSUM: Clinical Dementia Rating Sum of Boxes. MMSE: Mini Mental State Exam. <sup>a</sup>Means are adjusted for age at death (years), sex, Braak & Braak stage, semi-quantitative ratings of diffuse and neuritic plaques, and dummy indicators for microinfarcts, HS-aging, and Lewy body pathology. <sup>b</sup> $p < 0.05$  versus mild. <sup>c</sup> $p < 0.01$  versus none, mild, and moderate. <sup>d</sup> $p < 0.05$  versus none and mild. Bold values indicate significant finding.

(Table 2). In addition, the adjusted CDRSUM group mean score for cases with moderate B-ASC pathology was worse compared to that of cases with mild B-ASC pathology. These results indicate that after adjusting for age and co-morbid brain pathologies, individuals with moderate and severe B-ASC pathology had worse global cognition compared to those with none or mild B-ASC pathology.

#### Clinical vascular risk factors associated with B-ASC pathology

Bivariate analyses and regression modeling were used to assess the relationship between vascular risk factors and B-ASC pathology. Race and ethnicity were not adjusted for in the models because of the low sample size within each group (data not shown). In the < 80 age at death group, hypertension, diabetes, and hypercholesterolemia were associated with B-ASC severity (Supplementary Table 2). In the ≥ 80 age at death group, sex and smoking pack years were associated with B-ASC severity. With respect to clinically evident cerebrovascular disease, self-reported stroke history was associated with autopsied confirmed B-ASC pathology in the < 80 years age at death group ( $p = 0.003$ ) and the ≥ 80 years age at death group ( $p = 0.033$ ) (data not shown). However, there was no association between atrial fibrillation and B-ASC pathology (data not shown). Five vascular risk factors, along with age at death, were used in an ordinal logistic regression, with B-ASC severity as the ordinal outcome measure, to determine risk factors associated with B-ASC severity. In the < 80 age at death group, age at death, female sex, and hypertension were associated with predicting B-ASC severity. In the ≥ 80 age at death group, only age at death and female sex remained to be significant variables in the model but not hypertension (Table 3). These findings suggest that age at death and sex are associated with autopsy-proven B-ASC in both younger and older-aged individuals. However, hypertension may only be a risk factor for B-ASC pathology

only in young elderly individuals, raising the possibility of other B-ASC risk factors being important in more advanced old age.

#### Novel B-ASC genetic risk factor: ABCC9

Genetic and pathological information from NACC were used to assess the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology. Of the 2390 cases included in the analysis, a total of 925 persons had available *ABCC9* SNP information. Individuals with *ABCC9* genotype information were slightly more likely to have AD pathology (45% vs. 40%) than those lacking genotype data (data not shown). Among subjects with available *ABCC9* SNP data, bivariate analysis showed that the *ABCC9* HS-Aging risk genotype was associated with the presence of any B-ASC pathology (mild, moderate, and severe combined) in the ≥ 80 age at death group ( $p = 0.032$ ) (Figure 3). By contrast, *APOE* genotype status was strongly associated with CAA pathology as expected but not with B-ASC pathology (Figure 2(e)).

To account for relevant covariates, a logistic regression analysis was used, treating the presence of B-ASC pathology as a dependent variable, the *ABCC9* HS-Aging risk genotype as an independent variable, and age at death, sex, smoking pack years, and history of hypertension, diabetes, and hypercholesterolemia as covariates. Results from this model showed that individuals in the ≥ 80 years at death group with the *ABCC9* HS-Aging risk genotype were 1.9 times more likely to have a diagnosis any B-ASC pathology (mild, moderate, or severe) compared to individuals without the *ABCC9* HS-Aging risk genotype ( $p = 0.04$ ). There was no association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology in the < 80 age at death group. In a sensitivity analysis adjusting for research center identifications as a fixed effect, the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology was still observed ( $p = 0.04$ ) in the ≥ 80 years at death group.



**Table 3.** Brain arteriolosclerosis and vascular risk factors.<sup>a</sup>

	Beta coefficient ± S.E.	Odds ratio	95% C.I.	p
<b>&lt;80 years age at death group (n = 922)</b>				
Age at death (one-year increase)	0.04 ± 0.01	1.0	1.0–1.1	<0.0001 <sup>b</sup>
Gender (male vs. female)	−0.3 ± 0.1	0.8	0.7–0.8	0.029 <sup>b</sup>
Hypertension (vs. no hypertension)	0.3 ± 0.1	1.4	1.1–1.8	0.017 <sup>b</sup>
Diabetes (vs. no diabetes)	0.3 ± 0.2	1.4	0.9–2.1	0.120
Pack years (one-year increase)	0.0 ± 0.003	1.0	1.0–1.006	0.962
Hypercholesterolemia (vs. no hypercholesterolemia)	0.2 ± 0.1	1.3	1.0–1.6	0.067
<b>≥80 years age at death group (n = 1255)</b>				
Age at death (one-year increase)	0.02 ± 0.01	1.0	1.00–1.04	0.021 <sup>b</sup>
Gender (male vs. female)	−0.2 ± 0.1	0.8	0.7–1.0	0.041 <sup>b</sup>
Hypertension (vs. no hypertension)	0.2 ± 0.1	1.2	1.0–1.5	0.134
Diabetes (vs. no diabetes)	0.001 ± 0.2	1.0	0.7–1.4	0.995
Pack years (one-year increase)	0.003 ± 0.002	1.0	1.0–1.0	0.102
Hypercholesterolemia (vs. no hypercholesterolemia)	−0.1 ± 0.1	0.9	0.7–1.1	0.257

<sup>a</sup>An ordinal logistic regression model was applied in both age at death groups using vascular risk factors identified from exploratory analysis (see Supplementary Table 2). In both age at death groups, the statistical models included cases with available data on all six variables. <sup>b</sup>p-Value < 0.05.

### *ABCC9 and cerebral blood flow*

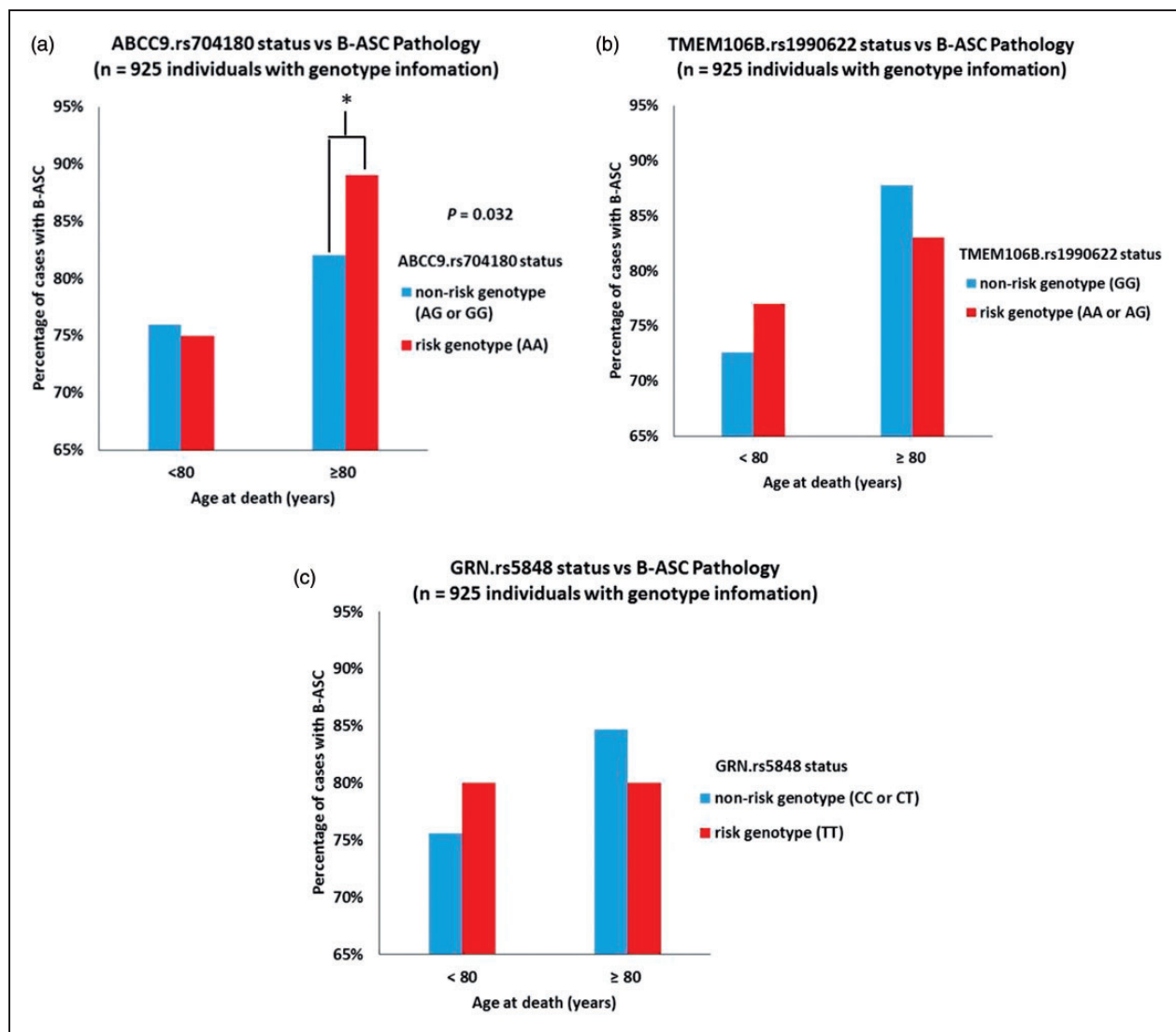
To provide further testing on the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology, we assessed a neuroimaging modality that we hypothesize detects a clinical manifestation of B-ASC pathology. This was performed by measuring CBF in convenience sample of individuals ( $n = 15$ ) from the ADNI data set comparing persons with A\_A versus G\_G *ABCC9*.rs704180 genotype. Individuals were on average 72 years of age at the time of scan and both groups of rs704180 homozygotes (A\_A and G\_G) were matched for cognitive status, *APOE* alleles, sex, and age (see Supplementary Table 5 for complete data on these parameters). Individuals with two *ABCC9* HS-Aging non-risk alleles ( $n = 7$  with rs704180 G\_G) showed higher global CBF measurements compared to those with two *ABCC9* HS-Aging risk alleles ( $n = 8$  with rs704180 A\_A) (Figure 4). The group level relative difference in CBF was 28%,  $p < 0.001$  (Figure 4).

### **Discussion**

In this study, we describe the global cognitive status, in addition to both clinical and genetic putative risk factors of B-ASC, in autopsied cases from the NACC data set. The presence of severe B-ASC pathology was associated with global cognitive impairment. In addition, a neuroimaging CBF experiment further supports the association between the *ABCC9* HS-Aging

risk genotype and B-ASC. Potential risk factors for B-ASC included advanced age at death, hypertension, and sex. In younger individuals (age at death < 80 years), hypertension was associated with B-ASC. However, this association was not observed in older individuals (age at death ≥ 80 years). In the ≥ 80 years age at death group, the *ABCC9* HS-Aging risk genotype was associated with B-ASC pathology. These findings suggest that B-ASC risk factors are age dependent. For example, hypertension appears to have a strong role in younger elderly individuals, while at least one genetic factor (*ABCC9*) may affect the B-ASC risk in the “oldest-old”.

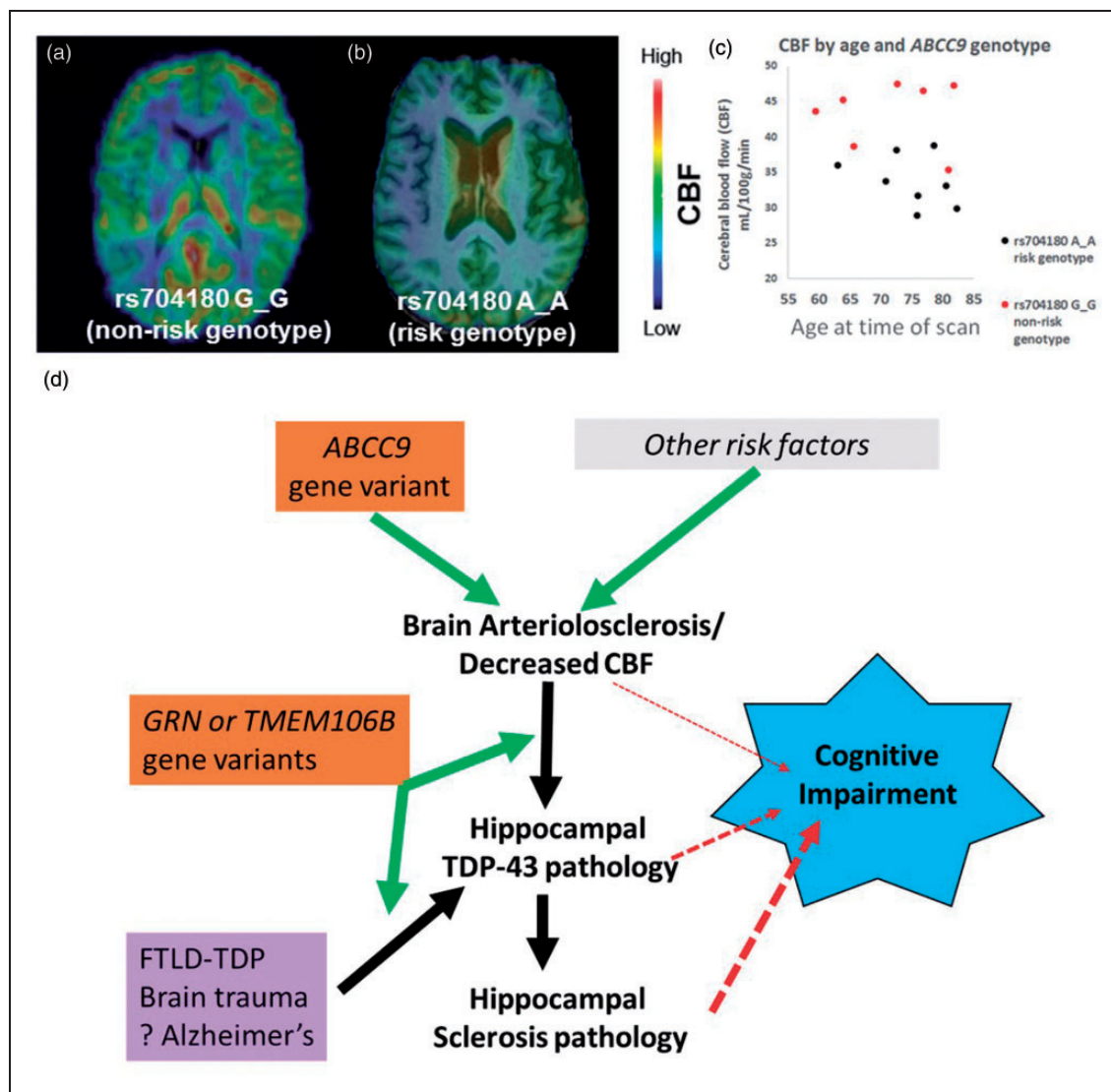
There are potential limitations in this study. The NACC data set is not a population-based data set; it is better characterized as a clinical series of persons enrolled at ADCs, and in addition, carries known biases associated with autopsy cohorts.<sup>42,43,53,61</sup> As a result, NACC participants are predominantly Caucasian, highly educated, and are drawn predominantly from dementia clinics.<sup>42,44</sup> Due to the lack of socioeconomic information and low sampling of individuals from different ethnic/racial groups, race and ethnicity were not included in the regression models. The data on clinical disease risk factors are largely self-reported, which can lead to an underestimation of the true disease frequencies.<sup>62</sup> In addition, duration of disease (i.e. hypertension) data was not available; therefore, it was not adjusted for in the regression models. We found that female sex was associated with



**Figure 3.** Relationship between any degree of brain arteriolosclerosis (B-ASC) pathology in NACC and HS-Aging risk genotypes. (a) The association between *ABCC9* HS-Aging risk genotype (rs704180 A\_A, as determined previously)<sup>24,25</sup> and B-ASC pathology, stratifying by age of death. \*Chi-square  $p$ -value = 0.032. By contrast, as shown in (b,c), neither the *TMEM106B* (rs1990622 A\_A or A\_G) nor *GRN* (rs5848 T\_T) risk genotypes are associated with B-ASC pathology.

B-ASC; this finding is potentially confounded by the increased longevity of women (age being a risk factor for B-ASC) and many other covariates that vary with sex. Although this association survived in a regression model that accounted for other factors including age at death, these data should be interpreted with caution and future work is merited in this area. The NACC neuropathology data set coding guidebook does not suggest optimal brain sections for B-ASC diagnosis. As a result, B-ASC diagnostic methods are inconsistent across ADCs. Although non-NACC guidelines exist for B-ASC diagnosis,<sup>63</sup> different ADCs have reported using the basal ganglia<sup>64</sup> or a “global”<sup>8</sup> criteria in the diagnosis of B-ASC. In a prior, non-ADC autopsy study with 135 vascular dementia brains, B-ASC was seen in

the frontal lobe (83.7% of cases), temporal lobe (80.7% of cases), and basal ganglia (89.6% of cases).<sup>65</sup> Because the diagnosis of B-ASC is presently based on H&E staining, other pathologies that lead to a distortion of vascular walls (i.e. CAA) may mistakenly be diagnosed as B-ASC, leading to a biased estimation of B-ASC frequency. However, we saw a strong correlation between *APOE* status and CAA severity, but no correlation between *APOE* status and B-ASC severity in our sample, indicating that these pathologies are at least partly independent. In the future, improved methodologies and consensus for B-ASC pathologic diagnosis should improve the specificity of B-ASC operationalization. Until then, it can be argued that a multicenter approach, combining data from dozens of



**Figure 4.** Arterial Spin labeling (ASL) neuroimaging indicates that *ABCC9* HS-Aging risk genotype is associated with decreased cerebral blood flow (CBF), compatible with a novel pathogenic mechanism. (a) A representative scan of a 77-year-old female with the *ABCC9* HS-Aging non-risk genotype: rs704180 G\_G. (b) A representative scan from a 76-year-old male with rs704180 A\_A. (c) Individuals with the rs704180 G\_G genotype showed significantly higher global CBF compared to those with the rs704180 A\_A genotype, group level relative difference in CBF is 28%,  $p < 0.001$ . Neither other scans nor SNPs were analyzed from the ADNI data set. (d) An interpretation of the results presented in this paper. We hypothesize that an *ABCC9* gene variant increases risk for B-ASC and/or decreased CBF, which, in turn, may cause or exacerbate hippocampal TDP-43 pathology and HS-Aging. All of these pathologies have been associated with cognitive impairment. Other variants in *GRN* and *TMEM106B* genes are not associated with B-ASC pathology and thus may represent downstream factors, perhaps more directly related to risk of hippocampal TDP-43 pathology. Presumed non-cerebrovascular pathologies (e.g. frontotemporal lobar degeneration (FTLD), brain trauma, and Alzheimer's disease) also are associated with hippocampal TDP-43 pathology, indicating that hippocampal TDP-43 pathology is a common downstream pathological phenomenon.

neuropathologists, each applying center-specific diagnostic rubrics, is the best way to achieve an outcome that is representative of what any given neuropathologist would define as “brain arteriolosclerosis.”

Despite the challenges inherent to a retrospective cross-sectional study, the NACC and ADNI databases provide relatively high-quality contexts to study

clinical, genetic, neuroimaging, and/or pathological correlations. Detailed cognitive assessments, genetic, and neuropathological data have allowed us to study associations of B-ASC with cognitive status and both clinical and genetic variables. These include both “traditional” ASC risk factors (hypertension and diabetes), as well as novel genetic aspects (*ABCC9* SNP). Mixed

pathologies are frequently seen in the aged brain<sup>30,66,67</sup> and detailed neuropathological data from NACC allowed us to adjust for other dementia-inducing pathologies in our analyses. B-ASC is a common cerebrovascular pathology that is often seen in a complex milieu along with other brain diseases<sup>30</sup> and has been associated with motor impairment in advanced old age.<sup>12</sup> In this NACC-ADC data set, the frequency of any B-ASC pathology (mild, moderate, and severe) was 75.3%, and became more severe with increasing age at death, which is consistent with findings from other autopsy cohorts.<sup>11,68,69</sup>

We showed that B-ASC pathology was associated with worse MMSE and CDRSUM scores after adjusting for co-morbid cognitive impairment-inducing pathologies. Prior studies reporting the cognitive profiles of B-ASC are limited, mostly focusing on patients diagnosed with vascular dementia (a very heterogeneous condition).<sup>5-7</sup> Although both age at death groups had MMSE and CDRSUM scores indicative of cognitive impairment, individuals in <80 age at death group were more globally impaired compared to individuals in the  $\geq 80$  years age at death group. This finding may be attributed to the differing frequencies of AD and B-ASC pathologies present in the two age at death groups: higher number of individuals with “high” AD pathology in <80 years age at death group and higher number of individuals with moderate or severe B-ASC pathology in  $\geq 80$  years age at death group. Individuals with AD pathology exhibit greater deficits in memory function and faster rates of information decay compared to individuals with cerebrovascular disease.<sup>70</sup> Our study provided quantitative evidence to support the hypothesis that B-ASC is associated with worse cognitive status independent of other brain changes. Furthermore, it underscores the importance of identifying risk factors, specific neuroimaging abnormalities, and potential treatments of B-ASC, in order to prevent or reverse its development later in life.

Hypertension is a major risk factor for B-ASC.<sup>16,71</sup> In an autopsy study, Moritz and Oldt<sup>16</sup> observed that B-ASC pathology was more severe in the hypertensive group compared to the non-hypertensive group.<sup>16</sup> In the <80 age at death group, we found hypertension to be associated with B-ASC. However, in the  $\geq 80$  age at death group, there was no association detected between B-ASC and hypertension, and these results did not change after adjusting for anti-hypertensive medication use. Similarly, in an autopsy study consisting of 70 cases with B-ASC, 31% of cases were normotensive with 10 of these cases having an age at death  $\geq 80$  years.<sup>71</sup> These results suggest that hypertension may not be the only risk factor for B-ASC pathology in older elderly individuals.

We hypothesize that the known strong impact(s) of diabetes on brain function may be mediated through a combination of vascular and metabolic etiologies.<sup>72</sup> We did not find support for the direct impact of diabetes on B-ASC, but there was a trend between diabetes and B-ASC in the younger cohort. We note that there was not a distinction between Type I or Type II diabetes in the NACC data set, although the majority is presumed to be Type II diabetes. Evidently, beyond the “usual suspects”, there are additional, currently unknown risk factors for B-ASC in advanced old age.

One category of risk factors that may be relevant to B-ASC pathology in the “oldest-old” is genetics, and we here provide support for a specific candidate risk allele. The *ABCC9* SNP rs704180 was previously associated with risk for HS-Aging,<sup>24,25</sup> a hippocampal pathology seen in  $\sim 10$ –25% of autopsied individuals beyond 80 years at death.<sup>26,73-75</sup> Recently, we found an association between HS-Aging and B-ASC in three separate cohorts, including the NACC data set.<sup>8</sup> Using digital image methods for analysis of arteriolar morphology, we found that HS-Aging cases had larger vessel areas, vessel perimeters, vascular areas, and vessel wall thicknesses compared to non HS-Aging cases.<sup>8</sup> Research from a different cohort, using different neuropathological scoring, and statistical methods, reported that moderate B-ASC (but not severe or mild) was associated with hippocampal atrophy.<sup>76</sup> Because of all these findings collectively, we hypothesized that the *ABCC9* HS-Aging risk genotype is associated with B-ASC in advanced old age, possibly upstream of the risk for HS-Aging (Figure 4(d)). The present study showed an association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology in cases with an age at death  $\geq 80$  years. There was no evidence of that association among individuals with age at death <80 years.

In order to test the association between the *ABCC9* HS-Aging risk genotype and B-ASC, we analyzed CBF in elderly individuals. The rationale for this experiment includes that (1) B-ASC is associated with white matter hyperintensities (WMHs) on MRI scans,<sup>77-81</sup> and (2) WMHs have been correlated with decreases in CBF<sup>82-84</sup> and cognitive impairment.<sup>85-89</sup> We found the *ABCC9* HS-Aging risk genotype to be associated with decreased CBF in elderly individuals. These findings support the hypothesis that the *ABCC9* HS-Aging risk genotype promotes B-ASC in the oldest-old with decreases in CBF on neuroimaging. *ABCC9* encodes a regulator of ATP-sensitive potassium channels that is expressed in vascular smooth muscle cells.<sup>90-93</sup> The protein is important for vascular tone regulation and reactivity to metabolic factors and oxidative stress.<sup>90,94-96</sup> We hypothesize that gene variants in *ABCC9* could result in chronic perturbations of the neurovascular unit leading to decrease in CBF and

B-ASC pathology. Therefore, the brain changes associated with *ABCC9* gene variants may be part of a “brain wide” disease characterized by HS-Aging, TDP-43 pathology, and B-ASC in elderly individuals.<sup>97,98</sup> Since *TMEM106B* and *GRN* SNPs were not associated with B-ASC pathology, we hypothesize that these are downstream in the pathological process of HS-Aging (Figure 4(b)). Further studies are warranted to test this hypothesis.

We conclude that B-ASC is a common vascular pathology with a deleterious impact on global cognition in elderly individuals. Risk factors for B-ASC include hypertension, which has long been considered to be a putative modifiable factor, as well as advanced age. Additional possibly targetable mechanisms involved in the B-ASC pathogenesis are mostly unknown, but the results of this study offer candidate pathways involving *ABCC9* gene products. Furthermore, we provide evidence that ASL neuroimaging is a potential candidate biomarker to indicate *ABCC9*-related variations in CBF that could be useful in a clinical setting. These findings may serve to increase awareness about B-ASC, a common cerebrovascular pathology associated with cognitive impairment.

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## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Authors’ contributions

ETI and PTN both conceived the study, wrote the manuscript, and were involved in all aspects of the study design. ELA helped conceive the study, performed statistical analysis, and provided editorial input. AJ performed neuroimaging analysis and provided editorial input. SEM and WAK

provided access to NACC data set and provided editorial input. FA provided assisted in statistical analysis and provided editorial input. DWF, YK, FAS, RJK, GAJ, JHN, SEM, WAK, FA, ADB, and LJV assisted in study design and/or clinical material, and provided editorial input.

### Supplementary material

Supplementary material for this paper can be found at <http://jcbfm.sagepub.com/content/by/supplemental-data>

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