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ORIGINAL ARTICLE

Age differences in arterial and venous extra-cerebral blood flow in healthy adults: contributions of vascular risk factors and genetic variants

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Abstract Sufficient cerebral blood flow (CBF) and venous drainage are critical for normal brain function, and their alterations can affect brain aging. However, to date, most studies focused on arterial CBF (inflow) with little attention paid to the age differences in venous outflow. We measured extra-cerebral arterial and venous blood flow rates with phase-contrast MRI and assessed the influence of vascular risk factors and genetic polymorphisms (*ACE* insertion/deletion, *COMT* val158met, and *APOE*ε4) in 73 adults (age 18–74 years). Advanced age, elevated vascular risk, *ACE* Deletion, and *COMT* met alleles were linked to lower in- and outflow, with no effects of *APOE* ε4 noted. Lower age-related CBF rate was unrelated to brain volume and was observed only in val homozygotes of *COMT*val-158met. Thus, in a disease-free population, age differences in CBF may be notable only in persons with high vascular risk and carriers of genetic variants associated with vasoconstriction and lower dopamine availability. It remains to be established if treatments targeting alleviation of the mutable factors can improve the course of cerebrovascular aging in spite of the immutable genetic influence.

Keywords Jugular vein · Internal carotid artery · Vertebral artery · Phase-contrast MRI · *COMT* · *ACE*

Introduction

The fact that the brain draws more than 20% of the body's blood supply while comprising only 2% of the total body weight (Attwell and Laughlin 2001) underscores the importance of studying cerebral blood flow (CBF). Investigating factors that influence CBF is particularly important in the context of brain aging. Several investigations have demonstrated with various in vivo methods that compared to young adults, healthy elderly have lower arterial CBF (Chen et al. 2011; Henriksen et al. 2014; Parkes et al. 2004; Shirahata et al. 1985; van Es et al. 2010; Zarrinkoob et al. 2015; Zhao et al. 2007), and a longitudinal investigation found arterial CBF declines over time (ten Dam et al. 2007).

Despite these descriptive findings, the mechanisms of age differences in CBF remain unclear. Age-related differences in the cerebrovascular system appear plausible candidates for explaining the reportedly diminished CBF in older adults. Advanced age is accompanied by progressively increasing risk for cardiovascular disease with prevalence, severity and cumulative detrimental effects of major vascular risk factors—such as hypertension, reduced cardiac output, obesity, sedentary lifestyle, and tobacco smoking

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(Mozaffarian et al. 2015)—steadily rising with advanced age. Indeed, in some extant studies, assorted vascular risk factors have been linked to lower arterial CBF and reduced cerebral perfusion beyond the influence of calendar age (ten Dam et al. 2007; Vernooij et al. 2008). Moreover, some findings suggest that although larger brains draw greater CBF (van Es et al. 2010; Vernooij et al. 2008; Zarrinkoob et al. 2015), age-related declines in CBF may exceed the magnitude expected from age-related parenchymal shrinkage (Chen et al. 2011).

Whereas arterial CBF in aging is relatively well described, significantly less is known about the venous drainage system that guides deoxygenated blood into the dural sinuses and then into the internal jugular veins (Doepp et al. 2004). The extant studies of age differences in brain drainage reveal slower internal jugular vein (IJV) flow and greater IJV lumen area (Chung et al. 2010; Magnano et al. 2016) with advanced age, and some have theorized that abnormalities of venous drainage might contribute to cerebral aging and cognitive declines (Zivadinov and Chung 2013). Thus, understanding the influences that shape CBF in both directions may elucidate structural and functional changes in the aging brain and show the way to possible mitigation thereof.

Blood flow to and from the brain is determined by multiple biomechanical, neurochemical, and physiological variables, and variations in CBF rate depend on blood viscosity, compliance of the blood vessels, structural remodeling of vascular beds as well as the levels and activity of multiple hormones and neurotransmitters that act as vasoconstrictors or vasodilators (Armulik et al. 2011; Casey et al. 2015; Hall et al. 2014; Henriksen et al. 2014; Nishijima et al. 2015). Dilation and constriction of the cerebral arteries and veins have a substantial effect on CBF (Armulik et al. 2011; Hall et al. 2014), and common genetic variants associated with vascular function may affect cerebral and extracerebral blood flow. Recent studies showed that motility of specialized contractile cells, and pericytes and contractility of the blood vessels are lower in carriers of a variant of the Apolipoprotein E gene, *APOEε4* (Casey et al. 2015; Hajjar et al. 2015). Although *APOEε4* is arguably the best known genetic risk factor for Alzheimer's disease (AD), it is also important in many vascular processes and the effects of its risky allele extend beyond AD (Song et al. 2004). Notably, CBF is lower in *APOE* ε4 carriers regardless of AD diagnosis (Kim et al. 2013; Thambisetty et al. 2010). In addition, *APOE* ε4 variant has been linked to venous thrombosis and flow abnormalities in lower extremities (Zhu et al. 2014), although at the time of this writing, its effect on cerebral outflow is not known.

Compliance of blood vessels is also affected by several hormones and neurotransmitters that act as vasoconstrictors or vasodilators, such as angiotensin II, bradykinin, norepinephrine, and dopamine (Greenfield and Tindall 1968; Messina et al. 1975). Availability of a potent endogenous vasoconstrictor, angiotensin II, is controlled by the angiotensin converting enzyme ACE (also known as dipeptidyl-carboxypeptidase I), which in addition to enabling its synthesis from angiotensin I inhibits vasodilator bradykinin (Vauquelin et al. 2002; von Bohlen und Halbach and Albrecht 2006). Notably, a higher concentration of ACE in the cerebro-spinal fluid has been linked to lower CBF (Janciauskiene et al. 2009). As is the case with many enzymes, ACE is under genetic control and the *ACE* gene that affects its production has a common functional polymorphism that is due to insertion (I) and deletion (D) of an Alu repetitive element in the intron of *ACE*. The change produces three genotypes: $Alu^{+/+}$, $Alu^{+/-}$, $Alu^{-/-}$, or *ACE* I/I, *ACE* I/D, and *ACE* D/D, respectively. The insertion allele is associated with dose-related reduction in ACE activity and by thus limiting synthesis of angiotensin II, exerts influence similar to that of an ACE inhibitor—i.e., limits vasoconstriction. Carriers of the D (high activity) allele evidence increased susceptibility to vascular disease (Lao et al. 2005), increased risk for hypertension (Bautistaet al. 2008), increased risk for stroke of large vessels (Saidi et al. 2009), and greater leukoaraiotic burden in the brain (Hassan et al. 2002). In some studies, ACE D/D homozygotes are less prevalent in older age groups, indicating a possible negative impact on survival (Morris et al. 1994). Carrying *ACE* I (low activity) allele has been associated with lesser arterial stiffness (Benetos et al. 1996; Mattace-Raso et al. 2004; Taniwaki et al. 1999), although in some samples, the effects are sex-specific (Lynch et al. 2007). At the time of this writing, there are no studies that examine *ACE* I/D differences in arterial or venous CBF in healthy adults.

An influential vasoactive hormone, dopamine, exerts its influence as a vasodilator via its effect on the smooth muscles of the blood vessels (Zeng et al. 2007). Dopamine availability is regulated by multiple means, including pre- and postsynaptic receptors and catabolizing enzymes, such as catechol-*O*-methyl transferase (COMT). The activity of COMT is controlled by an eponymous gene *COMT* that has a common single nucleotide polymorphism (SNP) *COMT* val158met. The mutant met allele of that polymorphism is associated with fourfold reduced activity of COMT and thereby greater availability of dopamine, which plays an important role in regulating arterial blood pressure (Jose et al. 2003). In adults, *COMT* val/val genotype has been linked to systolic hypertension (Hagen et al. 2007) increased rate of conversion to dementia (Dixon et al. 2014), and in children, resting CBF is reduced with increased dose of the val allele (Thomason et al. 2009). There is, however, no comparable study of the influence that SNP may exert on cerebrovascular function in adults. Because the dopaminergic system undergoes significant

and possibly nonlinear changes with age (Morgan et al. 1987), it is plausible that genetic propensity for higher dopamine availability may indirectly affect its correlates, including CBF, in an age-dependent manner.

Given the effect common vascular risk factors have on brain aging (Jagust 2013; Raz and Rodrigue 2006), and the calls to pay more attention to disentangling contributions of calendar age and accompanying vascular changes (e.g., Morra et al. 2013), it is surprising that the effects of genetic variants on CBF have not before been evaluated in conjunction with the influence of common age-related vascular risk factors, such as hypertension. The aim of this study was, therefore, to examine the effects of common vascular risk factors and two genetic variants known to affect brain vascular function on CBF inflow and outflow. We hypothesized that in addition to older adults having slower blood flow rate, carriers of *APOE* ε4, and homozygotes for *ACE* D or *COMT* val158 alleles would evidence additional reduction in arterial and venous blood flow.

Method

Participants

The sample was drawn from adult volunteers ($N=73$, 45 women, age 18–74 years), who took part in an ongoing longitudinal study and were recruited from the Detroit metropolitan area through advertisement in local printed and electronic media. Because blood flow assessment was introduced after commencement of the study and terminated before its conclusion, only a limited number of participants were recruited into this sub-study, based on their scheduled visits within a defined period, on a first-come, first-assessed basis. Thus, in the context of a larger study, this sample was not selected on any particular additional criteria and participants who were not included can be considered missing at random. The participants underwent phone interviews and completed a 66-item health questionnaire, from which information pertinent to their inclusion in the study was drawn. The reasons for exclusion were reported history of cardiovascular, neurological, and psychiatric conditions, head trauma with a loss of consciousness for more than 5 min, history of alcohol and drug abuse, thyroid problems, hypertension, and diabetes. The items used to screen for cardiovascular disease included any sort of "heart troubles" and cardiovascular complaints as well as taking specific medications prescribed for treatment of cardiovascular symptoms. The participants had corrected visual acuity of 50/20 or better (Optec 2000, Stereo Optical, Chicago, IL) and hearing of 40 dB or better for frequencies of 500–4000 Hz (Maico, MA27, Eden Prairie, MN). To screen for dementia and depression, we used the Mini-Mental State Examination (MMSE; Folstein et al. 1975) and the Center for Epidemiologic Studies Depression Scale (CES-D; Radloff 1977). Only persons who scored 26 or above on the MMSE and 15 or below on the CES-D were invited to participate. All participants provided written informed consent in accord with university and hospital review board guidelines. Because the phase-contrast MRI sequence was introduced late in the study, the participants were at various stages of longitudinal assessment and insufficient cognitive data common to all participants across the stages of study did not allow for testing cognitive correlates.

Vascular risk assessment

We measured blood pressure on four separate days by a mercury sphygmomanometer (BMS 12-S25) with a standard blood pressure cuff (Omron Professional) on the left arm, with participants seated in a comfortable chair. The systolic and diastolic measures were averaged for each individual across sessions. The data on exercise regimen, smoking, and history of hypertension as well as anti-hypertension medication were obtained through a questionnaire. A compound vascular risk index was designed to reflect recognized factors for cardiovascular disease (Mozaffarian et al. 2015), and was computed as VR = Hypertension + (((Systolic Blood Pressure − 120)/20)) + (BMI/20) + Smoking + Exercise + (Exercise Frequency/7)), where hypertension diagnosis (8 participants reported), history of smoking (5 participants reported), and reported engagement in physical exercise were dichotomous incidence variables (0 vs. 1, with 14 participants reporting no exercise-like physical activity); exercise frequency was the number of days exercising per week; BMI, body-mass index was computed as weight over squared height (kg/m^2) and scaled by 20; and the systolic blood pressure measure was an average computed over 2–4 (most commonly 4) occasions, referenced against the normative value of 120 mmHg and scaled by 20 to bring it to the common scale and avoid undue influence on the composite score.

Blood panel data

Hematocrit determines blood viscosity and therefore affects blood flow. In addition, older persons may have lower hematocrit (Aanerud et al. 2012; Henriksen et al. 2014); we examined if this was the case in our sample. We found that although, as expected, men had higher hematocrit than women [44.0 vs. 40.35%, $F(1,61)=32.158$, $p<.001$], within the normal range of hematocrit values sampled here, no differences were noted with respect to age, vascular risk, or genetic variants (all $F\leq 1$). As there was no association between hematocrit with age or blood flow rate ($r=-.02$

to 0.08, ns), we did not add hematocrit to the statistical model. Eight participants took anticoagulants but did not differ from their age-matched peers in blood biomarkers and blood flow values. They were retained in the study. Key descriptive statistics of the sample are presented in Table 1.

MRI data acquisition

All images were acquired on a 3 T Verio scanner (Siemens GmbH, Erlangen, Germany). The MR scanning protocol included conventional structural imaging and a comprehensive evaluation of the head and neck vasculature via 2D phase-contrast (PC) flow quantification. For brain volume estimation, the structural scans were acquired in the coronal plane using a 12-channel head coil. Structural imaging included an MP-RAGE sequence with the following parameters: echo time (TE) = 4.38 ms, repetition time (TR) = 1600 ms, inversion time (TI) = 800 ms, field of view (FOV) = 256 × 256 mm^2, resolution = .67 × 0.67 × 1.34 mm^3, matrix size = 384 × 384, flip angle (FA) = 8°, and GRAPPA factor = 2. To evaluate possible white matter lesions, a set of 50 contiguous axial slices of fluid-attenuated inversion recovery (FLAIR) images was acquired with the following parameters: TR = 8440 ms, TE = 112 ms, TI = 2200 ms, FA = 150°, FOV = 256 × 256 mm^2, in-plane resolution = 1 × 1 mm^2, slice thickness = 2 mm, and matrix size = 256 × 256. All MP-RAGE and FLAIR images were inspected for potential pathology, and possible incidental findings were reviewed by an experienced radiologist.

The PC-MRI images were acquired with a 16-channel head/neck coil perpendicular to the IJVs at the C2/C3 level, which is superior to the carotid bifurcation and intersects the IJV inferior to the jugular foramen. The participant's head was secured with head coil restraints resting on the subject's forehead and a neck pillow positioned to the participant's comfort. The procedure produces internally consistent measures of venous and arterial blood flow (Sethi et al. 2015). After a brief neck vessel localizer scan, the PC-MRI sequence was acquired with the following parameters: TR = 95.25 ms, TE = 10 ms, FA = 20°, FOV = 160 × 160 mm^2, in-plane resolution = 0.57 × 0.57 mm^2, slice thickness = 2.5 mm, matrix size = 448 × 448, bandwidth = 192 Hz/pix, GRAPPA = 2, and maximum encoding velocity (VENC) = 50 cm/s [see previous publications for details (Haacke et al. 2012, 2015; Feng et al. 2012)].

A note on parameter selection is in order. Higher VENC of 80 cm/s has been used in some studies for measuring large vessel flow in the extracranial head and neck (e.g., Stoquart-Elsankari et al. 2009). However, we focused on venous flow, which tend to be slower especially in the paraspinal veins. Therefore, we opted for a lower VENC, which ensured higher signal-to-noise ratio (SNR) in the venous flow measurements, so that only one scan was needed. The tradeoff for improved SNR is possible aliasing of arterial vessel flow, which exceeds the VENC value. This tradeoff is mitigated if the peak velocity stays within a reasonable range (e.g., smaller than three times the VENC) and by applying the automatic unwrapping software (Lotz et al. 2002). In the SPIN software used in this study, we implemented a robust automatic unwrapping algorithm that compares pixelwise phase values in x, y, and z directions and ensures that only pixels that are aliased are unwrapped (Jiang et al. 2015). Moreover, in our experience, the venous flow aliasing is rare and a VENC value of 50 cm/s is sufficiently high to handle it.

MRI data processing

The 2D PC-MRI images were processed with the in-house written software, SPIN (Signal Processing in NMR, Detroit, MI, USA; http://mrinnovations.com/index.php?site=spin, last accessed 12/07/2015). The software was used for quantifying blood flow rate through major arteries and veins, including internal carotid arteries (ICA) and vertebral arteries (VA) for in-flow and the internal jugular veins (IJV) for outflow. Phase unwrapping was performed when the maximum velocity exceeded 50 cm/s. Two trained raters (A.M.D. and S.S.) delineated vessel boundaries on magnitude images in reference to the processed phase images using a computer-assisted full-width half-maximum (FWHM) region growing threshold method with manual editing when necessary (see Sethi et al. 2015 for details). In this manner, six vessels were evaluated: left and right IJV, ICA, and VA. Flow rates (mL/s) were calculated from differences in phase contrast over time,

Table 1 Sample descriptors

	Range	Mean	SD
Age (years)	18–74	48.12	17.24
Systolic blood pressure (mmHg)	96.50–168.33	119.90	13.55
Diastolic blood pressure (mmHg)	59.50–125.33	75.96	9.96
Hematocrit (%)	35.50–48.20	41.70	2.99
Exercise frequency (days per week)	0–7	3.40	2.12
BMI	17.17–38.22	26.11	4.82
Total brain volume (cm^3)	895.48–1384.68	1113.71	99.71
Gray matter volume (cm^3)	461.69–741.12	580.43	59.76
White matter volume (cm^3)	426.13–650.00	533.28	46.98
MMSE	26–30	28.73	1.04
Education (years)	12–20	15.37	1.88
CES-D	0–15	5.11	4.38

BMI Body-Mass Index, *MMSE* Mini-Mental State Examination, *CES-D* Center for Epidemiologic Studies Depression Scale

taking into account the vessel area for each left and right sides, and were summed over the two sides to calculate total flow rate. All flow measures had high inter-rater reliability estimated via intraclass correlations computed with an assumption of random raters [formula ICC(2,1); Shrout and Fleiss 1979]. The reliability for each vessel type was as follows: IJV, ICC(2,1) = 0.99; ICA, ICC(2,1) = 0.99; VA, ICC(2,1) = 0.93; other smaller vessels (e.g., vertebral veins) could not be measured with comparably high reliability, and were thus omitted from analysis. For narrowing of the upper IJV body above C3/C4, a cross-sectional area of less than 12.5 mm^2 was considered stenotic (Haacke et al. 2015). Six participants met this criterion in at least one of their IJVs, and were, therefore, excluded from the analyses, as it was unclear if their flow was carried by collateral veins not measured in this study.

Total brain volumes, as well as volumes of the gray and white matter, were estimated from the MP-RAGE scans. The images were downsampled to 1 mm^3 isotropic voxels and were processed with the FSL brain extraction tool (BET; Smith 2002) for total brain volume, as well as tissue segmentation with the FMRIB's automated segmentation tool (FAST; Zhang et al. 2001) for total gray and white matter volumes. To avoid overestimation, we used a value of 0.3 for the fractional intensity threshold option (−f in FSL) instead of the default 0.5. To prevent overestimation, the mean partial volume fraction for gray and white matter was calculated using the fslstats option in FSL. The segmented images were then binarized, and the volume of each tissue type was determined. Finally, the mean partial volume fraction was multiplied by the volume of the binarized tissue regions to yield a volume for each tissue type.

Genomic analysis

The details of the genomic methods can be found in the previous publications (Raz et al. 2009, 2011). DNA was isolated from buccal cultures obtained in mouthwash samples, using a Gentra Autopure LS under the standard buccal cell protocol. After sequencing and purification, DNA was analyzed on an ABI PRISM 3700 DNA Analyzer. All 5′-nuclease assays adapted from a quantitative PCR method (Lo et al. 2000) were performed on an Applied Biosystems 7900.

APOE (rs429358 and rs7412) polymorphisms were preamplified with forward 5′-CAATGCTACCGAGTTTTC TTCC-3′ and reverse primers 5′-TTCAGATTCTTCACA GATGCGTA-3′ in a 25 µl reaction containing 2.5 mmol/l MgCl2, 0.5 µmol/l of the primers, 1.25 U AmpliTaq Gold polymerase, and 200 µmol/l dATP, dCTP, dGTP, and dTTP. The primers and probes for the rs7412 assay were 5′-TCC GCGATGCCGATGAC-3′, 5′-CCCCGGCCTGGTACAC-3′, VIC-CAGGCGCTTCTGC-NFQ, and FAM-CAGGCA CTTCGC-NFQ. The primers and probes for the rs429358 assay were 5′-GCGGGCACGGCTGT-3′, 5′-GCTTGC GCAGGTGGGA-3′, VIC-CATGGAGGACGTGTGC-NFQ, and FAM-ATGGAGGACGTGCGC-NFQ. *COMT* val158met (rs4680) polymorphism was interrogated using Taqman SNP Genotyping assays. For *ACE* I/D (rs4646994) genotyping, DNA was amplified using either ACE-1721F (insertion) or ACE-1428F (deletion) and ACE-1826R and interrogated using the TaqMan probe 1745T.

The allelic groups of *COMT* val158met were distributed as follows: 20 val homozygotes; 11 met homozygotes; and 42 heterozygotes. Hardy–Weinberg equilibrium test: $\chi^2 = 2.07$, $p = .15$. In the distribution of *ACE* I/D alleles, heterozygotes constituted approximately half of the sample (46%, $N = 36$), with 25 D/D and 17 I/I homozygotes. The distribution of *ACE* genotypes conformed to Hardy–Weinberg equilibrium: $\chi^2 = 0.35$, $p = .55$. For *APOE* polymorphism, the majority (73%, $N = 53$) were APOEε3 homozygotes, 5 were APOEε23 heterozygotes, 14 APOEε34 heterozygotes, and one APOEε4 homozygote. To avoid dealing with cells with $N < 5$, the allelic groups were combined and the sample was divided into 15 (21%) *APOE*ε4 carriers and those who did not carry that allele ($N = 58$). Because the sample was heterogeneous with respect to the population of origin, we tested for differences in allele frequency between African-Americans (21% of the sample) and European-Americans, and found no significant differences in allele distribution. For *APOE* ε4: $\chi^2 = 0.01$ $p = .93$; COMT: $\chi^2 = 4.12$, $p = .13$, and *ACE* I/D, $\chi^2 = 1.68$ $p = .43$, the allele distributions of two populations did not differ.

Statistical analyses

A single multivariate general linear model was fitted to the data. In the full model, in-flow (via ICA and VA, combined) and outflow (via IJV) served as two levels of a within-subject variable. Because sex composition of the sample was unbalanced (45 women to 28 men, $\chi^2 = 3.959$; $p = .047$), sex was included in the model as a categorical factor. Age (centered at its sample mean), total brain volumes, and vascular risk score were entered as continuous independent variables. Using brain volume as a covariate is preferred to taking a flow-to-brain volume ratio, as ratio variables have been demonstrated to be inferior to covariates or residualized variables for the purposes of correction (e.g., Arndt et al. 1991; Mathalon et al. 1993). For the analyses of genetic effects, the variants were dichotomized according to hypothetically "risky" alleles as two-level between-subject factors: ApoE (0 for no ε4 allele vs. 1 for carrier of ε4), ACE (0 for I carriers vs. 1 for D homozygotes), and COMT (0 for met allele carriers vs. 1 for val homozygotes). Type III error was used to allow for unbalanced categorical groupings, and Huynh–Feldt correction

was applied to correct for violation of sphericity assumption of the repeated measures analyses. The second-order interactions between pairs of independent variables were tested in a full model and if found non-significant ($p > .10$), removed from the model; reduced model was evaluated afterwards and its results reported.

Results

Descriptive statistics and preliminary analyses

The full correlation matrix for the brain variables, age, and vascular risk is presented in Table 2. Inspection of that matrix indicates high correlations between the arterial and venous flow rates, but revealed no associations of flow indices with parenchymal volume. There was no association between age and the total brain, gray or white matter volumes. As mentioned above, hematocrit was unrelated to the blood flow rate, age, or vascular risk. On the other hand, the vascular risk index positively correlated with age and negatively related to arterial blood flow rate.

To assess the need for separating variables by left vs. right side, we conducted a preliminary analysis, which revealed greater blood flow rate in the right IJV compared to the left IJV [5.16 ± 2.16 vs. 3.57 ± 2.11 mL/s; $F(1,69) = 16.96$, $p < .001$], but there were no interactions between the vein side and age, sex, or vascular risk (all $F < 1$, ns). ICA blood flow rate exhibited no lateral asymmetry: $F(1,69) = 2.26$, $p = .14$, nor there were lateral differences in VA blood flow rate: $F < 1$, ns. Interactions between the artery side and age, sex, or vascular risk were not significant (all $p > .20$). Therefore, to conserve degrees of freedom, for the main analyses, flow rate indicators were summed across right and left sides.

Effects of age and vascular risk

In the main analysis of the effects of age and vascular risk on blood flow rate, none of the interactions in the full model, except COMT × age, reached significance (all $p > .25$), and therefore, were removed from the model. Estimation of the reduced model revealed that in-flow was greater than outflow: $F(1,65) = 15.76$, $p < .001$; 10.28 ± 0.22 vs. 8.87 ± 0.22 mL/s (model adjusted means ± standard errors here and throughout). The main effect of age $F(1,65) = 9.44$, $p = .003$ reflected overall greater flow in the younger participants. The main effect of vascular risk $F(1,65) = 4.03$, $p = .049$ was due to lower flow in persons with higher risk scores. Although age × vessel type and vascular risk × vessel type interactions were not significant, simple effects analysis revealed that both effects were significant only for the arterial, and not venous, flow. For age the effects were $F(1,65) = 14.91$, $p < .001$ for arterial flow rate, and $F(1,65) = 2.79$, $p = .10$ for venous flow rate. Bootstrap analysis with 5000 replacement samples of the whole sample produced regression coefficient estimates of $b = -0.03$ mL/s/year, with 95% confidence interval (CI) −0.06/−0.01 for in-flow, and $b = -0.02$, 95% CI −0.05/0.02 mL/s/year for outflow. For vascular risk, simple effects were as follows: for in-flow: $r = -.30$, $b = -0.68$, bootstrapped 95% CI −1.25/−0.20 mL/s/unit of risk, and for outflow: $r = -.19$, $b = -0.46$, 95% CI −1.01 to 0.14 mL/s/unit of risk. Note that both bootstrap CIs for outflow included 0. These analyses are illustrated with regression plots in Fig. 1.

A significant main effect of *ACE* I/D: $F(1,65) = 6.56$, $p = .01$, reflected lower flow in D homozygotes compared to the I carriers: 10.85 ± 0.28 vs. 9.73 ± 0.38 mL/s for in-flow and 9.53 ± 0.35 vs. 8.66 ± 0.47 mL/s for outflow. The main effect of *COMT* val158 met [$F(1,65) = 12.61$, $p = .001$] was

Table 2 Correlation matrix for blood flow, brain volume, vascular risk, and age

	Age	Vascular risk	Brain	Gray	White	IJV flow	ICA flow	VA flow	BMI	Systolic	Diastolic
Vascular risk	**0.411**										
Brain volume	−0.172	0.093									
Gray matter volume	−0.177	0.057	**0.949**								
White matter volume	−0.140	0.124	**0.916**	**0.741**							
IJV flow	−0.122	−0.185	0.027	0.045	0.000						
ica flow	−0.262	−0.240	0.075	0.093	0.040	**0.546**					
va flow	−0.149	*−0.300*	0.164	0.203	0.090	**0.519**	**0.390**				
BMI	0.182	**0.624**	0.092	0.055	0.126	−0.010	*−0.300*	−0.222			
Systolic blood pressure	**0.406**	**0.940**	0.077	0.044	0.107	−0.162	−0.202	−0.262	**0.592**		
Diastolic blood pressure	0.163	**0.747**	0.171	0.144	0.179	−0.159	−0.097	−0.226	**0.559**	**0.811**	
Hematocrit	−0.060	0.055	0.032	−0.010	0.080	0.024	0.019	−0.180	0.217	0.097	0.103

Critical values: $r = .022$, $p \leq 0.05$; $r = 0.27$, $p < .01$; $r = .35$, $\mathbf{p < 0.001}$

BMI Body-Mass Index, *IJV* internal jugular vein, *VA* vertebral artery, *ICA* internal carotid artery, *VR* vascular risk score (see text for details)

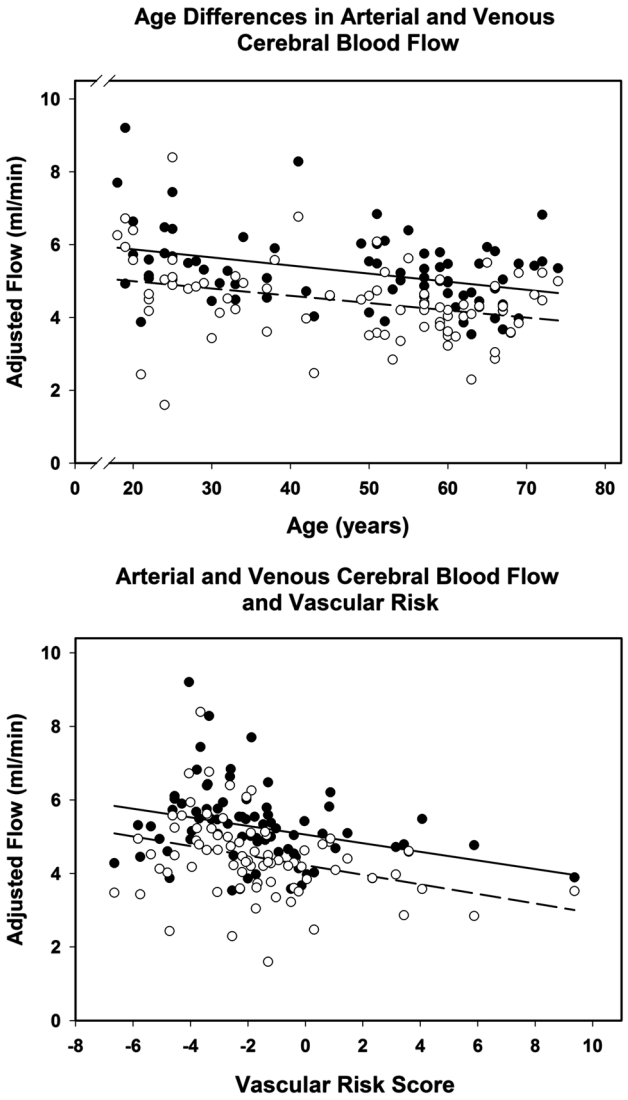

Fig. 1 Age- and vascular risk-related differences in the rate of cerebral blood flow: arterial (into the brain) and venous (out of the brain)

due to carriers of the met variant having lesser blood flow rate in both directions: 10.75 ± 0.40 vs. 9.83 ± 0.27 mL/s for in-flow and 9.96 ± 0.50 vs. 8.23 ± 0.33 mL/s for outflow. The differences between allelic groups are illustrated in Fig. 2.

Notably, age differences were modified by a significant age × *COMT* interaction: $F(1,65) = 14.76$, $p < .001$. Simple effects analyses with bootstrap estimates of regression coefficients indicated that age-related slowing of flow rate was observed only in *COMT* val homozygotes ($r = .72$; $b = -0.12$ mL/s per year, 95% CI −0.16/0.06 for in-flow and $r = .58$, $b = -0.09$ mL/s per year, 95% CI −0.16/−0.04 for outflow) and not in met carriers ($r = -.10$ for in-flow and $r = .06$ for outflow, both ns). The age × *COMT* interaction is depicted in Fig. 3.

Neither sex differences nor *APOE* effect were noted: both $F < 1$, ns. The addition of the brain volume as a covariate to the model did not affect the results except for moving the significance level for vascular risk from $p = .049$ to $p = .056$.

Discussion

The main novel finding in this study is that a genetic variant (*ACE* deletion) linked to increased propensity for vasoconstriction was associated with lower arterial and venous extra-cerebral flow. In addition, participants with higher composite load of vascular risk factors had lower extra-cerebral blood flow, although this association was more robust for in-flow than for outflow. Notably, a modest age-related reduction in arterial and venous flow was observed only in persons who did not carry the allele linked to greater availability of dopamine.

In contrast to reported higher arterial CBF in children who carried the met allele of *COMT* val158met (Thomason et al. 2009), we observed higher flow rates in adult val homozygotes. The observed interaction between genetic propensity for lesser dopamine availability and

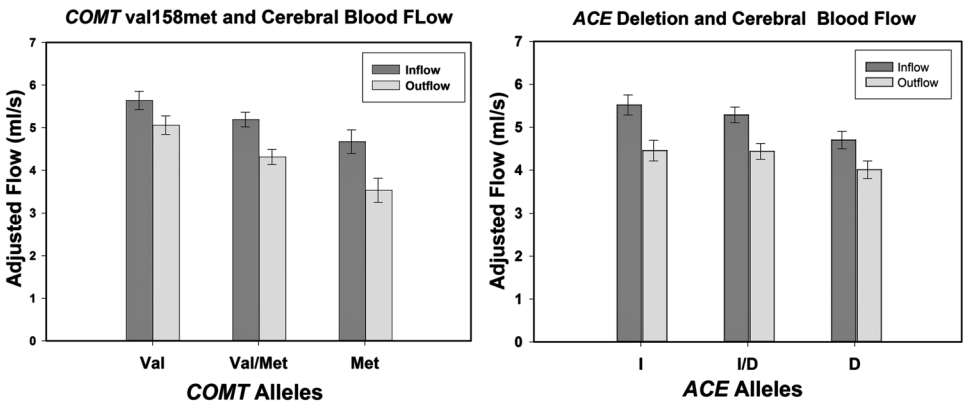

Fig. 2 Differences in blood flow rate between **a***COMT* val158met val homozygotes and met carriers and **b** between *ACE D* homozygotes and *I* carriers. The *bars* represent the least-square means from the general linear model (see text); the *error bars* are standard errors around the least-square means

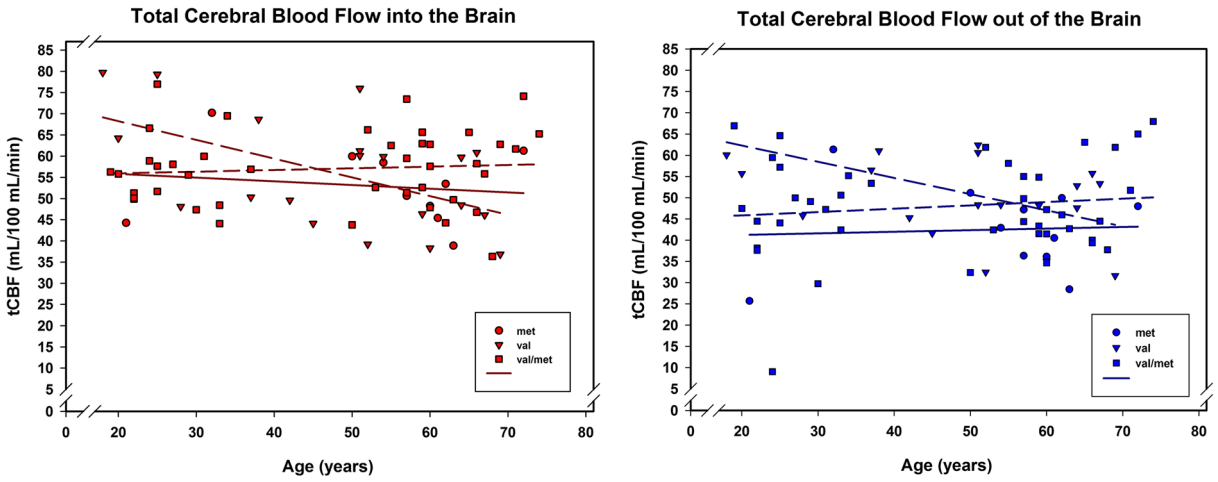

Fig. 3 Age-related differences in blood flow rate by *COMT* val158met genotype. The regression on age shows a significant negative slope only for val homozygotes (*solid line*) but not for met carriers (*dashed line*)

age differences in extra-cerebral blood flow rate mirrors the age-dopamine interaction in relation to cognitive performance as predicted by the inverted-U model (Bäckman et al. 2006, 2010). The inverted-U model posits that because of decline in dopamine in old age, the impact of genetic differences in dopamine availability on the brain and cognition increases with age. Persons who have lower amounts of dopamine due to genetic variation are more affected by age-related declines in the dopaminergic system than their counterparts who carry more dopamine-favorable genetic variants. Although the effects of genetic dopamine variation are not strong, they support the proposition that when it comes to aging, even the modest unfavorable genetic influence may provide a "nudge" in the direction of decline (Raz and Lustig 2014). It is possible that the effect of *COMT* val158 met on dopamine is pleiotropic with respect to age, i.e., that younger adult val homozygotes (lower available dopamine) evidence faster blood flow than met carriers, but children and older adults who have lower dopamine levels than young and middle age adults may have a particularly significant reduction in blood flow rate. The observed pattern of age and genetic differences in blood flow suggests a negative pleiotropism as observed in some previous studies that showed advantage for val/val homozygotes of COMT val158met in younger age but not among older adults (e.g., Lee and Qui 2016). A larger population-based study covering the lifespan age range is needed to test this hypothesis.

The venous outflow was lesser than arterial in-flow, which suggests that some of the venous blood could have been drained through a system of collateral vessels. The role of vertebral and collateral drainage system in reducing age and vascular risk effects on the outflow compared to the inflow is a plausible hypothesis but with the current methods that restrict reliable measures to only major vessels, it could not be tested. Evidence to date, however, does not support a major role for collateral contributions to drainage in healthy adults (Zamboni et al. 2013). Although in persons with stenosis, collateral drainage is likely, exclusion of such participants from this study made this a remote possibility (Sethi et al. unpublished data). Moreover, in supine position as employed in our study, most of venous drainage occurs through the jugular vein (Shenkin et al. 1949).

Notably, in this sample, total brain size was unrelated to the blood flow rate and calendar age. Although age-related differences in parenchymal volume with age are observed in many studies (Raz 2000; Raz and Rodrigue 2006), this is not necessarily a universal phenomenon. This finding may stem from the fact that in our sample, the participants were selected for optimal health. Thus, the lack of brain volume associations with age and blood flow serves to underscore the sensitivity of blood flow rate to even moderate health risks in a highly-selected cohort.

The mechanisms of the observed age-related differences in arterial blood flow rate are unclear. Although total and regional CBF were linked to increase of transition time between arteries and arterioles as assessed by the arterial spin labeling (ASL) technique (Liu et al. 2012), it is unclear how comparable ASL findings are to our results that were obtained with PC-MRI. The latter produces higher estimates of CBF and is imperfectly correlated with the former (Dolui et al. 2016).

The results reported here may have important implications for interpreting age differences in task-related activation observed in fMRI studies. Although imaging based on brain oxygen-level difference (BOLD) effect is a staple of cognitive neuroscience of aging, interpretation of activation

maps obtained in fMRI comparisons of young and older participants is not straightforward. BOLD is an essentially cerebrovascular phenomenon and its dependence on the amount and flow rate of arterial blood is crucial to understanding age differences in resting state, as well as task-related activation. With increase in vascular risk being an important characteristic of typical aging, and the observed association between physiological and genetic vascular risk factors with cerebral arterial blood flow rate, it is important to take age-related differences in blood flow into account when interpreting fMRI findings in age-heterogeneous samples. Recent research has shed some light on the relationship between CBF and the BOLD effect. The results of several investigations have shown that CBF response to cognitive load does not match changes in blood oxygen levels (Mohtasib et al. 2012), change in CBF does not support the required increase in oxygen metabolism during task performance (Hutchison et al. 2013) and lower baseline CBF is linked to reduced activation estimates in older adults (Ances et al. 2009).

The findings reported here may link individual differences in blood flow and BOLD through common genetic influence. Several characteristics of CBF are under substantial genetic control (Tarnoki et al. 2014), and so are the amplitude, latency, and duration of the hemodynamic response function (HRF), from which activation is inferred in fMRI (Shan et al. 2016). Identifying specific genetic variants that affect BOLD and CBF remains a task to be accomplished, although several candidate genes have been proposed. At the time of this writing, we have no knowledge about any studies of age differences in fMRI activation and ACE I/D variant. However, the literature on differences in BOLD that can be related to COMT val158met variant is quite sizeable, although somewhat contradictory (Papenberg et al. 2015).

In resting-state fMRI, COMT genotype modifies functional networks among brain regions, with low-dopamine, val homozygotes showing significantly lower functional connectivity in comparison with val/met heterozygotes and met homozygotes (Damoiseaux et al. 2016). In a sample of middle-aged and older adults, reduced task-related activation was noted in carriers of the val allele (Nyberg et al. 2014). Of note, in that study, participants with val/val genotype exhibited greater BOLD response on an easy task but not during demanding cognitive manipulation when their BOLD response was lower than that of the met carriers and in particular met/met homozygotes. Our finding of greater arterial flow rate in val/val homozygotes at rest makes lower resting flow in met/met homozygotes a plausible explanation of the reported differences in BOLD.

In comparing the results of this study to the extant literature, several characteristics should be taken into account. First, the participants were healthy volunteers who reported no history of cardiovascular or cerebrovascular diseases that are common on general population of older adults, particularly myocardial infarction, heart failure, or diabetes mellitus. Second, in this study, we restricted evaluation of the blood flow rate to extra-cerebral blood vessels, for which full detection and reliable measurements were confirmed and it is, therefore, unclear if the findings can be generalized to cerebral arteries and veins. Third, we examined hematocrit levels, which could affect blood flow velocity, and removed two participants with abnormally low values. We have thus eliminated blood viscosity as a potential confound, and in our sample, hematocrit was not associated with blood flow rate, total CBF, age or vascular risk. Although we replicated the previous findings of right-sided dominance in IJV flow (Ayanzen et al. 2000; ElSankari et al. 2013; Stoquart-Elsankari et al. 2009), we observed no asymmetry in the arterial flow to the brain and the importance of lateral dominance in the flow rate remains unclear.

The results of this study should be interpreted in the context of several caveats and limitations. The most important one is that this is a cross-sectional study on a sample with a wide age range. It cannot, therefore, inform about age-related change or individual variability therein (Lindenberger et al. 2011).

Second, as all blood flow measures were conducted in a conventional MRI scanner, while the participants were in supine position, age differences in arterial flow could be underestimated, because delivery of arterial blood to the brain in this position requires lesser anti-gravity cardiac effort and may reduce the impact of age-related declines in cardiac output on flow rate.

Third, the lack of *APOE* ε4 effect may reflect a selection bias predicated on rigorous health screening and an almost total absence of ε4 homozygotes. In contrast to *APOE ε4* and many other common genetic variants associated with health risks, *COMT* val158met and *ACE* I/D display a relatively balanced distribution of alleles, regardless of the health status of the sample.

Fourth, a moderate sample size precluded examination of additional genetic variants, such as polymorphisms of the *NOS* gene nitric oxide synthase that may have a significant effect on CBF (Dalkara and Alarcon-Martinez 2015) and longevity in general (Montesanto et al. 2013).

Fifth, the effects of additional age-linked cardiovascular factors that have a profound effect on cerebral blood flow (e.g., reduction in cardiac output, Meng et al. 2015) should be examined in future studies in an attempt to explain age-related variability in CBF.

Sixth, a possible limitation and concern may be that the arterial-venous flow mismatch observed here is an artifact of averaging across the heart beat and respiration. This is unlikely for the following reasons. Flow from the entire cardiac cycle was collected in this study over a

period of several minutes, so the cardiac data were averaged over many respiratory cycles. When measuring total flow, integrating the data over the cardiac cycle dramatically increases the SNR relative to a single timepoint measurement (Haacke and Patrick 1986; Nayak et al. 2015). Although the effect of respiration can cause a slight shift in the overall IJV flow (~2%) when a moving average over a given respiratory cycle is used (Billman 2011; Schrauben et al. 2015; Pucheu et al. 1994), the magnitude of this effect is unlikely to account for the observed arterial-venous mismatch.

Seventh, this study focused on IJV only because measurements of comparable reliability were not attained for the vertebral and smaller veins. Attention to rigorous reliability of venous flow assessment is important, especially in light of potentially misleading comparisons of vessels with differentially unreliable measures. Thus, future improvements in evaluation of venous flow in smaller vessels is important because of their measurable contribution to the outflow (Ciuti et al. 2013), although such measurements present an important challenge.

Conclusion

Even relatively minor elevation in vascular risk and propensity for greater vasoconstriction are linked to lower arterial blood flow and venous drainage rates. Age-related differences in flow rate were limited to a subgroup that was genetically predisposed to lesser synaptic dopamine availability. In contrast to stable and immutable genetic variations, vascular risk factors examined here can be modified and mitigated via life-style changes and pharmaceutical interventions (Yaffe et al. 2014). Thus, if indeed the differences in blood flow rate are linked to declines in cognitive performance, addressing such modifiable factors can be a valuable contribution to ameliorating the course of aging.

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