Risk Factors for β-Amyloid Deposition in Healthy Aging

Vascular and Genetic Effects

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Importance: Identifying risk factors for increased β-amyloid (Aβ) deposition is important for targeting individuals at risk for developing Alzheimer disease and informing clinical practice concerning prevention and early detection.

Objective: To investigate risk factors for Aβ deposition in cognitively healthy middle-aged and older adults. Specifically, we hypothesized that individuals with a vascular risk factor such as hypertension, in combination with a genetic risk factor for Alzheimer disease (apolipoprotein E ε4 allele), would show greater amyloid burden than those without such risk.

Design: Cross-sectional study.

Setting: General community.

Participants: One hundred eighteen well-screened and cognitively normal adults, aged 47 to 89 years. Participants were classified in the hypertension group if they reported a medical diagnosis of hypertension or if blood pressure exceeded 140 mm Hg systolic/90 mm Hg diastolic, as measured across 7 occasions at the time of study.

Intervention: Participants underwent Aβ positron emission tomography imaging with radiotracer fluorine 18-labeled florbetapir. Participants were genotyped for apolipoprotein E and were classified as ε4+ or ε4−.

Main Outcome Measure: Amyloid burden.

Results: Participants in the hypertension group with at least 1 ε4 allele showed significantly greater amyloid burden than those with only 1 risk factor or no risk factors. Furthermore, increased pulse pressure was strongly associated with increased mean cortical amyloid level for subjects with at least 1 ε4 allele.

Conclusions and Relevance: Vascular disease is a prevalent age-related condition that is highly responsive to both behavioral modification and medical treatment. Proper control and prevention of risk factors such as hypertension earlier in the life span may be one potential mechanism to ameliorate or delay neuropathological brain changes with aging.


One of the major research foci in the aging field today is centered on the neural and cognitive decline that occurs with Alzheimer disease (AD). Understanding disease etiology and progression and developing effective disease-modifying treatments of AD are among the key scientific goals of the 21st century. Recent attention to the lack of efficacious treatments, coupled with the knowledge that neurobiological changes precede behavioral expression and clinical diagnosis of AD by a decade or more, highlights the importance of studying fibrillar amyloid deposition in healthy life-span samples to identify those individuals most at risk for future neuropathological decline. 1

The primary neuropathological features of AD include the deposition of amyloid plaques and tau-driven neurofibrillary tangles. 2 Previous research from autopsy and in vivo imaging has estimated at least 20% of normal older adults carry elevated levels of β-amyloid (Aβ). Current theories have proposed that amyloid deposition is one of the earliest detectable changes in the neuropathology of AD. 6 Thus, identifying the most salient risk factors for Aβ deposition, especially modifiable environmental factors such as vascular health, can inform our understanding of individual differences in susceptibility to pathology as well as help direct medical efforts focused on prevention and early detection.

Although multiple genetic variants have been identified as risk factors for AD, 7 the apolipoprotein E ε4 genotype (APOE ε4) is perhaps the best verified genetic polymorphism associated with a significantly increased risk of cognitive decline and dementia. 8, 10 Individuals with 2 copies of an ε4 allele carry a 10- to 12-fold risk for AD...
in comparison with ε3 homozygotes. The APOE lipoprotein is involved in both cholesterol and Aβ transport and the ε4 polymorphism is additionally a risk factor for vascular disease.

One major environmental risk factor for dementia is cardiovascular and neurovascular health. Multiple epidemiological studies have shown that risk factors for vascular disease, such as diabetes mellitus and hypertension, are also risk factors for cognitive decline. Midlife elevations in blood pressure have been shown to predict diagnosis of dementia later in the life span, and hypertension in even healthy adults has been associated with poorer cognitive performance, increased rate of brain shrinkage, degraded white matter connectivity, and greater regional brain iron concentration compared with adults with normal blood pressure. Additionally, older adults with an APOE ε4 allele and cardiovascular disease may be at greater risk for cognitive decline than those without such factors, although high levels of atherosclerosis have been linked with increased cognitive decline independent of APOE genotype. Thus, genetic and vascular risk factors may work in synergy to bring about the neuropathological changes that lead to cognitive decline.

The goal of the current study was to examine the individual and combined impact of genetic (APOE ε4) and vascular (hypertension) risk factors on in vivo amyloid deposition in a healthy aging sample of middle-aged and older adults. We hypothesized that individuals with hypertension would show greater levels of Aβ deposition compared with their peers with normal blood pressure and that individuals with both genetic and vascular risk factors would show the greatest elevation in Aβ burden. We additionally tested the hypothesis that increased pulse pressure (PP) would be associated with higher levels of amyloid deposition, particularly in those participants with at least 1 APOE ε4 allele.

**METHODS**

**PARTICIPANTS**

We recruited 147 participants (aged 30-89 years) who underwent cognitive testing, magnetic resonance imaging, and fluorine 18 (18F)-labeled florbetapir positron emission tomography (PET) imaging as part of the Dallas Lifespan Brain study. Participants were well educated (mean, 16.23 years) native English speakers, and screened for neurological and psychiatric disorders, loss of consciousness more than 10 minutes from a traumatic insult to the head, drug/alcohol abuse, stroke and major heart surgery or chemotherapy within 5 years, and Mini-Mental State Examination score less than 26.

**PROCEDURES**

All participants completed four 2-hour visits: 2 for cognitive and neuropsychological testing, followed by 1 for magnetic resonance imaging scanning, and 1 for amyloid PET imaging. The institutional review board approved study procedures and all participants provided written informed consent prior to enrollment and were debriefed in accord with university human investigations committee guidelines.

**APOE Genotyping**

Venous blood was collected into EDTA anticoagulated tubes and genomic DNA was isolated by standard protocols. We typically isolated 30 to 70 μg of DNA from 2 mL of whole blood. APOE genotypes were determined by real-time polymerase chain reaction using TaqMan probes (Applied Biosystems Inc) unique for each APOE single-nucleotide polymorphism, rs429358 (assay ID C3084793 20) and rs7412 (assay ID C 904973 10), according to established protocols. APOE data were available for all but 3 of the participants, for a total sample of 144 with complete data.

**Blood Pressure Measurement**

Blood pressure was measured twice at each cognitive and PET imaging visit and once at the magnetic resonance imaging visit. All participants had blood pressure readings from at least 3 measurement points; however, 13 participants were missing 1 to 4 measurements. Systolic and diastolic pressure were averaged across all available occasions for each subject, and this mean blood pressure was used in part to determine vascular risk group membership.
ship (for categorical variable analyses). For continuous variable analyses of blood pressure effects, we computed PP (systolic minus diastolic) because it is a known correlate of arterial stiffness and may be a better predictor of coronary heart disease than either systolic or diastolic elevations in pressure.31

Vascular and Genetic Risk Group Classification

Participants were classified as having vascular risk if they reported a current physician diagnosis of hypertension or if their measured mean blood pressure over the 7 measurement occasions exceeded stage 1 criteria for diagnosis (ie, average systolic blood pressure greater than 140 mm Hg or average diastolic pressure greater than 90 mm Hg). A total of 75 participants from the sample of 144 were classified as having normal blood pressure, with a mean age of 54.3 years (range, 30-88 years). Because the groups differed in age considerably, we restricted the normotensive sample to adults 47 years and older (n = 118; mean age, 62.4 years), which was the age of the youngest person in the vascular risk group. In this reduced sample, 69 participants were classified as having hypertension and had a mean age of 74.0 years (range, 47-89 years). Fifty-four of those participants reported physician diagnosis and medication for hypertension and 15 reported no current diagnosis or history of hypertension but showed measured blood pressure elevations exceeding stage 1 criteria. Participants were classified as having genetic risk if they carried at least 1 ε4 allele. Among this age-restricted sample of 118, 22.8% were classified as having genetic risk (18.6% ε4 heterozygotes and 4.2% ε4 homozygotes). There was no significant effect of hypertension status ($F_{1,114} < 1; P = .52; F_{1,114} < 1; P = .99$) or genetic status ($F_{1,114} < 1; P = .63; F_{1,114} = 2.81; P = .10$) on education or Mini-Mental State Examination score, respectively (Table 1). However, both vascular risk groups (ie, hypertensive and either ε4+ or ε4−) were significantly older (P range <.05-.001) than both normotensive groups (Table 1). Age was entered into all statistical models to account for this difference.

Because our participants were prospectively selected for a study of healthy aging, we had insufficient information and variance to create a risk factor score (such as the Framingham index). Less than 3% of the sample reported regular smoking, less than 0.5% were diagnosed with type 1 diabetes, and approximately 4% of the sample reported a history of type 2 diabetes (all non-insulin dependent). Mean (SD) body mass index in the sample was 26.18 (3.48) (calculated as weight in kilograms divided by height in meters squared) and was not correlated with amyloid burden ($r = 0.03; P = .76$).

MEDICATIONS

Fifty-four of the participants in the vascular risk group reported taking antihypertensive medications: β-blockers (n = 9), ace inhibitors (n = 9), angiotensin receptor blockers (n = 4), calcium channel blockers (n = 4), potassium-sparing diuretics (n = 3), α-blockers (n = 1), or a combination of at least 2 of these medications (n = 24). These participants were classified in the medicated hypertensive group. The remaining 15 participants in the vascular risk group who were not taking antihypertensive medication were classified in the unmedicated hypertensive group.

IMAGING PROTOCOL

PET Acquisition

Participants were injected with a 370×10^-6 Bq bolus of 18F-florbetapir. At 30 minutes postinjection, participants were positioned on the imaging table of a Siemens ECAT EXACT HR PET scanner. A 2-minute scout was acquired to ensure the brain was completely in the field of view and there was no rotation in either plane. A 2-frame × 5-minute dynamic emission acquisition began 50 minutes postinjection and immediately after an internal rod source transmission scan was acquired for 7 minutes. The transmission image was reconstructed using back projection and a 6-mm full-width-at-half-maximum gaussian filter. Emission images were processed by iterative reconstruction, 4 iterations and 16 subsets with a 3-mm full-width-at-half-maximum ramp filter.

PET Data Processing

Each participant’s PET scan was spatially normalized to a florbetapir uptake template positioned in Montreal Neurological Institute space (2-mm3 voxels) using SPM8 (Wellcome Department of Cognitive Neurology) and in-house MATLAB (MathWorks Inc) scripts and visually inspected for registration quality. We computed standardized uptake value ratios (SUVRs) in 8 bilateral regions of interest, using a cerebellar reference region that excluded peduncles. Regions of interest and the reference region were defined by modifying automated anatomical labeling masks to minimize nonspecific white matter binding. Mean SUVR was calculated by averaging SUVRs in the 8 regions of interest: precuneus, posterior and anterior cingulate, temporal, dorsolateral prefrontal, orbital frontal, parietal, and occipital cortex.

RESULTS

EFFECT OF VASCULAR AND GENETIC RISK ON Aβ DEPOSITION

To determine the effect of hypertension and APOE ε4 on Aβ burden, we used a generalized linear model with age as a continuous predictor and vascular risk (normotensive vs hypertensive) and genetic risk (APOE ε4+ vs APOE ε4−) as categorical predictors of amyloid burden (mean cortical SUVR). All possible interactions were tested and systematically removed from the final model if nonsignificant (P > .10). There was a significant effect of age ($F_{1,113} = 5.22; P = .02$) as amyloid burden increased with older age. The main effects of hypertension ($F_{1,113} = 3.41; P = .06$) and APOE ε4 ($F_{1,113} = 3.52; P = .06$) showed trend levels of significance, whereas the presence of an APOE ε4 allele, or having hypertension, was associated with greater Aβ deposition. Importantly, the APOE ε4 × hypertension interaction was significant ($F_{1,113} = 3.90; P = .05$) (Figure 1 shows the combined effect of hypertension and APOE genotype on cortical amyloid burden). Individuals who were both APOE ε4+ and had hypertension showed greater levels of amyloid burden than subjects with either a single risk factor or none at all.

MEDICATED VS UNMEDICATED HYPERTENSION

To test the possibility that there were differing effects of medicated and unmedicated hypertension on Aβ deposition, we reconstructed the vascular risk variable into normotensive, medicated, and unmedicated hypertension and reestimated the effects on amyloid burden (see Table 2 for group demographics). We reproduced the
significant effect of age ($F_{1,109} = 12.98, P < .001$) and found a main effect for APOE ε4 ($F_{1,109} = 7.36, P < .001$). Moreover, the APOE ε4 × vascular risk group interaction was significant ($F_{1,109} = 4.01, P = .02$), reflecting that unmedicated participants with hypertension and genetic risk showed higher levels of amyloid burden than all other groups (Figure 2).

ASSOCIATION OF PP AND AMYLOID DEPOSITION

In addition to testing the categorical effects of hypertension, we also examined whether a dose-response effect existed between elevation in blood pressure and elevated cortical amyloid level. We repeated the generalized linear model approach using age and PP as continuous predictors and APOE status as a categorical predictor of mean cortical SUVR. We observed significant effects of APOE group ($F_{1,111} = 6.63; P = .01$), PP ($F_{1,111} = 6.40; P = .01$), and a trend level effect of age ($F_{1,111} = 3.54; P = .06$) on mean cortical SUVR. A significant interaction of APOE group × PP ($F_{1,111} = 9.52; P = .003$) indicated that increased PP was predictive of greater amyloid burden in the genetic risk group, whereas this effect was not apparent in individuals who were APOE ε4− (Figure 3).

COMMENT

Our study demonstrates that hypertension, a prevalent vascular risk factor in aging populations, interacts with APOE ε4 genotype to increase amyloid deposition in cognitively healthy middle-aged and older adults. Individuals with vascular or genetic risk alone do not show elevated mean cortical amyloid burden when compared with a group with neither risk factor. We additionally show that in healthy individuals with genetic risk for late-onset AD (ie, presence of an APOE ε4 allele), unmediated hypertension is associated with the greatest risk for accumulating Aβ. Participants with controlled hypertension, however, show significantly less amyloid burden than the unmedicated group and only a slight elevation compared with participants without hypertension. Beyond these categorical differences, we further demonstrate a dose-response effect of increases in PP with increases in cortical amyloid load. For ε4+ individuals, increases in PP reliably predicted elevated amyloid burden. All of these effects are beyond the significant effect of age on amyloid, underscoring the importance of individual differences in health and genetic risk factors in brain aging.

The present results are in accord with previous research demonstrating the impact of blood pressure elevations on AD diagnosis. Large-scale epidemiological studies have demonstrated that midlife increases in blood pressure predict the development of dementia later in the life span. Further, a recent report showed that individuals with the highest diastolic blood pressure elevations in midlife showed the strongest associations between plasma amyloid levels (Aβ1-40 and Aβ1-42) and risk for later AD. Thus, blood pressure elevation appears to increase the risk for AD. Our study extends this literature by directly measuring in vivo amyloid plaque in nonpathological aging and suggests that in persons with genetic risk for AD, blood pressure elevations are associated with increased amyloid accumulation even in cognitively normal adults.

Studies directly investigating the association of vascular health and in vivo amyloid deposition in the human brain are limited and findings thus far have been equivocal. One recent study investigating the link between cerebrovascular disease, defined by high white matter hyperintensities, and amyloid positivity in healthy adults found no relationship between the two, although individuals with both cerebrovascular disease and amyloid positivity showed poorer executive function performance. In agreement with the present findings, the Framingham Coronary Risk Profile score (an index of elevated cholesterol level, diabetes, hypertension, and smoking) has been associated with increased amyloid burden in a sample of normal controls, participants with mild cognitive impairment, and participants with AD. In a subset of participants with APOE genotyping, no interaction of Framingham Coronary Risk Profile score with APOE positivity was detected, although low power constrained interpretation of those findings.

Interestingly, the detrimental effect of hypertension and its interaction with APOE genotype on brain aging may not be limited to amyloid burden. A significant body of work has documented the deleterious effects of hypertension and other vascular risk factors on both normal and abnormal cognitive and brain aging, as well as its interaction with various genetic risk factors for cognitive decline beyond APOE. This literature highlights the complex nature of vascular and genetic effects on neurocognitive aging and suggests the synergistic effect of vascular compromise and genetic risk results in increased susceptibility to both the accumulation of neural insults and associated cognitive decline with aging. Understanding the time course, mechanism, and for whom the aging process becomes pathological is an open question in need of further study.

Figure 1. β-Amyloid deposition by vascular and genetic risk groups. Individuals with hypertension and at least 1 apolipoprotein E (APOE) ε4 allele have greater β-amyloid deposition than all other groups. SUVR indicates standardized uptake value ratio. *Significant interaction ($P = .05$) between vascular risk and genetic status, with ε4+ participants with hypertension showing the greatest amounts of β-amyloid deposition.
The neural mechanisms underlying the specific effect of hypertension and APOE ε4 on amyloid accumulation is unknown, although the animal literature offers some insight. Previous work in a mouse model of arterial hypertension suggested that chronic hypertension may increase blood-brain barrier permeability and result in Aβ deposition.38 Further examination of Aβ plaque in another murine model of hypertension showed that hypoperfusion and neuroinflammation in the cortex and hippocampus preceded the development of amyloid deposition.39 Mechanistically, APOE (a major cholesterol transport protein in the brain) has been linked to AD and Aβ deposition via cholesterol metabolism.40

The present study should be interpreted in the context of its limitations. The cross-sectional design of our study prohibits the delineation of cause-effect relationships among variables. The findings are additionally limited in their generalization to the full population of middle-aged and older adults by design, as we focus on a highly screened sample of healthy aging. Consequently, the sample is free from clinically significant vascular disease. While this does not allow us to study the spectrum of vascular effects, it does allow for a relatively focused study of the effects of hypertension compared with an index-based approach where the significance of individual factors is less clear. However, because the study was not prospectively selected to study hypertension directly, the findings should not be overinterpreted. Because the prevalence of hypertension increases with age, the normotensive group was younger than the hypertensive group. To address this possible age confound, we matched the age range of the groups and explicitly modeled age in all analyses. Additionally, given the relatively low prevalence of the APOE ε4 allele in the gen-

Table 2. Vascular Risk Group Demographics

<table>
<thead>
<tr>
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<th>Medicated</th>
<th>Unmedicated</th>
<th>Medicated</th>
<th>Unmedicated</th>
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<tbody>
<tr>
<td>Sample size</td>
<td>47</td>
<td>7</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Age, y</td>
<td>73.64 (10.15)</td>
<td>78.56 (8.37)</td>
<td>74.09 (9.88)</td>
<td>71.29 (12.85)</td>
</tr>
<tr>
<td>MMSE score</td>
<td>29.34 (0.94)</td>
<td>28.86 (1.22)</td>
<td>28.44 (1.33)</td>
<td>28.50 (1.23)</td>
</tr>
<tr>
<td>Education, y</td>
<td>15.87 (2.77)</td>
<td>16.14 (3.34)</td>
<td>16.22 (2.77)</td>
<td>16.50 (1.98)</td>
</tr>
<tr>
<td>SUVR</td>
<td>1.20 (0.15)</td>
<td>1.26 (0.19)</td>
<td>1.21 (0.21)</td>
<td>1.42 (0.28)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>137.72 (15.48)</td>
<td>135.15 (10.28)</td>
<td>154.17 (7.64)</td>
<td>161.83 (14.01)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>83.13 (9.05)</td>
<td>81.79 (8.89)</td>
<td>89.98 (6.60)</td>
<td>92.30 (9.66)</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>54.59 (10.71)</td>
<td>53.37 (7.20)</td>
<td>64.19 (10.41)</td>
<td>69.53 (16.44)</td>
</tr>
</tbody>
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Abbreviations: APOE ε4+, heterozygosity or homozygosity for apolipoprotein E ε4 allele; BP, blood pressure; MMSE, Mini-Mental State Examination; SUVR, standardized uptake value ratio.

Figure 2. Mean β-amyloid deposition by genetic risk and hypertensive subgroup. Individuals with uncontrolled hypertension and at least 1 apolipoprotein E (APOE) ε4 allele show greater β-amyloid deposition. SUVR indicates standardized uptake value ratio. *Significant interaction (P = .02) between vascular risk group and genetic status, with unmedicated ε4+ participants with hypertension showing the greatest amounts of β-amyloid deposition.

Figure 3. Association of pulse pressure and β-amyloid deposition. Increased pulse pressure is associated with increased β-amyloid deposition in individuals with genetic risk for Alzheimer disease (ie, apolipoprotein E (APOE) ε4+).
eral population (approximately 24%), which was mirrored in our sample, the analyses were differentially powered in comparisons of vascular and genetic risk groups. Future prospective studies with selective case-control recruiting on vascular and genetic risk factors are needed to replicate and extend the present findings.

Our findings advance the existing literature in several important ways. First, our study focuses on a group of healthy and cognitively normal middle-aged and older adults, which enables the examination of risk factors and amyloid burden before the development of mild cognitive impairment and possibly preclinical dementia. Of course, long-term longitudinal follow-up (which we are pursuing) is needed to determine which proportion of the sample will ultimately develop pathology. Second, we used a highly selected sample of adults screened against significant cardiovascular disease, which lends confidence to the specificity of the hypertension findings. Third, in addition to examining categorical factors, we substantiate and extend that approach with actual blood pressure measurements across multiple occasions, which allows for the examination of a dose-response relationship and provides a more direct and nuanced study of hypertension effects than diagnostic group comparisons alone.

In sum, our results show that hypertension may modify the impact of genetic risk for AD on Aβ accumulation in healthy adults. Interestingly, these initial findings suggest that individuals with an APOE ε4 allele may be able to attenuate their likelihood for amyloid accumulation through proper control of blood pressure. However, future studies with larger sample sizes that examine additional factors, such as duration of hypertension treatment across multiple occasions, which allows for the examination of a dose-response relationship and provides a more direct and nuanced study of hypertension effects than diagnostic group comparisons alone.

Accepted for Publication: October 9, 2012. Published Online: March 18, 2013. doi:10.1001/jamaneurol.2013.1342

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Author Contributions: Dr Rodrigue had full access to all the data in the study and takes responsibility for the integrity of the data. Study concept and design: Rodrigue, Kennedy, Devous, and Park. Acquisition of data: Rodrigue, Rieck, Kennedy, Devous, and Diaz-Arrastia. Analysis and interpretation of data: Rodrigue, Devous, Diaz-Arrastia, and Park. Drafting of the manuscript: Rodrigue, Rieck, and Devous. Critical revision of the manuscript for important intellectual content: Rodrigue, Kennedy, Devous, Diaz-Arrastia, and Park. Statistical analysis: Rodrigue and Devous. Obtained funding: Rodrigue, Kennedy, Diaz-Arrastia, and Park. Administrative, technical, and material support: Rieck and Diaz-Arrastia. Study supervision: Kennedy, Diaz-Arrastia, and Park.

Conflict of Interest Disclosures: The radiotracer was provided at no cost to the study by Avid Radiopharmaceuticals Inc. Dr Devous serves on the advisory board of Avid Radiopharmaceuticals. Avid Radiopharmaceuticals is supporting a longitudinal component of this study.

Funding/Support: This study was supported by the National Institutes of Health grants 5R37AG-006265-25, 3R37AG-006265-2551, and P30AG12300 and Alzheimer’s Association grant IIRG-09-135087 (Dr Park). Dr Rodrigue was supported in part by National Institute on Aging grant 1K99-AG-036848-2.

Additional Contributions: We thank Michael Viguets, BS, ARRT(N), for assistance with positron emission tomography scanning. Chris Paliotto, BA, for collecting blood samples, and Prasanna Tamil, MS, and Erin Wooden, BS, for participation testing and scheduling.

REFERENCES


20. Raz N, Rodrigue KM, Kennedy KM, Acker JD. Vascular health and longitudinal...