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Subgroup of ADNI Normal Controls Characterized by Atrophy and Cognitive Decline Associated With Vascular Damage

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Abstract

Previous work examining Alzheimer's Disease Neuroimaging Initiative (ADNI) normal controls using cluster analysis identified a subgroup characterized by substantial brain atrophy and white matter hyperintensities (WMH). We hypothesized that these effects could be related to vascular damage. Fifty-three individuals in the suspected vascular cluster (Normal 2) were compared with 31 individuals from the cluster characterized as healthy/typical (Normal 1) on a variety of outcomes, including magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) biomarkers, vascular risk factors and outcomes, cognitive trajectory, and medications for vascular conditions. Normal 2 was significantly older but did not differ on ApoE4+ prevalence. Normal 2 differed significantly from Normal 1 on all MRI measures but not on Amyloid-Beta$_{1\text{-}42}$ or total tau protein. Normal 2 had significantly higher body mass index (BMI), Hachinksi score, and creatinine levels, and took significantly more medications for vascular conditions. Normal 2 had marginally significantly higher triglycerides and blood glucose. Normal 2 had a worse cognitive trajectory on the Rey's Auditory Verbal Learning Test (RAVLT) 30-min delay test and the Functional Activity Questionnaire (FAQ). Cerebral atrophy associated with multiple vascular risks is common among cognitively normal individuals, forming a distinct subgroup with significantly increased cognitive decline. Further studies are needed to determine the clinical impact of these findings.

Keywords

ADNI; vascular; cognitive decline; biomarkers; cluster

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We previously explored dementia-related biological heterogeneity in the cognitively normal controls in the Alzheimer's Disease Neuroimaging Initiative (ADNI) via cluster analysis based on 11 magnetic resonance imaging (MRI), cerebrospinal fluid (CSF), and serum biomarkers (Nettiksimmons et al., 2010). The analysis yielded three subgroups: Normal 1 (33% of the total, $n = 31$), Normal 2 (57% of the total, $n = 53$), and Normal 3 (10% of the total, $n = 9$). Normal 1 was considered the typical, healthy group with high brain volumes and Amyloid-$\beta_{1\text{-}42}$ (Aβ) CSF concentrations. Normal 3 had levels of CSF Aβ, total tau, and phosphorylized tau that were on par with the average levels in the ADNI mild cognitive impairment (MCI) and Alzheimer's disease (AD) groups (see Figure 1). Regional brain volumes estimated from structural MRI in Normal 3 were also approaching the average levels observed in the MCI group. Longitudinal cognitive models found that the subjects in Normal 3 had lower baseline scores on two cognitive tests used for assessing AD-related cognitive decline and were deteriorating significantly faster than Normal 1. These findings led us to believe that the individuals in Normal 3 were likely in the very early stages of the pathological progression of AD. The remaining group, Normal 2, showed atrophy on structural MRI in multiple brain regions that exceeded the atrophy seen in the prodromal-AD subgroup (Normal 3), and approached the levels seen in the ADNI MCI subjects but lacked CSF patterns characteristic of AD (see Figure 1). The purpose of this article is to further characterize Normal 2 and test whether vascular damage could be an explanation for the atrophy.

AD is not an obvious suspect for the atrophy seen in Normal 2 because CSF amyloid and tau levels were much more similar to the healthy cluster than to the prodromal AD cluster (Normal 3) or the MCI group. The current amyloid cascade hypothesis dictates that significant CSF changes occur before changes in brain volume or cognition occur (Jack et al., 2010). If this hypothesis is correct, then the differences in cognition and brain volume between Normal 1 and Normal 2 are not likely to be explained by incipient AD. An alternate explanation for differences between Normal 1 and Normal 2 is the presence of greater vascular brain injury in Normal 2 subjects. Clinically silent vascular brain injury, such as brain infarction detected by MRI, is widespread among aging populations and is independently associated with cognitive decline as well as frequently cooccurring with other pathologies such as AD (DeCarli, Fletcher, Ramey, Harvey, & Jagust, 2005; Schneider, Arvanitakis, Bang, & Bennett, 2007). In addition, the volume of white matter hyperintensities (WMH) in the brain imaged with MRI is considered a surrogate measure of vascular brain injury that is similarly associated with advancing age, vascular risk factors, incident dementia, and stroke (Debette, Beiser, Decarli, et al., 2010, 2011; DeCarli, Massaro, et al., 2005). The primary risk factors for vascular brain injury are hypertension (with effects on brain atrophy occurring well before old age), hypercholesterolemia, obesity, diabetes, metabolic syndrome, smoking, and hyperhomocysteinemia (Kivipelto et al., 2001; Rusanen, Kivipelto, Quesenberry, Zhou, & Whitmer, 2011; Seshadri et al., 2004; Whitmer, 2007; Whitmer, Gunderson, Barrett-Connor, Quesenberry, & Yaffe, 2005; Whitmer, Karter, Yaffe, Quesenberry, & Selby, 2009; Whitmer, Sidney, Selby, Johnston, & Yaffe, 2005; Yaffe, Blackwell, Whitmer, Krueger, & Barrett-Connor, 2006; Yaffe et al., 2007).

We hypothesized, therefore, that the brain atrophy seen in Normal 2 relative to Normal 1 is the consequence of vascular brain injury in the absence of significant burden of amyloid and tau. To test this hypothesis, we first looked for evidence that could suggest or rebut an association between AD and cluster membership. Then we examined the evidence of an association between the Normal 1 and Normal 2 cluster assignments and vascular disease. This included examining differences in vascular risk factors, WMH, atrophy patterns, and clinically defined vascular disease outcomes. Next we compared the longitudinal performance of Normal 1 and Normal 2 on a wider range of cognitive tests than were

previously examined to determine whether the differences in baseline brain volumes subsequently translated into clinically relevant differences in cognition. Lastly, we assessed whether there are dose–response relationships that support the hypothesis that differences between Normal 1 and Normal 2 are due to vascular brain injury.

Method

Subjects

Data used in the preparation of this article were obtained from the ADNI database (http://www.loni.ucla.edu/ADNI).[1] The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations as a US$60-million, 5-year public–private partnership. The individuals studied were recruited between August 17, 2005, and September 4, 2007, as ADNI participants and were identified at baseline clinical evaluation as cognitively normal. Normal controls were frequency matched to MCI and AD participants by age group. Normal control participants underwent cognitive testing and clinical examination by a physician at baseline and every 6 months for the first year and then annually for the next 2 years. MRI scans (1.5 Tesla) were performed in each subject (http://www.loni.ucla.edu/ADNI/Research/Cores/index.shtml) at baseline, and repeated at 6, 12, 24, and 36 months. Approximately half of the participants also provided CSF at the baseline and 12-month visits. Additional details are given in Petersen et al. (2010). This study was approved by the institutional review boards of all participating institutions. Informed written consent was obtained from all participants at each site.

Biomarkers

The ADNI normal control diagnostic group contained 222 individuals. The previous analysis contained 97 subjects; however, five of the previous subjects were subsequently excluded from the ADNI MRI database due to quality concerns regarding baseline scans. One additional subject who was not included in the previous analysis had complete data by the time of this analysis and was therefore included in the present analysis. At the time of this analysis, there were 93 cognitively normal individuals with complete baseline data for all biomarkers necessary for clustering.

The biomarkers used for clustering were total brain volume, hippocampal volume, ventricle volume, entorhinal cortex thickness, WMH, CSF Amyloid-$\beta_{1\text{-}42}$ (Aβ), CSF total tau (tau), CSF tau protein phosphorylated at the 181 threonine position (P-tau), the ratio of tau to Aβ, the ratio of P-tau to Aβ, and serum homocysteine. All biomarkers used in clustering were standardized by subtracting the overall normal baseline mean and dividing by the overall normal baseline standard deviation in order to allow comparisons between biomarkers that exist on different scales. All MRI summary volumes are fractions of the total intracranial volume, which included the area occupied by the brain stem inside the skull, and were calculated using a modification of FreeSurfer implemented by the Anders Dale Laboratory at the University of California, San Diego, as part of the ADNI shared data set (Jack et al., 2008). In addition, segmentation of gray matter, white matter, and CSF was performed on native space T1 spoiled gradient echo (SPGR) images by an in-house computer program using Bayesian maximum likelihood expectation maximization (EM) computation (Rajapakse, Giedd, & Rapoport, 1997) at the Imaging of Dementia and Aging (IDeA) laboratory at the University of California, Davis, directed by Charles DeCarli. Tissue probabilities used a combination of Gaussian intensity distributions combined with a

[1]A detailed description of the study design and inclusion criteria are available from http://clinicaltrials.gov/show/NCT00106899. Data used in this analysis were downloaded from the ADNI database on December 14, 2010.

Markov random field (MRF) component for modeling the tissue classification of voxel neighborhoods. Two in-house enhancements included (a) automatic initialization of the EM step via a high-dimensional B-spline warp in which template-based tissue probability maps are fitted to the native T1 SPGR images, and (b) edge detection to dictate the appropriate neighborhood clique structure of the MRF for locations in homogeneous tissue or at tissue boundaries (Lee et al., 2010). WMH were also detected by IDeA Laboratory based on coregistered T1-, T2-, and proton density (PD) weighted images using an automated protocol described previously (Schwarz, Fletcher, DeCarli, & Carmichael, 2009). CSF samples were batch processed under the direction of Leslie Shaw and John Trojanowski of ADNI Biomarker Core at the University of Pennsylvania School of Medicine (Shaw, 2008).

Vascular risk factors available from ADNI medical histories and symptom checklists include total cholesterol, triglycerides, blood glucose, BMI, seated systolic and diastolic blood pressure, smoking, and history of alcohol abuse. Negative outcomes associated with vascular damage available for examination were modified Hachinski score; urea nitrogen; creatinine levels; history of cardiac, respiratory, or endocrine abnormalities; edema; peripheral vascular abnormality; and swollen ankles (Petersen et al., 2010). In addition, medications at each visit were recorded. Two of the authors (Jasmine Nettiksimmons and Charles DeCarli) reviewed the medication list at study screening to identify treatments related to vascular risk factors. The ADNI database includes indications for medications, allowing for further refinement of association with vascular disease. For example, diuretics were counted as vascular risk factors only if associated with hypertension; conversely, antihypertensives used for prostatic hypertrophy were excluded. The numbers of vascular associated medications for each subject identified at screening were compared between groups.

Clinical Outcomes

The original study only evaluated cluster differences on the Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog) and the Rey's Auditory Verbal Learning Test (RAVLT) sum of five trials. For this comparison of Normal 1 and Normal 2, a wider range of cognitive tests was used, including some more challenging subscales that may have fewer ceiling effects in normal subjects. Previous work has suggested that white matter damage is likely to impact functions of the frontal lobe, such as executive function, attention, and working memory (Au et al., 2006; Mayda, Westphal, Carter, & DeCarli, 2011; Nordahl et al., 2005). These domains, along with others, are explored in this analysis. A composite executive function score was created using several different cognitive tests that tap into executive function—digit span forward and backward, digit symbol substitution, Trails B, and category naming (sum of animals and vegetables; Wechsler, 1981, 1987; Partington & Leiter, 1949; Morris, Heyman, Mohs, & Hughes, 1989). These individual tests were transformed into z scores by subtracting the baseline mean and dividing by the baseline standard deviation, both calculated using data from all 222 normal controls. The z scores were then averaged after reversing the sign for Trails B to maintain consistency of interpretation (for Trails B, high scores indicate greater cognitive deficit). In addition to the tests used in the composite, we examined the ADAS cognitive subscale (ADAS-cog), RAVLT, and Logical Memory II (Rosen, Mohs, & Davis, 1984; Rey, 1964; Wechsler, 1987). Three subscales from the RAVLT were used—the sum of 5 trials, retention after a 30-min delay, and the percent savings calculated as the ratio of the 30-min delay score to the fifth trial. Comparisons were also made between Normal 1 and Normal 2 on the Geriatric Depression Scale (GDS) and the Functional Activity Questionnaire (FAQ), both of which measure noncognitive clinical function (Sheikh & Yesavage, 1986; Pfeffer, Kurosake, Harrah, Chance, & Filos, 1982).

Statistical Analysis

Agglomerative clustering using the predetermined set of clustering variables (listed in the Biomarkers section) was performed using Ward's method of minimum variance and the Euclidean distance metric, which is described in more detail in previous work (Nettiksimmons et al., 2010). This analysis resulted in three clusters; the number of clusters was chosen on the basis of analytic metrics, consistency between methods, and sample size considerations for subsequent analysis. As previously mentioned, Normal 3 fit the characteristics for the early stages of AD-pathology development and has been excluded from the current analysis. The current analysis focuses on Normal 2 and uses Normal 1, the typical healthy cluster, as a comparison. Baseline differences between Normal 1 and Normal 2 in relevant continuous variables were tested with *t* tests. Confidence intervals for individual clustering biomarkers were constructed by cluster to examine which biomarkers differed substantially between the clusters and which did not. Despite using these variables to create the clusters, pairwise differences are not a forgone conclusion, especially because the clustering algorithm resulted in a third cluster not examined here. Our previous work demonstrated substantial differences between Normal 1 and Normal 2 on several regional and global measures of gray matter volume. Elaborating on this comparison, white matter and gray matter from composite MRI images for each group were compared to determine whether there were differences in the spatial distribution of each tissue. For the gray matter comparison, a binary image of gray matter from each subject was mapped to a common template and smoothed using a 6-mm Gaussian smoothing kernel. This results in a distribution of intensities at each voxel location (probability density distribution) that can be statistically evaluated (Kiebel, Poline, Friston, Holmes, & Worsley, 1999). Then, for each voxel, a *t* statistic for the differences in means between Normal 1 and Normal 2 was calculated using nonparametric significance testing (1000 permutations), which is displayed on the images using a color map (Nichols & Holmes, 2002). For WMH, we created composite maps that display the frequency of WMH (treated as a categorical variable) at each voxel location for each group. The warmer the color, the more frequent the occurrence at a particular location. Due to the binary nature of this analysis and the relatively small sample sizes, formal statistical comparison could not be performed.

Differences between clusters in continuous and binary measures of vascular risk and disease were tested with linear regression, using the vascular associate as the outcome with age and cluster membership as predictors. Baseline and longitudinal differences in cognitive test scores and anatomical volumes were assessed with linear mixed effects models, including random effects for slope and intercept, where possible. If the model failed to converge, only random intercepts were used. Regression models for cognitive tests and anatomical regions were performed individually, each including the following predictors: cluster membership, time, age (at enrollment, centered), and the interaction of age and time. In addition, models for cognitive test scores included years of formal education, and the adjusted model for creatinine included gender.

The dose–response analysis used linear regression models with random effects for slope and intercept relating cognitive outcomes to continuous measures of vascular risk and diseases. This analysis was performed with and without outliers to determine whether a few outlying observations were unduly affecting conclusions. All analyses were performed using R version 2.10–2.13 (R Development Core Team, 2011).

Results

Differences between normal subjects with and without CSF testing were assessed to determine whether the group that consented to lumbar puncture was different in any significant way. There were no significant differences found for any of the variables listed in

Table 1, with the possible exception of education (15.7 years vs. 16.4 years, $p = .09$; see online supplemental material). Table 1 includes summary descriptions of all three clusters. Normal 3 is included to provide context, but the p values reported compare only the clusters of current interest, Normal 1 and Normal 2. T tests were performed on variables not included in the clustering set and 95% confidence intervals are reported for each of the clustering biomarkers. Although differences are to be expected in clustering biomarkers, as the machinery behind cluster analysis attempts to achieve maximal separation of clusters in biomarker-space, not all biomarkers were significantly separated. Examining which biomarkers are fully differentiated between the clusters and which are not helps to better characterize the clusters in relationship to each other. The proportion of ApoE4+ individuals in Normal 1 and Normal 2 were almost identical at 0.23 and 0.25, respectively, and roughly half of the prevalence of ApoE4+ in Normal 3. The confidence intervals for Normal 1 and Normal 2 did not overlap on measures of total brain volume, hippocampal volume, and ventricle volume, as well as entorhinal cortex thickness. Total WHM confidence intervals suggest that the differences between Normal 1 were suggestive but not significant (95% CI [2.13, 3.52] in Normal 2 vs. 95% CI [1.03, 2.85] in Normal 1). The confidence intervals show no significant differences between the two clusters on CSF amyloid levels, the presumed first detectable change in the cascade of AD processes (Jack et al., 2010). Likewise, the confidence intervals for tau and the tau/Aβ ratio show a great deal of overlap. The confidence intervals for P-tau and P-tau/Aβ ratio show a modest but significant difference.

Images showing the results of the white matter composite and gray matter t-test images are shown in Figures 2 and 3. Figure 2 depicts the frequency of WMH with a threshold greater than 2%. There are clear differences between Normal 1 and Normal 2 in the frequency of WMH located in the periventricular area, similar to that previously shown in association with vascular risk factors (DeCarli, Fletcher, et al., 2005). Figure 3 summarizes significant differences in gray matter atrophy and CSF expansion between Normal 1 and Normal 2. There were significant differences of greater atrophy localized to the hippocampi bilaterally as well as the parahippocampal gyri, inferior temporal, inferior parietal, posterior cingulate, and perisylvian regions.

Tests of vascular risk factors indicate that the Normal 2 group had significantly higher average BMI than Normal 1 (2.3 BMI units higher, $p = .04$; Table 2). Normal 2 also had marginally significantly higher triglycerides and blood glucose (42 mg/dL higher, $p = .07$; 8 mg/dL higher, $p = .06$). Importantly, Normal 2 also had a significantly higher mean Hachinski score, which is a summary index of vascular risk (0.8 in Normal 2 vs. 0.3 in Normal 1, $p = .01$). Average creatinine levels were significantly higher in Normal 2 with or without adjusting for age and gender. Homocysteine levels were also significantly different between groups, but this difference became nonsignificant after correcting for increased creatinine levels. Comparisons of categorical risk factors and outcomes between clusters were largely not significant. Membership in Normal 2 was associated with being male (OR = 2.8, $p = .05$). The number of medications used at screening visit for vascular disease trended toward significance, with Normal 2 (1.77 ± 1.72) taking more vascular medications than Normal 1 (1.16 ± 1.19, $p = .06$). The difference in the number of vascular medications was significant after controlling for age in a generalized linear model for a Poisson distribution, with Normal 2 taking an average of 0.6 more medications than Normal 1 ($p = .003$).

In addition, correlations were performed between vascular risk factors, vascular outcomes, and variables that constituted the various components of the initial cluster analysis, using only Normal 1 and Normal 2 clusters (Table 3). Whole-brain volume was generally inversely associated with all vascular risk factors, but the associations were only significant

for the Hachinski score, blood urea nitrogen levels, and serum creatinine levels. Similarly, ventricular volume was positively associated with blood urea nitrogen levels.

Neither baseline composite executive scores nor the individual tests that made up the executive function composite differed between Normal 1 and Normal 2 (see Table 4). There were, however, significant longitudinal differences between Normal 1 and Normal 2 on the RAVLT 30-min delay (0.5 points lost annually, $p = .04$) and marginally significant differences on RAVLT percent savings (4 percentage points lost annually, $p = .08$). Normal 2 showed significantly worse baseline GDS scores than Normal 1, with or without adjusting for age (0.9 point difference, $p = .01$). Normal 2 also had significantly increasing scores on the FAQ after controlling for age (higher scores indicate diminished capacity to perform every-day tasks; 0.2 points per year, $p = .01$). Although Normal 2 had significantly worse GDS scores at baseline, Normal 1 showed significant worsening over time in the GDS (0.2 points per year, $p = .05$). Normal 2 did not have a significantly different trajectory from Normal 1, but the estimate and relatively small p value suggest that similar decline in Normal 2 may not be occurring or not occurring as rapidly. The Trails B scores (time to completion) were somewhat skewed, but they have been modeled without transformation for ease of interpretation. The overall conclusions were unchanged when 1/time was modeled instead (results not shown).

Regression models examining possible dose–response effects in the relationship between cognitive change measured with the RAVLT 30-min delay and continuous measures of vascular risk and disease largely failed to find such effects, with the possible exception of BMI, which had a significant relationship with annualized change after controlling for age (−0.05 per BMI point, $p = .04$) and remained borderline significant after removing outliers ($p = .06$), suggesting that increased BMI may be associated with a small negative impact on memory over time in cognitively normal subjects. A 5-point increase in BMI resulted in a quarter of a point decrease per year on the RAVLT 30-min delay, which had an average score of 7 in the healthier group.

Discussion

In summary, despite the fact that the ADNI normal subjects are an extremely healthy group of individuals, we found that a large subgroup of the cognitively normal ADNI differed from a well-defined healthy brain group in terms of increased BMI, vascular disease outcomes, treatment for vascular disease, MRI-based evidence of brain injury, delayed memory decline, and independent functioning.

These findings are somewhat surprising, given that the ADNI normal subjects are a highly select group of healthy individuals. Exclusion criteria included any significant neurological disease other than AD, including stroke or any significant systemic illness or unstable medical condition. The average number of years of education in this population was approximately 16 (4 years beyond high school), which suggests a high level of health literacy, which has been shown to be associated with improved outcomes for multiple health conditions (Berkman, Sheridan, Donahue, Halpern, & Crotty, 2011). In addition, the subjects also were chosen to be unassailably normal in cognitive function at baseline. As such, we did not expect to identify a group with increased brain injury whose cognitive decline was significantly worse, particularly if trajectory differences were subtle, because the cognitive tests performed in ADNI are primarily used in the diagnosis of dementia and suffer from ceiling effects when applied to a cognitively normal population. Therefore, the differences detected in this study are likely to be more pronounced in the general aging population due to the fact that the cognitively normal subjects of the ADNI study are generally much healthier and wealthier than the population at large.

The major between-cluster differences in vascular risk factors were related to the body mass index (BMI). The associations between cluster membership and BMI were driven by a contrast between overweight and healthy weight, as no one in the group of healthy subjects was underweight. This is consistent with previous studies that demonstrated a relationship between BMI and brain structure (Ward, Carlsson, Trivedi, Sager, & Johnson, 2005; Gunstad et al., 2008). Differences at the trend level were also seen with fasting blood glucose and triglyceride levels. This constellation of vascular risk factors is part of the metabolic syndrome, which has been associated with cognitive decline and incident dementia (Yaffe, 2007). Moreover, obesity itself—a major risk factor for the metabolic syndrome—is also associated with later-life dementia (Debette, Beiser, Hoffmann, et al., 2010; Whitmer, Gunderson, et al., 2005, Whitmer, Sidney, et al. 2005; Whitmer et al., 2008). Importantly, insulin resistance, a common consequence of the metabolic syndrome, is associated with generalized brain atrophy (Tan et al., 2011), hippocampal atrophy (Rasgon et al., 2011), and WMH (Katsumata et al., 2010). Individuals in the Normal 2 group also had increased vascular consequences of these risk factors. For example, Hachinski ischemic scores were significantly higher in this group, despite restrictions imposed by the study design. In addition, serum creatinine and homocysteine were significantly elevated in the Normal 2 group. Both serum homocysteine and creatinine have been associated with brain injury (Khatri et al., 2007; Seshadri et al., 2008; Wright et al., 2005), and hyperhomocysteinemia may be a secondary cause for vascular brain injury in the setting of renal insufficiency. Finally, the Normal 2 group was also prescribed significantly more medications for vascular disease; therefore, the group differences discovered were likely attenuated by medication use.

Although this study, by including small numbers of generally healthy individuals, had limited power to directly study the relationship between vascular risk factors and brain injury, the imaging findings are consistent with vascular brain injury previously reported in a number of studies (Jeerakathil et al., 2004; Seshadri et al., 2004). The summary WMH measure was not significantly different between the two groups, although it trended in the direction of the hypothesis. The exclusion criteria for ADNI included any significant systemic illness or unstable condition, which would have excluded subjects with moderate to severe vascular diseases, thereby reducing the variability we would expect to see in global WMH measures. However, the pattern of WMH for the two groups did differ significantly and clearly shows extension of WMH around the ventricles in a manner associated with hypertension in a larger, previously reported study (Yoshita et al., 2006). The longitudinal trajectory of WMH in this group is an area for further study as ADNI follow-up continues. The magnitude of regional brain atrophy was either intermediate to or consistent with that seen in the MCI group, as previously reported (Nettiksimmons et al., 2010). The pattern of gray matter atrophy as summarized in Figure 3, however, appears more closely related to that expected to be seen with AD. This may suggest that the AD-like pattern of early brain atrophy may not be limited to damage due to AD, and this observation deserves further study.

Moreover, our findings suggest that the brain structural and cognitive differences in the Normal 2 group were not the consequence of an early AD process. The evidence that supports this assertion is as follows: (a) there was no significant difference between Normal 1 and Normal 2 in terms of Aβ, which is hypothesized to be the first detectable change in the early development of AD (Jack et al., 2010), and (b) there was no significant difference between Normal 1 and Normal 2 with respect to total tau, and both differ substantially from Normal 3. Furthermore, Normal 2 was significantly associated with a number of vascular associates, providing an alternate explanation for the atrophy. This, combined with the CSF evidence, suggests that the atrophy findings were related to vascular and not AD-mediated brain injury.

The biological differences between the two clusters were also associated with differences in cognitive performance over time. Normal 2, although not significantly worse at baseline on any of the cognitive tests, did have a significantly worse cognitive trajectory for RAVLT 30-min recall and a hint of poorer trajectory in RAVLT percent savings. Because memory deficits constitute the earliest cardinal sign of eventual clinical AD (Morris et al., 2001), the finding of significant memory decline in Normal 2 may appear to contrast with our hypothesis that vascular brain injury, not AD, is the driver of atrophy in Normal 2. However, vascular disease may directly cause memory decline through injury to memory-critical brain regions, and the executive dysfunction that is the more traditional indicator of vascular brain injury may itself exacerbate episodic memory declines by impairing memory encoding (Nordahl et al., 2005; Parks et al., 2011). Therefore, longitudinal change in memory may reflect declining executive function, although this may be sufficiently minor, so as to not be detected by our executive composite. Although nonsignificant, other cognitive tests such as digit span forward and Logical Memory II also suggested the possibility of poorer trajectories in the Normal 2 group. For example, the Normal 1 group showed significant learning effects over time—a phenomenon typical of healthy aging—on digit span forward and Logical Memory II, whereas the Normal 2 group estimates suggest lesser or nonexistent learning effects. Cognitively normal individuals often show improvement in task performance over short periods of repeated testing, and the absence of practice effects has been used to indicate incipient disease (Dodge, Wang, Chang, & Ganguli, 2011). Lack of further differences in cognitive performance may reflect the choices of test instruments, as the cognitive tests available in ADNI are broadly used clinical instruments whose measurement properties are not ideal for teasing out subtle differences in cognitive performance in a highly select group of cognitively normal individuals. Despite a lack of substantial cognitive differences, the FAQ, which is a measure of the subject's ability to carry out life tasks, such as paying bills, balancing a checkbook, preparing meals, and keeping track of current events, also showed significantly greater decline in the Normal 2 group, suggesting that the noted cognitive abnormalities may be clinically relevant.

The primary weakness of this study is the aforementioned difficulty in detecting cognitive differences in cognitively normal individuals with tests meant for subjects with dementia, as well as the highly selective population of cognitively normal individuals from the ADNI study, which are not representative of the aging population at large. Because the ADNI normal group was selected to underrepresent vascular disease burden, we believe that the strengths of association between vascular disease, brain injury, and cognition are likely to be much stronger within the general population. The other key weakness was a lack of detailed, sensitive, longitudinal measures of many vascular risks and outcomes that would be required to accurately capture vascular disease processes that culminate over the course of multiple decades (Carmelli et al., 1998; Launer, Masaki, Petrovich, Foley, & Havlik, 1995; Swan et al., 1998). Of course, a larger sample also would have been preferable. Unsupervised cluster analysis has inherent limitations due to its unsupervised nature, which does not allow for features like cross-validation. However, this analysis has identified an unexpected biomarker pattern in an otherwise extremely healthy group of older individuals, demonstrating its utility as a hypothesis-generating tool.

The primary strength of this study was the availability of an unusually diverse group of clinical and biological measures (MRI, CSF, serum, health history, medications, and indication), which allowed a particularly informative unsupervised cluster analysis. The combination of diverse and informative measures with longitudinal psychometric follow-up provided a unique venue to examine multifaceted, longitudinal differences between subgroups. The only assumptions made by the authors were the variables selected for the clustering and the number of clusters. The individual cluster assignments were driven entirely by biomarker profile differences between study subjects.

It remains to be seen whether the individuals in Normal 2 are currently in a preclinical state and will go on to develop dementia—vascular dementia, AD, or otherwise. Extended ADNI follow-up, along with other longitudinal aging studies, may be able to determine whether pronounced atrophy in the absence of CSF abnormality in the cognitively normal aged represents the early period of a recognized clinical entity. The results from this study, although sometimes subtle, are particularly provocative because of the very healthy group of people from which they were drawn and the relative size of Normal 2, which contained over half of the normal subjects who had the complete data necessary for clustering. These findings challenge any presumptions that all older individuals somehow sit solely along the continuum of Alzheimer's pathology by demonstrating the degree of heterogeneity of pathologies among cognitively normal individuals and suggesting the presence of at least one common alternate pathway to brain atrophy. It is possible that some of what is currently considered "normal aging" is actually the result of pathological vascular processes (Mayda et al., 2011). From a public health perspective, this would present an enormous opportunity to target treatment of vascular disease, given that medical practitioners already have a wide variety of tools at their disposal to prevent and treat vascular disease. These results could also be used to encourage more middle-aged and older people to adopt healthier lifestyles and be more willing to aggressively treat conditions like hypertension, because the prospect of cognitive decline with advancing age is a tangible threat.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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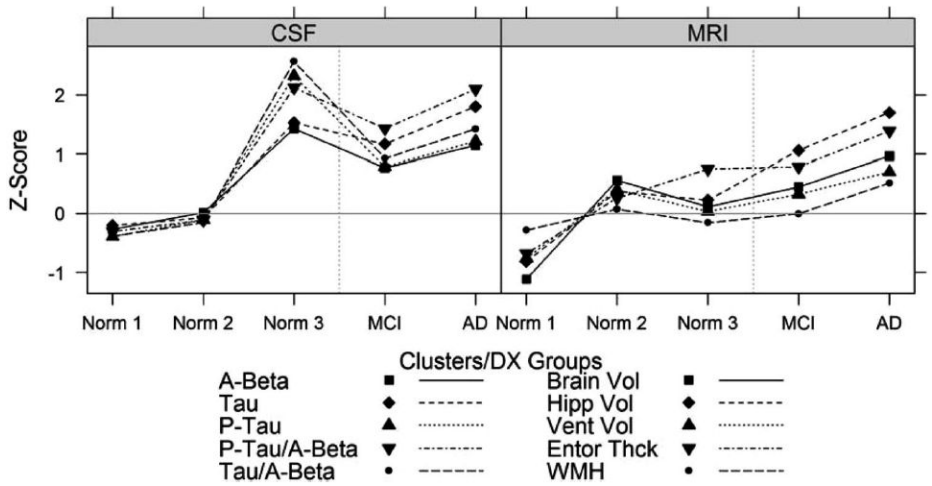

Figure 1.

Z Scores for CSF and MRI clustering biomarkers showing relative abnormality. Signs have been reversed, where necessary, so that high values always indicate abnormality. *Z* scores were created using the means and standard deviations from all available baseline normal control data. This graphic clearly illustrates the relatively healthy CSF levels in Normal 2 accompanied by fairly abnormal MRI measures. Adapted from "Subtypes based on CSF and MRI markers in normal elderly predict cognitive decline" by J. N. Nettiksimmons, D. Harvey, J. Brewer, O. Carmichael, C. DeCarli, C. R. Jack, R. Petersen, L. M. Shaw, J. Q. Trojanowski, M. W. Weiner, L. Beckett, and The Alzheimer's Disease Neuroimaging Initiative. 2010, *Neurobiology of Aging, 31*(8), p. 1425. Copyright 2010 by Elsevier Ltd.

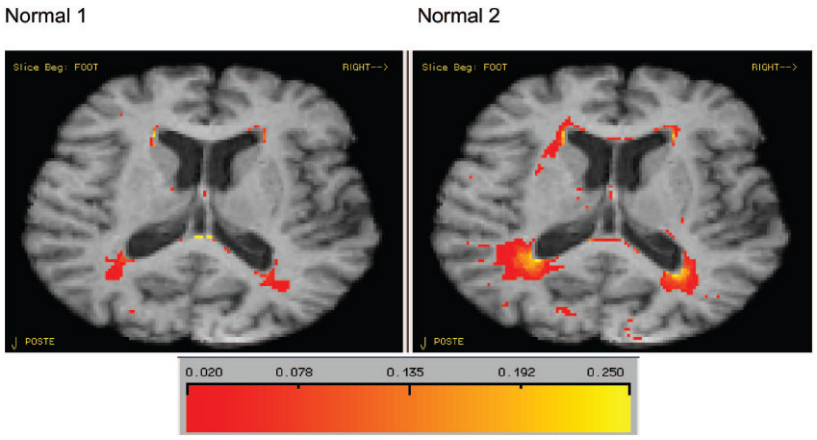

Figure 2.

Frequency of WMH in Normal 1 versus Normal 2 as a percentage of total subject group, ranging from red at 2% (0.02) to yellow at 25% (0.25). Frequency indicates the percentage of subjects having a WMH voxel at that particular image location. The voxel locations are more broadly distributed in group Normal 2, and the number of individuals at each voxel is also increased.

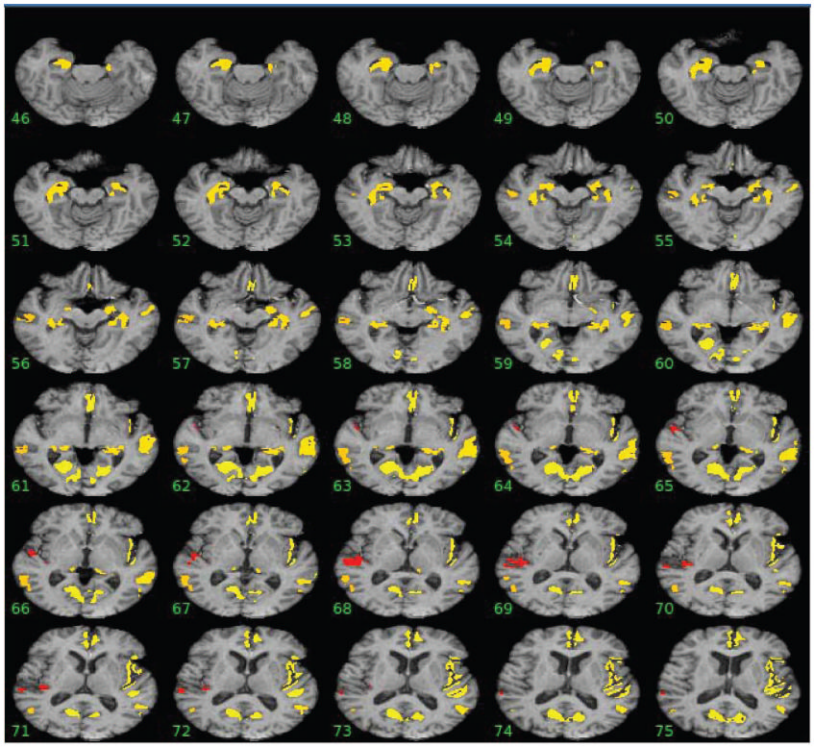

Figure 3.
Significant group differences in cerebral atrophy. Yellow denotes clusters of significantly lower gray matter volumes in Normal 2 and red denotes clusters of significantly higher CSF volume in Normal 2. Yellow regions indicate where cluster-level gray matter density differs significantly between groups after correction from multiple comparisons using permutation testing. Significant group differences in atrophy can be seen in the bilateral hippocampus, insula, posterior cingulate, and superior temporal regions.

Table 1

Comparison of General Characteristics and Clustering Biomarkers Between Normal 1 and Normal 2

Measure	Normal 1 (*n* = 31)	Normal 2 (*n* = 53)	Normal 3 (*n* = 9)	*p* value (Normal 1 vs. Normal 2)
Nonclustering variables[a]				
Age (years)	72.5 ± 5.9	76.7 ± 4.6	76.4 ± 3.5	**0.001**
Gender (male)	42%	60%	67%	0.11
Education (years)	15.3 ± 2.7	16.0 ± 3.0	16.1 ± 2.8	0.25
ApoE4+[b]	23%	25%	53%	0.84
Follow-up (years)	2.9 ± 0.3	2.8 ± 0.4	2.8 ± 0.4	0.63
Clustering variables[c]				
Whole brain volume[d]	0.712 (0.7058, 0.7182)	0.6705 (0.6657, 0.6752)	0.6815 (0.67, 0.693)	
Ventricle volume[d]	0.0162 (0.0128, 0.0196)	0.0307 (0.0281, 0.0333)	0.0261 (0.0198, 0.0324)	
Hippocampal volume[d]	0.0054 (0.0053, 0.0056)	0.0048 (0.0047, 0.0049)	0.0049 (0.0046, 0.0051)	
Entorhinal thickness (mm)	3.3814 (3.2656, 3.4972)	3.1583 (3.0697, 3.2468)	3.0807 (2.8657, 3.2956)	
WMH (cm^3)	1.944 (1.034, 2.854)	2.825 (2.129, 3.521)	2.261 (0.572, 3.950)	
Homocysteine (μmol/L)	8.86 (7.9, 9.83)	10.65 (9.91, 11.39)	9.94 (8.15, 11.74)	
Amyloid-$\beta_{1\text{-}42}$ (pg/mL)	221.0 (203.6, 238.4)	205.7 (192.4, 219)	127.1 (94.8, 159.4)	
Total Tau (pg/mL)	63.1 (54.6, 71.6)	66.7 (60.2, 73.2)	111.9 (96.1, 127.6)	
P-Tau_{181} (pg/mL)	19.1 (15.8, 22.5)	23.0 (20.5, 25.5)	55.9 (49.7, 62.1)	
Tau/$A\beta_{1\text{-}42}$ (pg/mL)	0.30 (0.24, 0.37)	0.35 (0.30, 0.40)	0.91 (0.79, 1.03)	
P-Tau_{181}/$A\beta_{1\text{-}42}$ (pg/mL)	0.09 (0.07, 0.12)	0.12 (0.10, 0.14)	0.46 (0.41, 0.51)	

[a] Means and standard deviations are reported for nonclustering variables and differences were assessed with *t* tests.

[b] Proportion with at least one E4 allele.

[c] Means and 95% confidence intervals are reported for clustering variables

[d] Presented as fraction of ICV.

P-values <0.05 are indicated in bold type.

Table 2

Means, Standard Errors, and P Values From Linear Regression Models Testing the Association of Continuous Vascular Risk Factors and Conditions With Cluster Membership While Controlling for Age

Measure type	Measure	Normal 1	Normal 2	*p* value
Vascular risk factors	Cholesterol (mg/dL)	190.9 ± 7.1	187.3 ± 6.6	0.70
	BMI	25.4 ± 0.9	27.7 ± 0.8	**0.04**
	Trigylcerides (mg/dL)	114.6 ± 17.6	157.2 ± 16.5	0.07
	Blood glucose (mg/dL)	93.9 ± 3.3	101.9 ± 3.1	0.06
	Systolic BP (mmHG)	133 ± 3.4	131.1 ± 3.1	0.66
	Diastolic BP (mmHG)	74.2 ± 1.6	74.9 ± 1.5	0.73
Vascular outcomes	Urea nitogren (mg/dL)	17.6 ± 1.2	19.5 ± 1.1	0.23
	Hachinski	0.3 ± 0.1	0.8 ± 0.1	**0.01**
	Creatinine (mg/dL)	0.8 ± 0.05	1.02 ± 0.04	**0.002**
	Creatinine (mg/dL) (adjusted for gender)	0.7 ± 0.04	0.86 ± 0.05	**0.01**

Note. The adjusted creatinine model has been adjusted for gender; the coefficients shown correspond to female gender.

BMI = body mass index; BP= blood pressure.

P-values <0.05 are indicated in bold type.

Table 3

Correlations Between Clustering Biomarkers and Continuous Vascular Risk Factors and Outcomes Calculated on Combined Normal 1 and Normal 2 Data

	Continuous vascular-associated variables								
Clustering biomarkers	**Cholesterol**	**BMI**	**Triglycerides**	**Serum glucose**	**Systolic BP**	**Diastolic BP**	**Urea nitrogen**	**Hachinski**	**Creatinine**
Whole brain volume	−0.08	−0.07	−0.18	−0.17	−0.01	−0.10	**−0.22**	**−0.26**	**−0.35**
Ventricle volume	0.01	−0.06	0.04	0.07	−0.06	0.00	**0.25**	0.14	0.20
Hippocampal volume	0.13	−0.03	−0.05	−0.05	−0.01	0.08	−0.12	−0.12	−0.08
Entorhinal thickness	0.15	0.00	−0.10	−0.09	−0.07	−0.09	0.09	−0.15	−0.11
WMH	0.00	−0.01	0.15	0.12	0.04	−0.11	−0.17	0.16	−0.09
Homocysteine	−0.02	0.12	0.21	−0.03	0.00	−0.10	**0.52**	0.15	**0.53**
Amyloid-$\beta_{1\text{-}42}$	−0.12	0.10	0.14	0.00	−0.02	−0.04	0.04	0.06	0.10
Total Tau	0.10	−0.07	0.02	0.03	0.05	**−0.23**	−0.14	0.04	−0.08
P-Tau_{181}	0.19	−0.05	0.09	0.10	0.09	−0.06	0.03	**0.26**	0.08
Tau/$A\beta_{1\text{-}42}$	0.12	−0.14	−0.11	0.02	0.08	−0.13	−0.13	0.01	−0.10
P-Tau_{181}/$A\beta_{1\text{-}42}$	0.17	−0.11	−0.06	0.10	0.09	−0.02	−0.03	0.17	−0.01

Note. Correlations meeting or exceeding a magnitude of 0.22 have been marked in bold type.

BMI = body mass index; BP = blood pressure; WMH = white matter hyperintensities.

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Table 4

Baseline and Annual Change Estimates From Regression Models of Cognitive Scores Using Cluster as a Predictor

	Estimated mean value for Normal 1	Normal 2 estimated difference from Normal 1	*p* value
Baseline			
Executive/Attention			
Digit span forward	8.4 (0.3)	0.4 (0.4)	0.29
Digit span backward	7.4 (0.4)	−0.3 (0.5)	0.48
Digit symbol	48.8 (1.5)	−1.5 (1.8)	0.43
Trails B	85.9 (4.8)	−8.0 (6.0)	0.19
Category naming	34.8 (1.4)	0.3 (1.8)	0.88
Executive composite	0.1 (0.1)	0.02 (0.1)	0.90
Memory			
ADAS-cog	5.6 (0.5)	0.7 (0.6)	0.25
RAVLT sum of 5 trials	44.7 (1.6)	−1.8 (2.0)	0.38
RAVLT 30 min	7.3 (0.6)	0.1 (0.8)	0.95
RAVLT savings	62.3 (4.5)	4.0 (5.6)	0.47
Logical Memory II	13.0 (0.7)	0.2 (0.9)	0.84
Other			
FAQ	0.1 (0.1)	0.1 (0.2)	0.55
GDS	0.5 (0.3)	0.9 (0.3)	**0.01**

	Estimated annual change in Normal 1	*p* value	Normal 2 estimated difference from Normal 1	*p* value
Annual Change				
Executive/Attention				
Digit span forward	0.2 (0.1)	**0.02**	−0.1 (0.1)	0.32
Digit span backward	0.02 (0.1)	0.89	0.04 (0.2)	0.80
Digit symbol	0.3 (0.4)	0.47	−0.5 (0.5)	0.31
Trails B	−0.6 (2.8)	0.84	2.4 (3.6)	0.50
Category naming	0.1 (0.4)	0.87	0.1 (0.4)	0.77
Executive composite	0.03 (0.02)	0.21	−0.02 (0.03)	0.51
Memory				
ADAS-cog	−0.1 (0.2)	0.66	0.1 (0.2)	0.80
RAVLT sum of 5 trials	−0.8 (0.4)	0.07	0.2 (0.5)	0.68
RAVLT 30 min	0.3 (0.2)	0.13	−0.5 (0.2)	**0.04**
RAVLT savings	3.0 (1.9)	0.12	−4.2 (2.4)	0.08
Logical Memory II	0.5 (0.2)	**0.03**	−0.2 (0.3)	0.49
Other				
FAQ	−0.1 (0.1)	0.36	0.2 (0.1)	**0.01**
GDS	0.2 (0.1)	0.05	−0.2 (0.1)	0.14

Note. All models control for education, age, and the interaction of age and time, with the exception of the FAQ and GDS, for which education was not controlled. Age and education were centered, so the estimates correspond with individuals with average age and education. The executive

composite is the average of the *z* scores for digit span forward and backward, digit symbol substitution, Trails b, and the Category Naming Test, with the sign reversed for Trails b so that the meaning of the *z* scores in the composite is consistent. Means and standard errors are provided.

ADAS-cog = Alzheimer's Disease Assessment Scale cognitive subscale; FAQ = Functional Activity Questionnaire; GDS = Geriatric Depression Scale; RAVLT = Rey's Auditory Verbal Learning Test.

P-values <0.05 are indicated in bold type.