PRECLINICAL EVIDENCE OF ALZHEIMER’S DISEASE IN PERSONS HOMOZYGOUS FOR THE ε4 ALLELE FOR APOLIPOPROTEIN E

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Abstract Background. Variants of the apolipoprotein E gene appear to account for most cases of late-onset Alzheimer’s disease, and persons with two copies of the ε4 allele appear to have an especially high risk of dementia. Positron-emission tomography (PET) has identified specific regions of the brain in which the rate of glucose metabolism declines progressively in patients with probable Alzheimer’s disease. We used PET to investigate whether these same regions of the brain are affected in subjects homozygous for the ε4 allele before the onset of cognitive impairment.

Methods. Apolipoprotein E genotypes were established in 235 volunteers 50 to 65 years of age who reported a family history of probable Alzheimer’s disease. Neurologic and psychiatric evaluations, a battery of neuropsychological tests, magnetic resonance imaging, and PET were performed in 11 ε4 homozygotes and 22 controls without the ε4 allele who were matched for sex, age, and level of education. An automated method was used to generate an aggregate surface-projection map that compared regional rates of glucose metabolism in the two groups.

Results. The ε4 homozygotes were cognitively normal. They had significantly reduced rates of glucose metabolism in the same posterior cingulate, parietal, temporal, and prefrontal regions as in previously studied patients with probable Alzheimer’s disease. They also had reduced rates of glucose metabolism in additional prefrontal regions, which may be preferentially affected during normal aging.

Conclusions. In late middle age, cognitively normal subjects who are homozygous for the ε4 allele for apolipoprotein E have reduced glucose metabolism in the same regions of the brain as in patients with probable Alzheimer’s disease. These findings provide preclinical evidence that the presence of the ε4 allele is a risk factor for Alzheimer’s disease. PET may offer a relatively rapid way of testing future treatments to prevent Alzheimer’s disease.

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VARIANTS of the apolipoprotein E gene appear to account for the majority of cases of late-onset Alzheimer’s disease (i.e., those involving the onset of dementia after the age of 60).1,2 The gene, located on chromosome 19, has three major alleles: ε2, ε3, and ε4.3 The ε2 allele appears to be protective, decreasing the risk of Alzheimer’s disease and delaying the onset of dementia.4 In contrast, the ε4 allele appears to be harmful, increasing the risk of Alzheimer’s disease and hastening the onset of dementia.5,6 If, as case-control studies suggest, persons with two copies of the ε4 allele (the ε4/ε4 genotype) have an especially high risk of Alzheimer’s disease, the study of presymptomatic subjects who are homozygous for the ε4 allele could provide additional support for this genetic risk factor, produce new information about the pathophysiology of the disorder, and identify biologic markers that may be very useful in monitoring future disease-prevention therapies.

Positron-emission tomography (PET) is a brain-imaging technique that can be used to study the physiologic processes that herald the onset of dementia. When used to measure cerebral glucose metabolism, PET reveals characteristic abnormalities in patients with probable and definite Alzheimer’s disease, including abnormally low parietal, temporal, and posterior cingulate levels; abnormally low prefrontal and whole-brain levels in more severely affected patients; and a progressive decline in these levels over time.7,8 Case series suggest that abnormalities in glucose metabolism can be detected by PET before substantial impairment occurs in persons at risk for Alzheimer’s disease9,10 and certain other neurodegenerative disorders.11,12 In a recent study, subjects with the apolipoprotein E ε3/ε4 genotype, age-associated memory impairment, and a family history of Alzheimer’s disease had abnormally low and asymmetric rates of glucose metabolism in a preselected parietal region before the onset of dementia.13

We used PET to investigate regions of the brain that are affected before the onset of cognitive decline in persons homozygous for the apolipoprotein E ε4 allele. We sought to test the hypothesis that such persons have abnormally low rates of glucose metabolism in the same brain regions as previously studied patients with probable Alzheimer’s disease, explore the possibility that they also have abnormally low rates in other regions of the brain, and begin to fashion a way to test possible preventive therapies for Alzheimer’s disease.

Methods

Subjects

To identify a relatively large number of persons homozygous for the apolipoprotein E ε4 allele, we used newspaper advertisements to recruit 235 volunteers (172 women and 63 men) 50 to 65 years of age who reported a family history of probable Alzheimer’s disease in at
less than one first-degree relative. The participants agreed that they would not be given information about their apolipoprotein E genotype, provided informed consent, and were studied under guidelines approved by local review committees at Good Samaritan Regional Medical Center (Phoenix, Ariz.) and the Mayo Clinic (Rochester, Minn.). Venous blood samples were drawn, leukocytes isolated, and apolipoprotein E genotypes characterized with analysis involving restriction-fragment–length polymorphisms.

Twelve subjects who were homozygous for the ε4 allele were identified. One declined to participate in the imaging studies of the brain. For each of the 11 ε4 homozygotes who agreed to participate in the imaging studies, 2 control subjects without this allele (6 with the ε2/ε3 genotype and 16 with the ε3/ε3 genotype) were matched for sex, age (within three years), and level of education (within two years). Investigators who were unaware of the subjects’ apolipoprotein E genotypes obtained data from medical and family histories, a neurologic examination, a structured psychiatric interview, the Folstein modified Mini–Mental State Examination (MMSE), the Hamilton Depression Rating Scale, a battery of neuropsychological tests, and brain-imaging studies.

For 10 ε4 homozygotes and 20 controls, the affected first-degree relative was a parent. The study subjects denied having an impairment in memory or other cognitive skills, did not satisfy criteria for a current psychiatric disorder, had no known cardiovascular or cerebrovascular disease, and did not use centrally acting medications for at least two weeks before their PET session. However, one ε4 homozygote and two controls reported a mild hearing impairment; one ε4 homozygote and two controls reported a brief loss of consciousness due to a closed head injury in the remote past; one control had had an episode of amaurosis fugax 14 years before the study; one ε4 homozygote and two controls had taken an antihistamine on the night before the PET session; three ε4 homozygotes and three controls reported a history of hypertension; and one ε4 homozygote reported a history of hypercholesterolemia. All had a normal neurologic examination.

Neuropsychological Tests

Each subject completed a one-hour battery of neuropsychological tests at the Mayo Clinic (Scottsdale, Ariz.), including the Auditory Verbal Learning Test, which assesses verbal learning and recall; the Complex Figure Test, which assesses constructive praxis and visuospatial memory; the Boston Naming Test, which assesses visual naming; the Information, Digit Span, Mental Arithmetic, Similarities, and Block Design subtests of the Wechsler Adult Intelligence Scale–Revised, which assess general intellect, attention, abstraction skills, psychomotor speed, and spatial skills; the Controlled Oral Word Association Test, which assesses verbal associative fluency and psychomotor speed; and the Orientation subtest of the Wechsler Memory Scale–Revised.

Brain Imaging

T₁-weighted, volumetric magnetic resonance imaging (MRI) was performed with a 1.5-T Signa system (General Electric, Milwaukee) at Good Samaritan Regional Medical Center to rule out gross anatomical abnormalities, to facilitate comparisons between brain function and structure when improved image-analysis techniques become available, and ultimately to characterize morphometric abnormalities in the ε4 homozygotes. PET was also performed at the same institution with a 951/31 ECAT scanner (Siemens, Knoxville, Tenn.), a 20-minute transmission scan, the intravenous injection of 10 mCi of [¹⁸F]fluorodeoxyglucose, a 60-minute dynamic sequence of emission scans, and frequent sampling of radial-artery blood as the subjects, who had fasted for at least 4 hours, lay quietly in a darkened room with their eyes closed and directed forward. A 15-cm back-projection method with a Hanning filter of 0.44 cycle per pixel, and a procedure to correct for radiation attenuation were used to reconstruct PET images consisting of 31 horizontal slices with a resolution in the plane of section of about 9.5 mm, full width at half maximum; a resolution in the axial direction of 5.0 to 7.1 mm, full width at half maximum; and a distance of 3.375 mm between slices. In these images, the rate of glucose metabolism (expressed as milligrams per minute per 100 g of tissue) was calculated with the use of arterial activity measurements, plasma glucose levels, and a graphic method. Glucose metabolism in the whole brain was calculated in each subject as the average measurement from all intracerebral voxels (including those of ventricles) inferior to a horizontal slice through the falx and superior to a horizontal slice through the mid-thalamus. No attempt was made to address the combined effect of atrophy and partial-volume averaging on whole-brain or regional measurements.

Image Analysis

To characterize regions of the brain with abnormally low rates of glucose metabolism in Alzheimer’s disease, a fully automated algorithm was initially used to compare PET images acquired at the University of Michigan in a group of 37 patients with probable Alzheimer’s disease (mean ± 5SD age, 64 ± 7.3 years) and a group of 22 normal controls (mean age, 64 ± 7.3 years). Each subject’s PET image was linearly and nonlinearly deformed according to the coordinates of a standard atlas of the brain.[21,26] Measurements in each voxel were normalized to that in the pons, which appears to be the region least affected in patients with Alzheimer’s disease. Data on the outer and medial surface of each hemisphere were extracted.[13] A three-dimensional stereotactic surface-projection z-score map of reductions in the metabolic rate in the group with probable Alzheimer’s disease was computed as the difference between group means divided by the standard deviation for the control group in each voxel, and the map (z score, >2.58; P ≤ 0.005, uncorrected for multiple comparisons) was then superimposed on a spatially standardized and volume-rendered MRI of the brain (Fig. 1).

To test the hypothesis that the presymptomatic ε4 homozygotes had abnormally low rates of glucose metabolism in the same brain regions as the patients with probable Alzheimer’s disease, the same algorithm was used to compare PET images from the ε4 homozygotes and their age-matched controls. The three-dimensional stereotactic surface-projection z-score map of metabolic reductions in the homozygous group (z score, >2.58; P ≤ 0.005, uncorrected for multiple comparisons) was superimposed on the map of metabolic reductions in the group with probable Alzheimer’s disease and the spatially standardized, volume-rendered MRI (Fig. 2). A critical z score of 4.32 was used to identify voxels in which the homozygous group had significant reductions in glucose metabolism in the same regions as the patients with probable Alzheimer’s disease (P < 0.05 after correction for the number of comparisons [i.e., the 278 resolution elements] in the searched volume) as well as in additional regions. Unpaired two-tailed t-tests were performed post hoc to confirm the consistency of the reductions in glucose metabolism in voxels with maximal z scores.

Results

The distribution of apolipoprotein E genotypes in the 253 subjects who reported a family history of probable Alzheimer’s disease is shown in Table 1. The percentage of ε4 homozygotes in this sample was higher than that in the general population (5.1 percent vs. 2 to 3 percent), a finding consistent with our expectation of an increased frequency of the ε4 allele in the study subjects’ affected first-degree relatives. The characteristics of the ε4 homozygotes and control subjects are shown in Table 2. There were no significant differences in age, sex, handedness, years of education, age of the affected family member at the onset of dementia, scores on the Hamilton Depression Rating Scale, or scores on the Mini–Mental State Examination (range, 28 to 30 in both groups).

Neuropsychological Tests

The neuropsychological scores of the ε4 homozygotes and control subjects are shown in Table 3. There were no significant differences between groups in verbal memory (as measured by the Auditory Verbal Learning Test), visual memory (as measured by the recall portion of the Complex Figure Test), naming (as assessed by the Boston Naming Test), or visuospatial and constructional
skills (as assessed by the copy portion of the Complex Figure Test and the Block Design subtest of the Wechsler Adult Intelligence Scale–Revised), all of which are characteristically impaired in persons with Alzheimer’s disease. There were no significant differences in language skills or psychomotor speed (as measured by the Controlled Oral Word Association Test), estimates of premorbid intellectual function (as assessed by the Information subtest of the Wechsler Adult Intelligence Scale–Revised), or directed attention span (as assessed by the Digit Span subtest). As compared with the control subjects, the $e_4$ homozygotes had slightly lower scores on the Mental Arithmetic test, Similarities test, and Freedom from Distractibility factor (measures of concentration and abstract reasoning), which could reflect cognitive predictors of dementia, some of the PET abnormalities described below, or false positive findings.

Although one 62-year-old $e_4$ homozygote denied having impairment in memory and had a score of 28 on the Mini–Mental State Examination, his scores on both the Auditory Verbal Learning and Boston Naming tests were more than 1 SD below the mean established for young adults, suggesting the presence of mild cognitive impairment. The exclusion of his data in a subsequent analysis only minimally affected the mean scores in the group of $e_4$ homozygotes.

**PET**

There were no significant differences between the $e_4$ homozygotes and control subjects in the rates of whole-brain glucose metabolism (mean $\pm$ SD), $5.1\pm1.0$ vs. $5.2\pm0.8$ mg per minute per 100 g; $P=0.62$ by two-tailed, unpaired t-test) or pontine glucose metabolism ($5.0\pm1.0$ vs. $5.0\pm0.7$ mg per minute per 100 g, $P=0.90$).

![Figure 1. Regions of the Brain with Reduced Rates of Glucose Metabolism in 37 Patients with Probable Alzheimer’s Disease.](image-url)
the measurement of which was used to normalize PET data for the variation in absolute measurements.

The group of ε4 homozygotes had significant bilateral reductions in glucose metabolism in the same posterior cingulate, parietal, temporal, and prefrontal regions as the group with probable Alzheimer’s disease (Fig. 2 and Table 4); the maximal reduction in glucose metabolism in the posterior cingulate cortex was significantly greater than those in the other regions (z score, 8.26; P<0.001).

The ε4 homozygotes also had significant reductions in glucose metabolism in additional prefrontal regions (Fig. 2) (maximal z score, 4.76; P<0.001 without correction for multiple comparisons), which PET, MRI, and neuropathological studies suggest are preferentially affected during normal aging. Although these findings should be considered preliminary, their spatial extent and bilateral nature suggest that they are not due to type I statistical errors.

The ε4 homozygote with neuropsychological evidence of mild cognitive impairment had the greatest reductions in glucose metabolism in each of the locations listed in Table 4. When his data were excluded in a subsequent analysis, glucose metabolism remained reduced...
in these locations in the ε4 homoygotes; again, the greatest reduction was observed in the posterior cingulate region (maximal z score, 5.23; P<0.05 after correction for multiple comparisons).

**DISCUSSION**

We identified regions of the brain that are affected before the onset of cognitive impairment in persons who, according to case-control studies, have a very high risk of Alzheimer’s disease. These data provide preclinical evidence that the apolipoprotein E ε4 allele is a risk factor for Alzheimer’s disease and support the possibility that this allele accelerates certain aging processes.

Using a brain-mapping algorithm that characterizes differences between groups in regional PET measurements, we found that the presymptomatic ε4 homoygotes had significantly reduced rates of glucose metabolism in the same parietal, temporal, prefrontal, and posterior cingulate regions as patients with probable Alzheimer’s disease. The largest reduction was in the posterior cingulate cortex, which is affected in Alzheimer’s disease.34,35 could be affected relatively early,34 and might provide the most sensitive metabolic evidence of the pathologic changes that herald the onset of dementia. The metabolic reductions were greatest in an ε4 homoygote with neuropsychological evidence of mild cognitive impairment, but were also apparent in the remaining, cognitively intact ε4 homoygotes. This observation is consistent with reports that reductions in regional glucose metabolism increase as Alzheimer’s disease progresses from a presymptomatic stage to one characterized by mild symptoms, and ultimately to increasingly severe stages.36,37 Although the reductions in regional glucose metabolism could reflect decreased activity of terminal neuronal fields,36 decreased density of terminal neuronal fields, atrophy, or some combination of these factors, they appear to be markers of the pathologic changes that precede the onset of Alzheimer’s dementia.

In a recent study,15 patients who presented to a clinic with reports of memory impairment, satisfied the proposed criteria for age-associated memory impairment,37 did not satisfy criteria for dementia,38 had a well-documented family history of probable Alzheimer’s disease in at least two first-degree relatives, and had undergone PET previously were divided into two groups according to their ε4-allele status: ε4 heterozygotes and noncarriers of the allele. As compared with the noncarriers of the allele, the ε4 homoygotes had abnormally low and asymmetric rates of glucose metabolism in a preselect-ed parietal region; measurements in other regions and the whole brain were not compared. In our study, ε4 ho-
association between the ε4 allele and Alzheimer’s disease — an association that has now been observed internationally in about 100 clinics.

The ε4 homozygotes had abnormally low rates of glucose metabolism bilaterally in additional prefrontal regions that numerous PET, MRI, and neuropathological studies suggest are preferentially affected during normal aging.29,33 A comparison of patients with probable Alzheimer’s disease with no copies, one copy, or two copies of the ε4 allele is needed to address the possibility that this allele is simply related to a form of Alzheimer’s dementia that preferentially affects the frontal lobes. The prefrontal abnormalities do not appear to be related to other factors known to affect frontal-lobe function, such as certain psychiatric disorders, medications, severity of depressive symptoms, or differences in the subjects’ behavioral state during the PET session.

Considering that older age is an important risk factor for Alzheimer’s disease, we propose that the additional reductions in prefrontal glucose metabolism in the ε4 homozygotes reflect an acceleration in certain aging processes that herald the onset of Alzheimer’s dementia. (If so, the failure to find a difference in glucose metabolism in these prefrontal regions between the older patients with probable Alzheimer’s disease and their controls could reflect the occurrence of a similar decline in both older age groups.) The idea that variants of the apolipoprotein E allele advance or retard certain aging processes is consistent with several observations: the ε4 allele has been associated with an increased risk of Alzheimer’s disease,1-7 a younger age at the onset of dementia,2,5 a faster rate of Alzheimer’s disease giving rise to optimism that an intervention might be developed that could slow the progression, delay the onset, or even prevent Alzheimer’s disease.1-5 If, for instance, the E4 isoform increases the risk of Alzheimer’s disease by binding to the β-amyloid protein and accelerating the deposition of amyloid (the main constituent of senile plaques),46,47 a drug or gene therapy that inhibits binding to the β-amyloid protein might interfere with the progression or onset of Alzheimer’s disease in persons with one or two copies of the ε4 allele. If, instead, the E2 isoform decreases the risk of Alzheimer’s disease by increasing the binding of apolipoprotein E to the microtubule-associated protein τ, interfering with hyperphosphorylation of the protein, and inhibiting its assembly into the paired helical filaments that make up neurofibrillary tangles,48,49 a drug or gene therapy50 that promotes these effects might interfere with the progression or onset of Alzheimer’s disease regardless of the apolipoprotein E genotype.5 If, as we postulate, the reductions in glucose metabolism observed in presymptomatic ε4 homozygotes progress, PET could provide a relatively rapid way to test treatments to prevent the disease.

We are indebted to Judy Lawrence, Carolyn Barbieri, Robin Holm- gren, Sandra Goodwin, Christopher Cordaro, Leslie Mullen, Carol Chapman, and Anita Palani for technical assistance; to David Kuhl, M.D., for his permission to use PET data from the University of Michigan; to Lawrence Mayer, M.D., Ph.D., and Amy Weaver, M.S., for statistical advice; and to Michael Lawson, M.D., Joe Rogers, Ph.D., and Mony DeLeon, Ph.D., for their encouragement.

REFERENCES


