

## PRECLINICAL EVIDENCE OF ALZHEIMER'S DISEASE IN PERSONS HOMOZYGOUS FOR THE $\epsilon 4$ ALLELE FOR APOLIPOPROTEIN E

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**Abstract** *Background.* Variants of the apolipoprotein E allele appear to account for most cases of late-onset Alzheimer's disease, and persons with two copies of the  $\epsilon 4$  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  $\epsilon 4$  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  $\epsilon 4$  homozygotes and 22 controls without the  $\epsilon 4$  allele who were matched for sex, age, and level of education. An automated method was used to generate an aggregate surface-projection map that com-

pared regional rates of glucose metabolism in the two groups.

*Results.* The  $\epsilon 4$  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  $\epsilon 4$  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  $\epsilon 4$  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. (N Engl J Med 1996;334:752-8.)

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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).<sup>1-7</sup> The gene, located on chromosome 19, has three major alleles:  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ .<sup>8</sup> The  $\epsilon 2$  allele appears to be protective, decreasing the risk of Alzheimer's disease and delaying the onset of dementia.<sup>4</sup> In contrast, the  $\epsilon 4$  allele appears to be harmful, increasing the risk of Alzheimer's disease and hastening the onset of dementia.<sup>2,5</sup> If, as case-control studies suggest, persons with two copies of the  $\epsilon 4$  allele (the  $\epsilon 4/\epsilon 4$  genotype) have an especially high risk of Alzheimer's disease, the study of presymptomatic subjects who are homozygous for the  $\epsilon 4$  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.<sup>9-14</sup> Case series suggest that abnormalities in glucose metabolism can be detected by PET before substantial impairment occurs in persons at risk for Alzheimer's disease<sup>14-16</sup> and certain other neurodegenerative disorders.<sup>17,18</sup> In a recent study, subjects with the apolipoprotein E  $\epsilon 3/\epsilon 4$  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.<sup>15</sup>

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  $\epsilon 4$  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  $\epsilon 4$  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

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least 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 human-subjects 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.<sup>19</sup>

Twelve subjects who were homozygous for the  $\epsilon 4$  allele were identified. One declined to participate in the imaging studies of the brain. For each of the 11  $\epsilon 4$  homozygotes who agreed to participate in the imaging studies, 2 control subjects without this allele (6 with the  $\epsilon 2/\epsilon 3$  genotype and 16 with the  $\epsilon 3/\epsilon 3$  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,<sup>20</sup> the Folstein modified Mini-Mental State Examination (MMSE),<sup>21</sup> the Hamilton Depression Rating Scale,<sup>22</sup> a battery of neuropsychological tests, and brain-imaging studies.

For 10  $\epsilon 4$  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  $\epsilon 4$  homozygote and two controls reported a mild hearing impairment; one  $\epsilon 4$  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  $\epsilon 4$  homozygote and two controls had taken an antihistamine on the night before the PET session; three  $\epsilon 4$  homozygotes and three controls reported a history of hypertension; and one  $\epsilon 4$  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 constructional 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.<sup>23</sup>

### Brain Imaging

$T_1$ -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  $\epsilon 4$  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 [ $^{18}\text{F}$ ]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 back-projection method, a Hanning filter of 0.40 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.<sup>24</sup> 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<sup>13</sup> 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 [ $\pm$ SD] age,  $64 \pm 7.5$  years) and a group of 22 normal controls (mean age,  $64 \pm 7.5$  years).<sup>12,13</sup> Each subject's PET image was linearly and nonlinearly deformed according to the coordinates of a standard atlas of the brain.<sup>25,26</sup> 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.<sup>27</sup> Data on the outer and medial surface of each hemisphere were extracted.<sup>13</sup> 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,  $\geq 2.58$ ;  $P \leq 0.005$ , 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  $\epsilon 4$  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  $\epsilon 4$  homozygotes and their age-matched controls. The three-dimensional stereotactic surface-projection z-score map of metabolic reductions in the homozygous group (z score,  $\geq 2.58$ ;  $P \leq 0.005$ , 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<sup>28</sup>) 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 235 subjects who reported a family history of probable Alzheimer's disease is shown in Table 1. The percentage of  $\epsilon 4$  homozygotes in this sample was higher than that in the general population (5.1 percent vs. 2 to 3 percent),<sup>8</sup> a finding consistent with our expectation of an increased frequency of the  $\epsilon 4$  allele in the study subjects' affected first-degree relatives.<sup>1,2</sup>

The characteristics of the  $\epsilon 4$  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  $\epsilon 4$  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

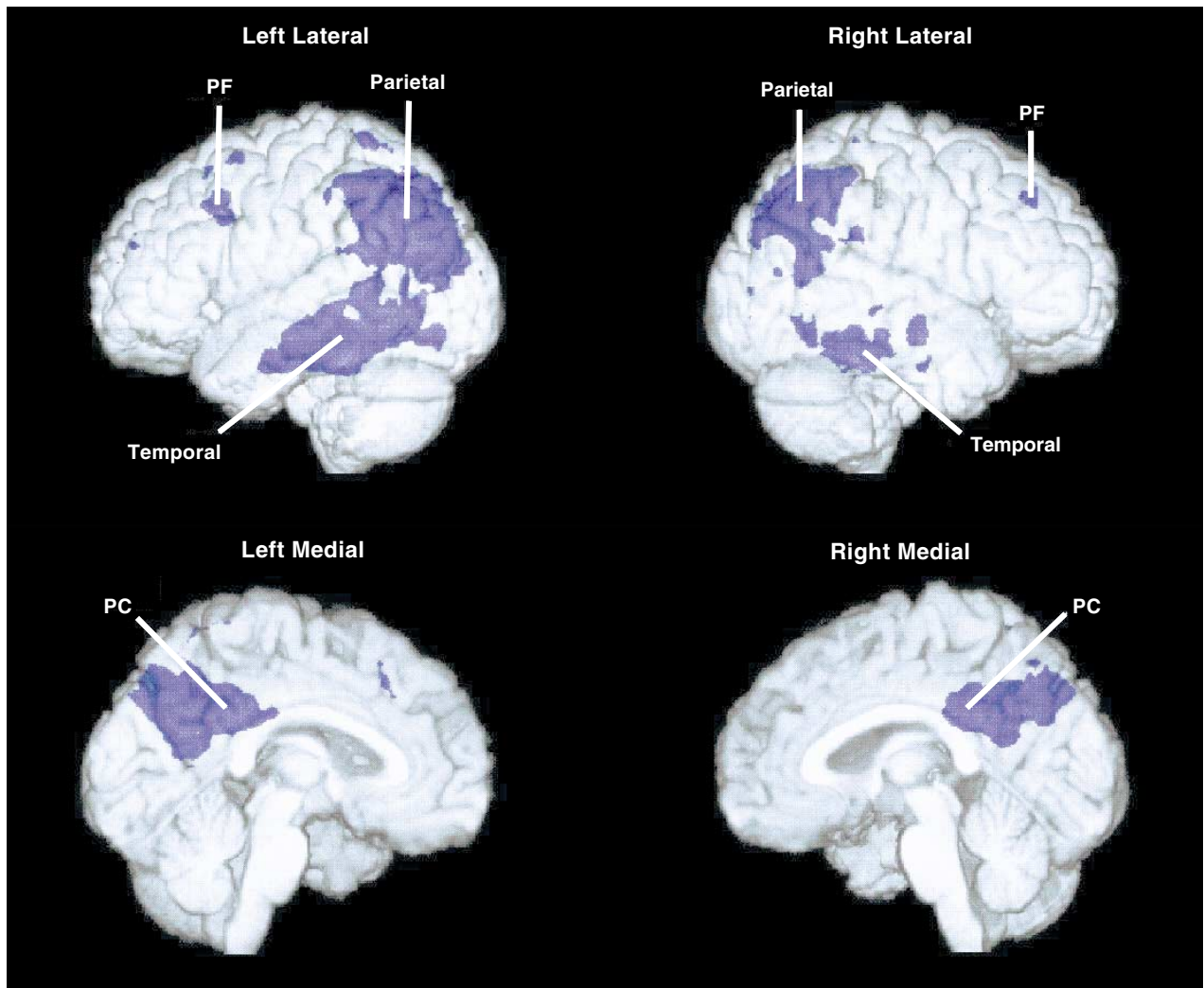


Figure 1. Regions of the Brain with Reduced Rates of Glucose Metabolism in 37 Patients with Probable Alzheimer's Disease.

In a preliminary analysis, an automated algorithm was used to compare PET images of cerebral glucose metabolism in a group of 37 patients with probable Alzheimer's disease (mean age, 64 years) and a group of 22 normal controls (mean age, 64). The three-dimensional surface-projection map of reductions in glucose metabolism (indicated in purple) in the group with probable Alzheimer's disease ( $z$  score,  $\geq 2.58$ ;  $P \leq 0.005$ ) was superimposed on the left lateral, right lateral, left medial, and right medial surfaces of a spatially standardized and volume-rendered MRI of the brain. In comparison with the control group, the group with probable Alzheimer's disease had significantly reduced rates of glucose metabolism bilaterally in prefrontal (PF), parietal, temporal, and posterior cingulate (PC) regions.<sup>12,13</sup>

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  $\epsilon 4$  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  $\epsilon 4$  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  $\epsilon 4$  homozygotes.

#### PET

There were no significant differences between the  $\epsilon 4$  homozygotes and control subjects in the rates of whole-brain glucose metabolism (mean [ $\pm$ SD],  $5.1 \pm 1.0$  vs.  $5.2 \pm 0.8$  mg per minute per 100 g;  $P = 0.62$  by two-tailed, unpaired  $t$ -test) or pontine glucose metabolism ( $5.0 \pm 1.0$  vs.  $5.0 \pm 0.7$  mg per minute per 100 g,  $P = 0.90$ ),

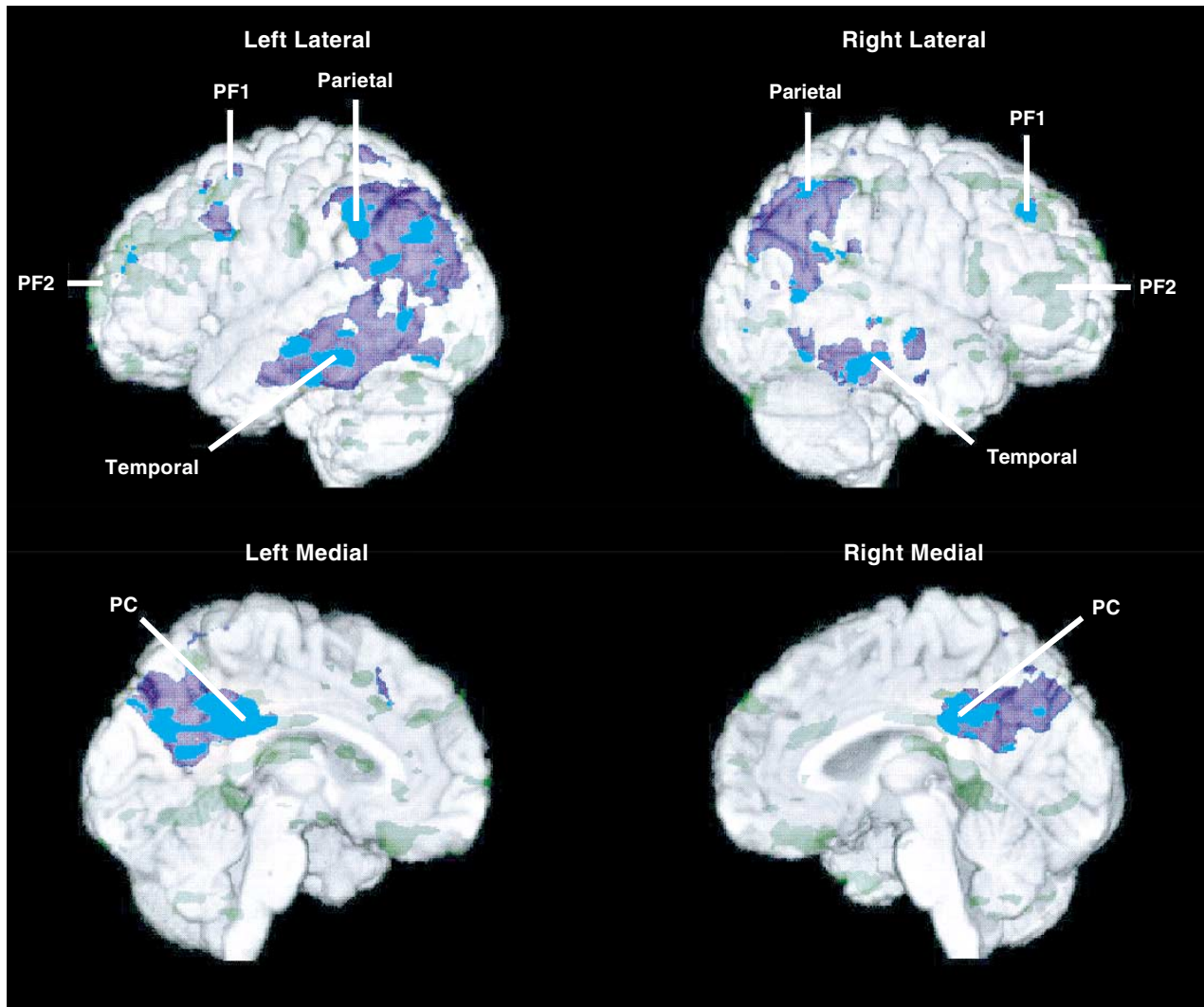


Figure 2. Regions of the Brain with Reduced Rates of Glucose Metabolism in 11  $\epsilon 4$  Homozygotes and Their Relation to Brain Regions with Reduced Glucose Metabolism in 37 Patients with Probable Alzheimer's Disease.

In this analysis, an automated algorithm was used to compare PET images of cerebral glucose metabolism in 11  $\epsilon 4$  homozygotes (mean age, 55 years) and 22 controls who did not carry the  $\epsilon 4$  allele who were matched for sex, age, and level of education (mean age, 56). The three-dimensional surface-projection map of reductions in glucose metabolism in the  $\epsilon 4$  homozygotes ( $z$  score,  $\geq 2.58$ ;  $P \leq 0.005$ ) was superimposed on the composite image shown in Figure 1. The purple areas are regions in which glucose metabolism was significantly reduced only in the group with probable Alzheimer's disease, the blue areas regions in which glucose metabolism was significantly reduced in both the  $\epsilon 4$  homozygotes and the patients with probable Alzheimer's disease, and the green areas regions in which glucose metabolism was significantly reduced only in the  $\epsilon 4$  homozygotes. In comparison with the controls, the  $\epsilon 4$  homozygotes had significantly reduced rates of glucose metabolism bilaterally in the same posterior cingulate (PC), parietal, temporal, and prefrontal (PF1) regions as the patients with probable Alzheimer's disease, as well as in additional prefrontal (PF2) regions, a finding that could reflect accelerated aging in this group.

the measurement of which was used to normalize PET data for the variation in absolute measurements.

The group of  $\epsilon 4$  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  $\epsilon 4$  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,<sup>29-31</sup> MRI,<sup>32</sup> and neuropathological studies<sup>33</sup> 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  $\epsilon 4$  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

Table 1. Distribution of Apolipoprotein E Genotypes in 235 Subjects Who Were 50 to 65 Years Old and Reported a Family History of Probable Alzheimer's Disease.

GENOTYPE	NO. OF SUBJECTS (%)
$\epsilon 2/\epsilon 2$	2 (0.9)
$\epsilon 2/\epsilon 3$	19 (8.1)
$\epsilon 2/\epsilon 4$	5 (2.1)
$\epsilon 3/\epsilon 3$	124 (52.8)
$\epsilon 3/\epsilon 4$	73 (31.1)
$\epsilon 4/\epsilon 4$	12 (5.1)

in these locations in the  $\epsilon 4$  homozygotes; 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  $\epsilon 4$  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  $\epsilon 4$  homozygotes 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,<sup>34,35</sup> could be affected relatively early,<sup>12</sup> 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  $\epsilon 4$  homozygote with neuropsychological evidence of mild cognitive impairment, but were also apparent in the remaining, cognitively intact  $\epsilon 4$  homozygotes. 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.<sup>9-13</sup> Although the reductions in regional

Table 2. Characteristics of the  $\epsilon 4$  Homozygotes and Control Subjects.\*

CHARACTERISTIC	HOMOZYGOTES (N = 11)	CONTROLS (N = 22)
Age (yr)	55.4 ± 4.3	56.3 ± 4.6
Sex (F/M)	8/3	16/6
Handedness (right/left)	11/0	21/1
Years of education	16.5 ± 2.4	15.6 ± 2.4
Age of relative at onset of dementia (yr)	69.6 ± 6.3	72.9 ± 7.1
Score on Hamilton Depression Scale	5.9 ± 4.1	3.9 ± 4.2
Score on Mini-Mental State Examination	29.4 ± 0.8	29.8 ± 0.6

\*Plus-minus values are means ± SD.

glucose metabolism could reflect decreased activity of terminal neuronal fields,<sup>36</sup> 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,<sup>15</sup> patients who presented to a clinic with reports of memory impairment, satisfied the proposed criteria for age-associated memory impairment,<sup>37</sup> did not satisfy criteria for dementia,<sup>38</sup> 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  $\epsilon 4$ -allele status:  $\epsilon 4$  heterozygotes and noncarriers of the allele. As compared with the noncarriers of the allele, the  $\epsilon 4$  heterozygotes had abnormally low and asymmetric rates of glucose metabolism in a preselected parietal region; measurements in other regions and the whole brain were not compared. In our study,  $\epsilon 4$  ho-

Table 3. Neuropsychological Scores in the  $\epsilon 4$  Homozygotes and Control Subjects.

TEST*	$\epsilon 4$ HOMOZYGOTES	CONTROLS	P VALUE†
	mean (±SD) score		
AVLT			
Total Learning	47.6 ± 11.3	49.9 ± 6.1	0.46
Short-Term Recall	9.5 ± 3.7	9.7 ± 1.8	0.78
Long-Term Recall	8.7 ± 3.9	9.4 ± 2.3	0.54
Complex Figure Test			
Copy	33.9 ± 2.7	34.7 ± 1.9	0.32
Recall	16.2 ± 6.4	18.6 ± 6.6	0.34
Boston Naming Test	56.5 ± 4.7	56.9 ± 3.1	0.82
WAIS-R			
Information	10.8 ± 1.5	11.5 ± 1.9	0.28
Digit Span	11.0 ± 2.4	11.5 ± 1.7	0.50
Block Design	11.5 ± 2.6	11.2 ± 2.4	0.80
Mental Arithmetic	10.2 ± 1.9	11.7 ± 2.7	0.12
Similarities	11.0 ± 1.8	12.4 ± 2.6	0.12
Freedom from Distractibility	103.2 ± 8.8	108.9 ± 10.0	0.12
Controlled Oral Word Association Test	47.3 ± 11.7	43.5 ± 11.3	0.38
WMS-R Orientation subtest	13.7 ± 0.5	13.7 ± 0.6	0.82

\*AVLT denotes the Auditory Verbal Learning Test, WAIS-R the Wechsler Adult Intelligence Scale-Revised, and WMS-R the Wechsler Memory Scale-Revised.

†The P value was calculated with unpaired two-tailed t-tests, uncorrected for multiple comparisons.

mozygotes and noncarriers matched for sex, age, and level of education were recruited from the general population. All reported a family history of probable Alzheimer's disease in at least one first-degree relative, reported no impairment in memory, did not satisfy criteria for age-associated memory impairment or dementia, and had slightly higher scores on the Mini-Mental State Examination and a battery of neuropsychological tests than those in the earlier study.<sup>15</sup> The  $\epsilon 4$  homozygotes had abnormally low rates of glucose metabolism in each of the same regions of the brain as patients with probable Alzheimer's disease as well as in additional prefrontal regions. Together, these studies provide preclinical evidence that the  $\epsilon 4$  allele is a risk factor for Alzheimer's disease. They challenge the suggestion<sup>39</sup> that a differential survival bias (i.e., the selection of a distinctive subgroup of surviving carriers of the  $\epsilon 4$  allele in case-control studies) contributed to, or even produced, the

association between the  $\epsilon 4$  allele and Alzheimer's disease — an association that has now been observed internationally in about 100 clinics.

The  $\epsilon 4$  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.<sup>29-33</sup> A comparison of patients with probable Alzheimer's disease with no copies, one copy, or two copies of the  $\epsilon 4$  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  $\epsilon 4$  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  $\epsilon 4$  allele has been associated with an increased risk of Alzheimer's disease,<sup>1-7</sup> a younger age at the onset of dementia,<sup>2,3</sup> a faster rate of  $\beta$ -amyloid protein deposition,<sup>7,40</sup> an increased risk of coronary artery disease and fatal myocardial infarctions,<sup>41-43</sup> and decreased longevity.<sup>41,44,45</sup> In contrast, the  $\epsilon 2$  allele has been associated with a decreased risk of Alzheimer's disease,<sup>4</sup> an older age at the onset of dementia,<sup>5</sup> slower rates of  $\beta$ -amyloid protein and neurofibrillary-tangle deposition,<sup>6,40</sup> a decreased risk of coronary artery disease (unless it is associated with type III hyperlipoproteinemia),<sup>42,43</sup> and increased longevity.<sup>41,44,45</sup>

As our investigation illustrates, studies of presymptomatic subjects with two copies of the  $\epsilon 4$  allele promise to provide new information about the risk factors, physiologic processes, and cognitive impairments that herald the onset of dementia, the sensitivity of new diagnostic tests, and the efficacy of future preventive therapies for Alzheimer's disease. However, apolipoprotein E genotypes cannot be used to predict whether or when Alzheimer's disease will develop in an unaffected person. Prospective, longitudinal studies of the general population have not yet specified the risk of Alzheimer's disease associated with each apolipoprotein E genotype, the mean age and variety of ages at the onset of dementia for each genotype, or the percentage of cases of Alzheimer's disease that are attributable to variants of the apolipoprotein E allele. Genotype-specific preventive therapies have not yet been identified that might outweigh the psychological and social risks involved in making predictions about such a catastrophic illness.

The remarkable series of recent studies that associated variants of the apolipoprotein E allele with the risk of Alzheimer's disease give rise to optimism that an

**Table 4. Location and Magnitude of Greatest Reductions in the Rates of Glucose Metabolism in the  $\epsilon 4$  Homozygotes.\***

REGION	ATLAS COORDINATE†			z SCORE‡
	x	y	z	
	<i>millimeters</i>			
Posterior cingulate	0.0	-32.3	31.5	6.58
Right parietal	42.8	-52.5	54.0	4.01
Left parietal	-60.8	-41.3	38.3	4.30
Right temporal	60.8	-27.8	-13.5	4.47
Left temporal	-58.5	-30.0	-13.5	4.71
Right prefrontal	40.5	37.5	40.5	4.67
Left prefrontal	-42.8	19.5	47.3	4.50

\*The reductions were identified by an automated search of the regions of the brain found to be affected in the previously studied patients with probable Alzheimer's disease.

†The coordinates were obtained from Talairach and Tournoux<sup>26</sup>; x is the distance in millimeters to the right (+) or left (-) of the midline, y is the distance anterior (+) or posterior (-) to the anterior commissure, and z is the distance superior (+) or inferior (-) to a horizontal plane through the anterior and posterior commissures.

‡P<0.001 before correction for multiple comparisons.

intervention might be developed that could slow the progression, delay the onset, or even prevent Alzheimer's disease.<sup>1-5</sup> If, for instance, the E4 isoform increases the risk of Alzheimer's disease by binding to the  $\beta$ -amyloid protein and accelerating the deposition of amyloid (the main constituent of senile plaques),<sup>46,47</sup> a drug or gene therapy that inhibits binding to the  $\beta$ -amyloid protein might interfere with the progression or onset of Alzheimer's disease in persons with one or two copies of the  $\epsilon 4$  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  $\tau$ , interfering with hyperphosphorylation of the protein, and inhibiting its assembly into the paired helical filaments that make up neurofibrillary tangles,<sup>48,49</sup> a drug or gene therapy<sup>50</sup> that promotes these effects might interfere with the progression or onset of Alzheimer's disease regardless of the apolipoprotein E genotype.<sup>5</sup> If, as we postulate, the reductions in glucose metabolism observed in presymptomatic  $\epsilon 4$  homozygotes progress, PET could provide a relatively rapid way to test treatments to prevent the disease.

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