Apolipoprotein E Type 4 Allele and Cerebral Glucose Metabolism in Relatives at Risk for Familial Alzheimer Disease

Gary W. Small, MD; John C. Mazziotta, MD, PhD; Mark T. Collins; Lewis R. Baxter, MD; Michael E. Phelps, PhD; Mark A. Mandelkern, MD, PhD; Andrea Kaplan; Asenath La Rue, PhD; Cara F. Adamson; Linda Chang, MD; Barry H. Guze, MD; Elizabeth H. Corder, PhD; Ann M. Saunders, PhD; Jonathan L. Haines, PhD; Margaret A. Pericak-Vance, PhD; Allen D. Roses, MD

Objective.—Cerebral parietal hypometabolism and left-right asymmetry occur early in the course of Alzheimer disease (AD), and the apolipoprotein E type 4 allele (APOE ε4) is a risk factor for familial AD. To determine if APOE ε4 is associated with lowered brain function in nondemented relatives at risk for familial AD, we studied 12 relatives with APOE ε4 and 19 relatives without APOE ε4. We also compared them with seven patients with probable AD.

Design.—After grouping subjects according to diagnosis and genotype, brain function measures were compared among groups.

Setting.—University medical center.

Patients.—At-risk subjects had mild memory complaints, normal cognitive performance, and at least two relatives with AD. Subjects with APOE ε4 did not differ from those without APOE ε4 in mean age at examination (56.4 vs 55.5 years) or in neuropsychological performance (mean Mini-Mental State Examination score, 28.8 vs 29.3).

Main Outcome Measures.—Cerebral glucose metabolism was measured using positron emission tomography and fludeoxyglucose F 18.

Results.—Parietal metabolism was significantly lower and left-right parietal asymmetry was significantly higher in at-risk subjects with APOE ε4 compared with those without APOE ε4. Patients with dementia had significantly lower parietal metabolism than did at-risk subjects with APOE ε4.

Conclusions.—These results suggest that the inheritance of APOE ε4 is associated with reduced cerebral parietal metabolism and increased asymmetry in nondemented relatives at risk for probable AD. Longitudinal study will determine if glucose metabolic measures provide a means to monitor experimental treatment responses during the early phases of the disorder.

From the Departments of Psychiatry and Biobehavioral Sciences (Dr Small, Baxter, La Rue, and Guze; Mr Collins, and Ms Kaplan and Adamson), Neurology (Dr Mazziotta and Chang), Pharmacology (Dr Mazziotta, Baxter, and Phelps), and Radiological Sciences (Dr Mazziotta), the Brain Mapping Division (Dr Mazziotta), and the Alzheimer’s Disease Center (Dr Small and Mazziotta), University of California at Los Angeles School of Medicine, the Veterans Affairs Medical Center, West Los Angeles, Calif (Dr Small and Mandelkern); the Department of Physics, University of California, Irvine (Dr Mandelkern); the Department of Psychiatry, University of New Mexico, Albuquerque (Dr La Rue); the Divisions of Neurology and Neurobiology, and the Joseph and Kathleen Bryan Alzheimer’s Disease Research Center, Duke University Medical Center, Durham, NC (Drs Corder, Saunders, Pericak-Vance, and Roses); and the Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown (Dr Haines). The views expressed are those of the authors and do not necessarily represent those of the Department of Veterans Affairs.

Reprint requests to UCLA Neuropsychiatric Institute, 760 Westwood Plaza, Los Angeles, CA 90024-1759 (Dr Small).

ALZHEIMER disease (AD) is the most common form of mental impairment in old age, with community prevalence rates between 5% and 10% for those aged 65 years and older and as high as 47% for those aged 85 years and older. Alzheimer disease and related dementias cost society each year an estimated $20 billion in direct costs (ie, actual dollar expenditures) and $88 billion in indirect costs (ie, resource losses not involving dollar expenditures). The disorder progresses insidiously, altering memory, higher intellectual function, language, praxis, and visual-spatial and other cognitive abilities. Patients eventually become bedridden and require total care. Neuropathological hallmarks include neurofibrillary tangles and amyloid deposition in senile plaques. Cerebral tissue disease is widespread, involving the parietal, temporal, and frontal lobes and sparing subcortical and primary sensory and motor cortical regions. Investigators have focused on both environmental and genetic factors as possible causes. A mutation in the amyloid precursor protein gene located on chromosome 21, or, more often, an unidentified gene on chromosome 14 is linked to the rare form of familial AD with onset before age 60 years. A strong association between the apolipoprotein E (APOE) locus on chromosome 19 and the more common late-onset disease form has confirmed previous evidence for chromosome 19 involvement. Apolipoprotein E has three allelic variants (types 2, 3, and 4) and five common genotypes (2/2, 2/3, 2/4, 3/4, and 4/4).
The APOE type 4 allele (APOE e4) appears to confer substantial risk of late-onset AD in a dose-related fashion, while the type 2 allele appears to confer substantial protection. The patterns of risk and protection are common to both sporadic AD and AD in "AD families," though the high percentage of APOE e4 and the low percentage of the type 2 allele in the "AD families" suggests that familial aggregation involves, in part, APOE genotype. No study has yet demonstrated abnormalities in presymptomatic carriers of APOE e4, and previous attempts at presymptomatic diagnosis of AD, using a variety of biochemical and neuroimaging approaches, have yielded conflicting results.

Positron emission tomography (PET) with fluordeoxyglucose F 18 allows the noninvasive determination of the local cerebral metabolic rate for glucose in humans through the use of a tracer kinetic model. Positron emission tomography determinations of glucose metabolism in AD have demonstrated a consistent pattern of reduced glucose use typically beginning in the superior parietal cortex and spreading inferiorly and anteriorly to involve the inferior parietal, superior temporal, and prefrontal cortices. Several groups have found parietal hypometabolism or hemispheric asymmetry in patients early in the course of a dementing illness before confirmation of a clinical diagnosis of probable AD. A previous PET study of people with mild memory complaints and familial risk for AD showed normal patterns of cerebral glucose metabolism, but at-risk relatives were not defined according to genotype. In the present study, glucose metabolism was measured in 38 subjects and compared among three groups: 12 at-risk relatives with APOE e4, 19 at-risk relatives without APOE e4, and seven patients with probable AD.

METHODS

Subjects

To be enrolled in the study, nonde-mented relatives at risk for dementia and those with dementia had to have a documented family history that included at least two relatives with AD. At-risk relatives had mild memory complaints (eg, misplacing familiar objects or difficulty remembering telephone numbers) and met modified diagnostic criteria for age-associated memory impairment. These criteria indicate perceived decrease in daily memory functioning verified by standardized self-report memory questionnaires; memory test performance less than or equal to 1 SD below the mean established for young adults; and verbal and performance IQ scores between 90 and 130. Because dementia may begin before 50 years of age, we modified these criteria at the beginning of the study to include subjects between ages 40 and 90 years, rather than 50 and 90 years. Eleven subjects were between ages 40 and 50 years (one with dementia, four at-risk subjects with APOE e4, and six at-risk subjects without APOE e4). Subjects with any neurological, medical, or psychological conditions that could affect memory or cognitive processing were excluded. Volunteers using nonpsychotropic drugs that do not alter mental or cerebral metabolic function (eg, low-dose aspirin) were included; subjects using psychotropic drugs were excluded. Subjects classified as having dementia met criteria for probable AD, which include the following: clinical examination, standardized rating scales, and neuropsychological tests documenting dementia; cognitive deficits in two or more areas; progressive memory and other cognitive losses; no disturbances of consciousness; and absence of systemic or other disorders that could cause deficits. Subjects were recruited from advertisements, media coverage, and referrals from physicians and patients. Of the 912 persons who volunteered to participate because of memory concerns, 874 were excluded for a variety of reasons (eg, medication use, inadequate family history, and concurrent medical or psychiatric illness). The 38 subjects who met inclusion criteria and received APOE genotyping and PET scanning are described in the Table.

Five of the 15 families included in the study had autopsy confirmation of AD in one or more relatives. Two of the families had a mean age at dementia onset of less than 60 years (34.0 and 57.5 years). In addition to PET studies, all subjects had psychiatric and neurological evaluations, and 29 had magnetic resonance imaging (MRI) scans (eight subjects were unavailable for MRI scans, and one subject de-veloped claustrophobia that interrupted the scan). One subject with dementia who did not receive an MRI scan later had autopsy confirmation of AD. Subjects also had routine screening laboratory tests to rule out treatable causes of mental impairment and were administered the Mini-Mental State Examination and Hamilton Rating Scale for Depression.

Additional neuropsychological evaluations were performed on 29 of the at-risk subjects (10 with APOE e4, 19 without APOE e4). Subjects with dementia did not receive the more extensive neuropsychological assessments because of difficulties completing the examinations. Measures sensitive to age-related cognitive losses were chosen for comparisons of the two at-risk subject groups and included the following measures: verbal fluency (Controlled Oral Word Association Test); verbal memory (Buschke-Fuld Selective Reminding Test); visual-motor coordination, associative memory, and motor speed (Digit Symbol subtest of the Wechsler Adult Intelligence Scale); and visual spatial memory (Rey-Osterrieth Complex Figure Test with a 3-minute delay).

All clinical assessments were performed within 3 weeks of PET scanning. Informed consent was obtained in accordance with the procedures set by two institutional review boards: the Human Subjects Protection Committees of the University of California, Los Angeles, and the Department of Veterans Affairs Medical Center, West Los Angeles. The results were not released to the participants.

MIR Scan Measurements

To determine presence and degree of cerebral atrophy and white matter hy-
perintensity, MRI scans were performed on several scanners, each with a 1.5-Tesla superconducting magnet, and included T1- and T2-weighted transaxial images, with 5-mm slice thickness and 2.5-mm gap. As described elsewhere, modified criteria from the Consortium to Establish a Registry for Alzheimer’s Disease Neuroimaging Task Force were used to rate degree of atrophy from a 4-point scale. Deep white matter and periventricular hyperintensities also were rated using a 4-point scale, as described elsewhere.

**PET Scan Measurements**

During the PET scanning, the subjects’ eyes and ears were not occluded, and they were examined in the supine position with low ambient noise. All intravenous lines were placed 10 to 15 minutes before tracer injection. Six to 10 mCi of fludeoxyglucose F 18 was administered intravenously, and imaging began 30 to 40 minutes thereafter. Fludeoxyglucose F 18 preparation, blood sampling, scanning methods, and determinations of plasma glucose and fludeoxyglucose F 18 concentrations have been detailed elsewhere. The CTI/Siemens 831-08 tomograph (CTI, Knoxville, Tenn; image resolution, 6.5 mm in the plane of section and 6.5 mm in the axial direction) was used to study 13 at-risk subjects (nine with APOE ε4, four without APOE ε4) and four subjects with dementia. Because this scanner was unavailable throughout the study, we scanned 18 at-risk subjects (three with APOE ε4, 15 without APOE ε4) and three subjects with dementia using a CTI/Siemens ECAT 953 (image resolution, 6.5 mm in the plane of section and 5.4 mm in the axial direction). Images contained 2 million to 3 million counts. Other than the use of the two tomographs, methodological approaches were held constant over the course of the study.

Right and left parietal, caudate, and thalamic regions were identified in all tomographic planes, and the metabolic rates were determined as previously described. This process involved comparing each scan with template sets obtained from normal anatomical and PET studies. The size and site of the regions of interest were then copied from these templates in a standardized fashion, as previously described. For each subject, an average metabolic rate value (MR) for these regions of interest was determined by weighting each structure’s planar metabolic value by its cross-sectional area with the use of the following equation:

$$\text{MR} = \frac{\sum (\text{MR}_i) A_i}{\sum A_i}$$

where $N$=number of planes that include the structure, $A_i$=the cross-sectional area of the structure in the plane $i$, and $\text{MR}_i$=the mean metabolic rate of the structure in the plane $i$. The mean left and right parietal metabolic values for each subject were averaged and normalized by dividing each by the average of the two unaffected ipsilateral regions of interest, the caudate and thalamus, as described elsewhere. Parietal asymmetry scores were calculated as absolute values, using the following formula, which provides a measure of percentage asymmetry:

$$\left(\frac{|L-R|}{L+R}\right)\times200$$

where $L$ refers to a left parietal ratio, and $R$ refers to a right parietal ratio. Because spatial resolution and resultant partial volume effects may have differed between scanners, we compared parietal ratios from a control subject and a subject with dementia using both the CTI/Siemens 831-08 and the CTI/Siemens ECAT 953 and found that such differences were less than 1% for the control subject and less than 2% for the subject with dementia.

**Genetic Analysis**

Blood samples were sent by overnight mail to Duke University Medical Center, Durham, NC, for APOE genotyping. DNA was amplified by polymerase chain reaction. After amplification, 5 U of Hha I (Gibco) was directly added to each well, and the plates were incubated at least 3 hours at 37°C. Fifteen microliters of 2× type III stop dye was added to each well, and 3 µL of each reaction
was loaded on a 6% nondenaturing polyacrylamide gel (0.4 mm thick × 43 cm long) and electrophoresed for 1 hour under constant current (45 mA). After electrophoresis, the gel was transferred to Whatman 3M chromatography paper, dried, and autoradiographed for 1 hour using Kodak XAR-5 film. Each autoradiogram was read independently by two different observers. The full protocol for amplification and restriction isotyping of APOE is described elsewhere.\textsuperscript{52,53} Clinical diagnoses were made with investigators blind to genetic data; image data were analyzed and regions of interest determined with investigators blind to clinical and genetic findings.

### Statistical Analysis

Analysis of variance was used for group comparisons of demographic and neuropsychological data, unless nonparametric tests were indicated. Because metabolic and MRI measures were not normally distributed, we used a nonparametric test, the Van der Waerden Test, for group comparisons. For comparisons involving small cell sizes, we used Fisher’s Exact Test. The significance level was predetermined as \( P<.05 \) (two-tailed). Although the direction of metabolic differences was predicted a priori, warranting one-tailed tests, we used the more conservative two-tailed tests throughout. Spearman’s correlation coefficient was used to detect a significant correlation between metabolism and age at examination.

### RESULTS

Subjects were right-handed and ranged in age from 40 to 85 years. The 31 at-risk relatives were divided into two groups: 12 at-risk subjects with \textit{APOE} \( e4 \) (all with genotype 2/3), and 19 at-risk relatives without \textit{APOE} \( e4 \) (17 with genotype 3/2 and two with genotype 2/3). At-risk subjects with \textit{APOE} \( e4 \) did not differ from those without \textit{APOE} \( e4 \) in mean age at examination, family age at dementia onset, years of education, or neuropsychological test performance, although the group with \textit{APOE} \( e4 \) had a nonsignificantly higher proportion of women (Table). The neuropsychological test results indicated that at-risk subjects did not have dementia and performed similarly to normal persons of the same age and educational level. As expected, subjects with dementia had significantly lower scores on the Mini-Mental State Examination compared with the at-risk groups. The group with dementia was also older (Table).

Comparisons among the three subject groups showed significant differences in parietal ratios for both the left \((P<.001)\) and right \((P<.001)\) hemispheres and asymmetry scores \((P<.002)\) (Figures 1 and 2). Comparisons between the two at-risk groups showed the subjects with \textit{APOE} \( e4 \) had significantly lower left \((P=.009)\) and right \((P=.02)\) parietal metabolism and significantly higher parietal asymmetry \((P=.019)\) than those without \textit{APOE} \( e4 \). These differences in parietal metabolic measures remained significant even when corrected for multiple statistical comparisons. The seven patients with probable AD had significantly lower left \((P=.009)\) and right \((P=.003)\) parietal metabolism than the at-risk group with \textit{APOE} \( e4 \), but asymmetry scores were not significantly different between these two groups \((P=.16)\). Four of the seven patients with dementia had \textit{APOE} \( e4 \) (genotype 4/4 \([n=1]\) or 3/4 \([n=3]\)). The one subject with dementia with genotype 4/4 had the lowest left (Figure 1) and right parietal metabolism. The two at-risk subjects with genotype 2/3 had relatively low asymmetry scores (Figure 2). One of the two subjects from early onset families had \textit{APOE} \( e4 \) (Figures 1 and 2). We found no significant correlations between metabolic measures and age at examination (right parietal, \( r_s = -0.24, P=.15; \) left parietal, \( r_s = -0.23, P=.15; \) asymmetry, \( r_s = 0.25, P=.13)\).

MRI scans were available on 29 subjects (10 in patients with \textit{APOE} \( e4 \), 15 in patients without \textit{APOE} \( e4 \), and four in patients with dementia). The at-risk groups did not differ significantly in atrophy ratings: four of 10 subjects in the group with \textit{APOE} \( e4 \) had mild atrophy, compared with five of 15 subjects in the group without \textit{APOE} \( e4 \). Atrophy ratings were significantly greater in the group with dementia (four of four subjects had moderate atrophy and one had mild atrophy) compared with the at-risk group without \textit{APOE} \( e4 \) \((P=.03, \) Fisher’s Exact Test). MRI scans showed no
evidence of deep white matter disease, hydrocephalus, or other lesions except for mild periventricular white matter hyperintensities in seven subjects (two at-risk subjects with APOE ε4, two at-risk subjects without APOE ε4, and three with dementia). The at-risk groups with and without APOE ε4 showed no significant differences in white matter disease ratings.

**COMMENT**

This is the first study comparing cerebral metabolism according to APOE allelic variant in nondemented relatives at risk for familial AD. Our results suggest that APOE ε4 is associated with lower cerebral parietal metabolism in people with only mild memory complaints and normal neuropsychological profiles. The small sample size and modest group differences, however, indicate the preliminary nature of these results. Longitudinal follow-up will determine if such hypometabolic patterns in pre-symptomatic persons indicate that the pathophysiological process begins well before even mild or questionable dementia is recognized clinically. PET measures of hypometabolism reflect decreased synaptic activity caused by loss or dysfunction of synapses. Our finding of lowered cerebral metabolism in subjects with normal neuropsychological function is consistent with other research suggesting that the brain compensates for regional neuronal dysfunction so that clinical neuropsychological measures remain normal. However, additional study of younger and older asymptomatic subjects with APOE ε4 is needed to establish the specificity of these findings.

Some methodological issues deserve comment. PET studies comparing metabolic rates between women and men yielded higher global rates for women, especially premenopausal women. In the present study, the at-risk subjects with APOE ε4 had a higher percentage of women compared with the at-risk subjects without APOE ε4. However, because sex differences in metabolic rates are relatively consistent across brain regions, such differences would be minimized by using metabolic ratios rather than rates. Moreover, if the higher proportion of women in the at-risk group with APOE ε4 influenced the metabolic differences between at-risk groups, they would tend to minimize rather than enhance such differences. Another potential limitation is that MRI scans were not available on all subjects. Thus, partial volume effects exaggerated by atrophy could explain the metabolic differences. If we include in the analysis only those subjects with MRI scan results in comparisons between the at-risk groups with and without APOE ε4, the parietal metabolic differences approached significant levels for left (P = .055) and right (P = .067) hemispheres and remained significant for asymmetry scores (P = .038) if we use conservative two-tailed tests.

Other considerations warrant caution about current clinical applications of these results. Because of the relatively small sample size, we cannot as yet identify a specific “cutoff” defining an abnormal parietal metabolic ratio. In fact, most individual data points between at-risk groups overlap (Figures 1 and 2). Moreover, tomographic resolution that differs among laboratories using different instruments will affect actual metabolic rates obtained from PET studies, although a recent study using metabolic ratios of affected versus unaffected regions showed closely comparable fludeoxyglucose F 18 PET data from different instruments of several laboratories. Development of clinically useful metabolic ratios or rates also will require additional studies of the effect of atrophy, which increases with age and exacerbates partial volume effects on metabolic rate. Such age-associated atrophy could partially explain the lower parietal ratios we found in the patients with dementia. Studying relatively large regions such as parietal rather than small regions such as hippocampus and the use of high resolution scanners will minimize partial volume effects. Registration of PET and MRI data (i.e., superimposing the same subject's functional and structural data) will improve small region definition, and further studies using PET-MRI registration and atrophy correction for PET data would address such issues.

Serial PET studies of subjects at risk will determine the rate of glucose metabolic change in the parietal lobe as well as other brain structures. This information will indicate the change rate of metabolic activity for specific individuals with different genotypes. It will also allow the correlation of the actual metabolic values of brain structures and the time of onset of clinical symptoms. Currently, the course of parietal metabolism according to genotype for at-risk subjects remains hypothetical. Even if PET does not become a widely available modality, the data derived from these methods could lead to other phenotypic screening tests once epidemiological risk estimates are available. In conjunction with APOE and other genotypic data, PET may assist in determining the time course for cerebral metabolic progression of the disease, provide homogeneous subject groups for potential study in experimental therapy protocols, and offer an objective and noninvasive approach to metabolic monitoring during experimental therapeutic trials.

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**References**


