THE ELEVATED risk of Alzheimer disease (AD) among individuals with the ε4 allele of the apolipoprotein E gene (APOE indicates the gene, APOE indicates the protein) has been confirmed in many populations. Among patients with dementia and stroke, the role of APOE remains uncertain. Many studies have been limited by small numbers of patients and other methodological issues. The aim of this population-based, case-control study was to examine the relationship between the APOE genotypes and dementia with stroke or its subtypes, vascular dementia (VaD) and AD with cerebrovascular disease (AD with CVD), in Rotterdam, the Netherlands, and New York City, while considering the effects of age, sex, and ethnic origin.

METHODS

Data were pooled from individuals participating in 2 population-based studies: Rotterdam and New York City (the Washington Heights neighborhood). Informed consent was obtained from all participants, and the study was approved by the local medical ethics committees and the institutional review board.

The Rotterdam study was a population-based cohort study of the total population in a suburb, aged 55 years and older, including institutionalized persons. The objective was to investigate determinants of chronic disabling diseases of the cardiovascular and nervous system. The cohort included 7983 subjects (response rate, 78%) who were examined from 1990 to 1993. In 1993 and 1994, 88% of the participants who were alive (n=6315) were reexamined. Cognitive performance was assessed by the Mini-Mental State Examination and by the Geriatric Mental State schedule. Dementia was diagnosed using a 3-phase design, as described elsewhere. Diagnosis included an interview with a relative, neuropsychological testing, an examination by a behavioral neurologist, and a magnetic resonance imaging scan.

The Washington Heights study consisted of a random sample of Medicare recipients in northern New York City provided by the Health Care Financing Administration. The objective of this investigation was to estimate the frequency of various age-related diseases...
of the nervous system and identify determinants of disease. The response rate was 72% and did not differ from nonresidents by ethnic origin. The 2250 participants underwent an annual assessment. A physician elicited the medical history and conducted a standardized neurological examination. A standardized neuropsychological battery\textsuperscript{16,17} and assessment of activities of daily living were used to ascertain cognitive and functional criteria for dementia.

Diagnosis

All information was reviewed at consensus conferences in either Rotterdam or New York. A panel of clinicians arrived at consensus for diagnosis of dementia according to the Diagnostic and Statistical Manual of Mental Disorders definition.\textsuperscript{2} The diagnosis of stroke was implemented similarly in Rotterdam and New York using the World Health Organization criteria.\textsuperscript{15} At both sites, direct questioning of the next of kin or caregiver established the diagnosis of stroke, supplemented by neurological examination and brain imaging. The diagnosis of AD was based on the National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.\textsuperscript{19} A diagnosis of dementia with stroke was considered for all patients with dementia with a history or clinical evidence of stroke, and further subdivided into the following groups: VaD, AD with CVD, or dementia with stroke-unclassified. Vascular dementia was based on the criteria of the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) and was distinguished from AD when the onset of dementia occurred within 3 months after the stroke or when there was an abrupt change or a stepwise decline in cognitive function.\textsuperscript{20}

A diagnosis of AD with CVD was based on a history or clinical evidence of stroke (including brain imaging), judged not to be the cause of dementia, in patients who would have otherwise met criteria for AD. A diagnosis of dementia with stroke-unclassified was made when limited information was available, such as the absence of brain imaging (n=3), or in the absence of a temporal relationship between dementia and stroke (n=24). All diagnoses were made without knowledge of the APOE genotype.

Study Population

Included were patients with dementia and stroke. The participants from the Washington Heights study also included 2 patients with VaD confirmed by autopsy. Excluded were patients with AD alone or dementia due to other causes. The comparison group consisted of a random sample of individuals without dementia in the Rotterdam and Washington Heights studies for whom DNA samples were available. For every case, approximately 3 controls were matched on both age (10-year interval) and ethnic group. Among Hispanics, there were too few controls older than 85 years; thus, all available controls were included with adjustments made in the analysis. This resulted in a study population of 197 patients with dementia and stroke and 507 controls.

There were no African Americans or Hispanics in the Rotterdam study. In Washington Heights, individuals were classified as African American, white (both non-Hispanic), or Hispanic groups by standardized, direct interview\textsuperscript{21} with the individual or a family member.


table

<table>
<thead>
<tr>
<th>Variables</th>
<th>Dementia With Stroke (n=167)†</th>
<th>Vascular Dementia (n=90)</th>
<th>AD With CVD (n=70)</th>
<th>Controls (n=507)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>80.1 (7.6)</td>
<td>79.1 (8.1)</td>
<td>80.2 (7.1)</td>
<td>76.8 (7.1)</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>51 (27)</td>
<td>29 (32)</td>
<td>16 (23)†</td>
<td>202 (39.8)</td>
</tr>
<tr>
<td>Women</td>
<td>136 (73)</td>
<td>61 (68)</td>
<td>54 (77)†</td>
<td>305 (60.2)</td>
</tr>
<tr>
<td>Residency, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New York</td>
<td>96 (51)</td>
<td>44 (49)</td>
<td>40 (57)</td>
<td>301 (59)</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>91 (49)</td>
<td>46 (51)</td>
<td>30 (43)</td>
<td>206 (41)</td>
</tr>
<tr>
<td>Ethnicity, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>97 (51.5)</td>
<td>48 (53)</td>
<td>34 (48.6)</td>
<td>292 (57.6)</td>
</tr>
<tr>
<td>African American</td>
<td>35 (19)</td>
<td>15 (17)</td>
<td>19 (27)</td>
<td>77 (15.2)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>54 (29)</td>
<td>27 (30)</td>
<td>16 (23)</td>
<td>138 (27.2)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (0.5)</td>
<td>0 (0)</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Education, mean (SD), y</td>
<td>7.2 (3.6)</td>
<td>7.0 (4.0)</td>
<td>7.8 (3.4)</td>
<td>9.2 (3.9)†</td>
</tr>
</tbody>
</table>

\footnote{AD with CVD indicates possible Alzheimer disease with cerebrovascular disease.†Includes 26 patients with “unclassified” dementia with stroke.
\footnote{The controls were younger and better educated than the cases. P<.001.
\footnote{The proportion of men and women were slightly different in the AD with CVD group compared with the others, P<.05.}

RESULTS

Characteristics of the patients and controls are outlined in Table 1 (Rotterdam, N=297; New York, N=397). The distribution of the APOE alleles in the controls from Rotterdam did not differ from those in New York (Rotterdam: APOE e2, 0.092; APOE e3, 0.769; and APOE e4, 0.138 vs New York: APOE e2, 0.081; APOE e3, 0.784, and APOE e4, 0.135; \(x^2 = 0.43, df = 2, P = .81\), nor did the distribution of the APOE alleles differ among patients with dementia and stroke (Rotterdam: APOE e2, 0.071; APOE e3, 0.709; and APOE e4, 0.220 vs New York: APOE e2, 0.078; APOE e3, 0.729, and APOE e4, 0.193; \(x^2 = 0.4, df = 2, P = .83\)). The distribution of patients with dementia and stroke, as well as the subtypes, did not differ significantly by location (Rotterdam: AD with CVD, 33%; VaD, 51%; unclassified, 16% vs New York: AD with CVD, 41%; VaD, 46%; unclassified, 13%; \(x^2 = 1.7, df = 2, P = .45\) or by ethnic group (white: AD with CVD, 35%; VaD, 50%; unclassified, 15%; African American: AD with CVD, 54%; VaD, 43%; unclassified, 3%; Hispanic: AD with CVD, 30%; VaD, 50%; unclassified, 20%; \(x^2 = 10.3, df = 6, P = .28\)). Moreover, the APOE allele frequencies did not differ by subtype of dementia (AD with CVD: APOE e2, 0.071; APOE e3, 0.729; and APOE e4, 0.203 vs VaD: APOE e2, 0.072; APOE e3, 0.722; and APOE e4, 0.206; \(x^2 = 0.5, df = 4, P = .83\)). Since allele frequencies did not differ by site and diagnosis and did not differ by race (which was unevenly distributed be-
between sites), we pooled the data from the 2 sites.

The distribution of APOE genotypes was similar in patients with prevalent and incident disease. Distribution of prevalent (n=126) and incident (n=61) APOE alleles were as follows: e4/e4: 3.9% and 3.3%; e3/e4: 32.5% and 29.5%; e3/e3: 46.1% and 49.2%; e2/e4: 2.4% and 3.3%; e2/e3: 15.1% and 14.7%; and e2/e2: 0% and 0%, respectively (x^2=2.1, df=4, P=.72). Since these groups were significantly distributed, these groups were also combined. This resulted in a study population of 187 patients with dementia and stroke, subclassified as follows: 2 patients (1%) with definite VaD, 18 (10%) with probable VaD, 72 (39%) with possible VaD, 70 (37%) with AD with CVD, and 27 (13%) who could not be classified as either VaD or AD with CVD. The control group consisted of 507 nondemented individuals frequency matched by 10-year age interval and ethnic group (Table 1). However, patients with dementia were still older and less well educated than controls. There were also more women among the patients than controls. The distribution of the APOE genotypes in patients and controls were in Hardy-Weinberg equilibrium suggesting no selective inbreeding or survival.

The frequency of the APOE e4 allele was significantly higher in patients with dementia with stroke compared with the controls (APOE e2, 0.886, e3, 0.778, e4, 0.186 vs dementia with stroke: APOE e2, 0.075, e3, 0.719, e4, 0.206; x^2=10.2, df=2, P=.006). Compared with APOE e3/e3, the OR for dementia with stroke, including VaD and AD with CVD, associated with APOE e4 homozygosity was increased nearly 7-fold (OR=6.9; 95% CI, 1.6-29.4, P<.006 [Table 2]), while the OR associated with APOE e4 heterozygosity was increased nearly 2-fold (OR=1.8; 95% CI, 1.2-2.7; P=.004 [Table 2]). Adjustment for age, ethnic group, or study site did not change the ORs.

Overall, the percent attributable risk or proportion of dementia with stroke among individuals with an APOE e4 allele was 41% (95% CI, 37%-44%); in VaD the percent attributable risk was 33% (95% CI, 29%-38%); and in AD with CVD the percent attributable risk was 44% (95% CI, 39%-48%).

APOE e4 allele frequency and RR associated with at least 1 APOE e4 allele did not change with increasing age. The APOE e4 frequency among demented patients with stroke was similar in women (0.210) and men (0.196). No effects of the APOE e2/e3 genotype were observed.

The relationship between the APOE e4 allele and dementia in patients with stroke did not vary by study site or subtype of dementia (VaD or AD with CVD [Table 2]). However, among African Americans and Hispanics, the OR for dementia with stroke associated with APOE e4 homozygosity was high (OR=10.7; 95% CI, 1.1-80.4, P<.002), while the OR associated with APOE e4 heterozygosity was only slightly increased (OR=1.3; 95% CI, 0.7-2.4). Among whites, the OR for dementia with stroke associated with APOE e4 homozygosity and heterozygosity were both increased (OR=3.9 and 2.2, respectively), but only APOE e4 heterozygosity was statistically significant (OR=2.2; 95% CI, 1.8-3.9; P<.007) due to the small number of white homozygotes (OR=3.9; 95% CI, 0.6-28.7).

**COMMENT**

An increased risk of dementia with stroke, which included patients with either VaD or AD with CVD, was found to be associated with the APOE e4 allele. The association between the APOE e4 allele and dementia with stroke was similar in women and men and did not vary with increasing age. The APOE e4 allele was associated with a higher RR of dementia with stroke in both the homozygous and heterozygous configuration, but among African Americans and Hispanics the strongest effect was observed in those individuals homozygous for APOE e4. No effects of the APOE e2 allele were apparent.

We pooled data from 2 population-based studies because prior studies had included too few patients and controls to investigate the relationship between APOE and dementia with stroke. The distribution of the APOE alleles and the subtypes of dementia with stroke were similar in 2 cities, lessening the possibility that the results reflect admixture of genetically distinct populations. Nevertheless, all analyses were adjusted for residency and ethnic group. Although autopsy confirmation was limited to 2 patients, the diagnostic workup in both populations was relatively complete. More extensive neuropathological examination will be essential to increase the diagnostic certainty and to further clarify the association between APOE e4 and dementia with stroke.

Recent investigations used criteria other than the NINDS-AIREN. The inconsistency of the association in earlier studies may also have been related to the use of hospitalized patients and controls that did not reflect the patient population. One study was population-based and limited the subject pool to stroke survivors as was done in this study. Alternatively, some of the differences between this study and others might reflect the presence of other genetic or environmental risk factors that modify APOE e4. APOE e2 frequency was previously associated with VaD, but several studies have found no effect of this allele.

Studies of stroke and APOE have also been inconsistent. Patients with ischemic stroke have been reported to have both a higher APOE e2 frequency and APOE e4 frequency than controls, but others have found no association between the APOE genotype and stroke. Although the occurrence of a stroke seems unrelated to APOE genotype, the outcome after intracerebral hemorrhage may be worse for individuals with an APOE e4 allele.

Our results imply that individuals who develop dementia with stroke, either as VaD or AD with CVD, and those who develop AD may share genetic susceptibility. The APOE protein may be activated in the response to cerebral ischemia and it may function in compensatory synapticogenesis. Alternatively, the APOE protein may be involved in the pathogenesis of VaD or AD with CVD through its effects on lipids. Compared with APOE3, APOE4 increases total cholesterol and low-density lipoprotein cholesterol levels, which could increase the risk of atherosclerotic vascular disease; APOE2 has the opposite effect.

Cerebrovascular disease and AD are common disorders and it is not surpris-
We conclude that APOE e4 is a genetic risk factor for dementia in patients with stroke, including the subcategories of VaD and AD with CVD. The degree of association may vary by ethnic group. To further elucidate the pathogenesis of these types of dementia, the role of vascular risk factors and disease should be studied in relation to the APOE genotype. Furthermore, additional genetic susceptibility or modifier loci as well as environmental factors should be investigated.

This research was made possible by financial support from the Netherlands Organization for Scientific Research (NWO). The Rotterdam study was supported by the NESTOR Stimulation Program for Geriatric Research.

The Rotterdam study was supported by the Netherlands Organization for Scientific Research (NWO). The Rotterdam study was supported by the NESTOR Stimulation Program for Geriatric Research.

The Washington Heights study was supported by the National Institutes of Health, the Charles S. Robertson Memorial Fund, and the Blanchette Hooker Rockefeller Fund.

The authors thank Harry Gurland, MD, David Wilder, PhD, and Rafael Lantigua, MD, for their work in the study design and statistical analysis. The authors also wish to thank the staff of the Rotterdam study and the Washington Heights study for their contribution to data collection.

References