D₂ Dopamine Receptor A1 Allele in Alzheimer Disease and Aging

Gary W. Small, MD; Ernest P. Noble, PhD, MD; Steven S. Matsuyama, PhD; Lissy F. Jarvik, MD, PhD; Scott Komo, MS; Andrea Kaplan; Terry Ritchie, PhD; Meredyth L. Pritchard, MS; Ann M. Saunders, PhD; P. Michael Conneally, PhD; Allen D. Roses, MD; Jonathan L. Haines, PhD; Margaret A. Pericak-Vance, PhD

**Background:** The apolipoprotein E4 (APOE*4) allele is a major risk factor for the common forms of late-onset Alzheimer disease (AD), but does not account for all the genetic variation in late-onset AD; hence, other genetic markers must be examined. The D₂ dopamine receptor (DRD2) A1 allele is associated with abnormal brain function and decreased DRD2s. These receptors are decreased in hippocampus and amygdala in AD, and allele frequencies may vary with age.

**Objective:** To study APOE and DRD2 genotypes in patients with AD and cognitively intact controls of varying ages.

**Design:** The DRD2 and APOE genotypes were examined in 832 unrelated white subjects, including 554 patients with AD (486 sporadic; 68 familial) and 278 controls. Logistic regressions tested A1 allele effects on disease status and age, and DRD2 linkage with AD was investigated in 60 families with late-onset AD.

**Setting:** University medical centers.

**Subjects:** Patients (mean ± SD age, 74.6 ± 8.1 years; range, 52-98 years) had probable AD, according to standard consensus diagnostic criteria; controls (mean ± SD age, 69.2 ± 8.6 years; range, 50-93 years) were cognitively intact.

**Main Outcome Measures:** Disease status, age, and DRD2 linkage with AD.

**Results:** No association between the DRD2 and APOE alleles was found, and the presence of the A1 allele did not increase the risk for AD. There was also no evidence of linkage between DRD2 and AD. Age analyses, including both patients and controls, indicated a decrease in A1 allele frequency with age.

**Conclusions:** The A1 allele does not contribute to AD risk, alone or in combination with the APOE*4 allele. The DRD2 A1 allele frequencies decrease with age in both patients and controls. Thus, studies of DRD2 disease association need to control for age.

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**ALZHEIMER DISEASE (AD),** the most common form of cognitive decline among elderly persons, afflicts 5% of people older than 65 years and accounts for the most striking rise in dementia incidence in the very old. In the search for causes, many investigations have focused on genetic factors (Table 1). Discoveries to date include findings of mutations of genes on chromosome 1, chromosome 14, or less commonly, chromosome 21 as causes of the familial form of AD that begins before the age of 60 years. For the more common late-onset AD, evidence for chromosome 19 involvement has been confirmed by studies showing a strong association between the apolipoprotein (APOE) locus on chromosome 19 and familial and sporadic AD. The APOE*4 allele confers a major dose-related risk for late-onset familial and sporadic AD, while APOE*2 confers protection. The APOE*4 associations also have been reported for early-onset AD.

Although the evidence supports APOE as a major risk factor in late-onset AD, some large, genetically informative, late-onset families have affected members without APOE*4, pointing to other genetic risk sources. In fact, the available data indicate that APOE accounts for only 45% to 55% of the genetic variation observed in late-onset AD. Additional genetic markers, therefore, require examination.

Several lines of evidence suggest possible involvement of the D₂ dopamine receptor (DRD2) gene (located in 11q23) in AD. The number of DRD2s in hippocampus and amygdala is decreased in brains with AD, and studies of the TaqI polymorphism of the DRD2 gene indicate that the less frequent A1 allele is associated with decreased DRD2 binding sites. Other studies suggest that the A1 allele is associated with reduced visual spatial function, as well as prolonged P300 latency, a cognitive, event-related brain potential also prolonged in AD. To our knowledge, we report herein the first study...
Subjects and Methods

Subjects for the association study were 554 patients with probable AD, according to the standard consensus diagnostic criteria.21 No reported family history of AD or dementia was present in 486 patients (AD, sporadic cases), and another 68 unrelated patients had a family history of 2 or more first-degree relatives with dementia (AI, familial cases). The average (±SD) age at examination was 74.6±8.1 years (range, 52-98 years) for all patients (sporadic, 74.1±8.1; familial, 78.5±7.3) and the average (±SD) age at onset of dementia was 69.0±8.6 years (range, 44-94 years) for all patients (sporadic, 68.9±8.5; familial, 69.9±9.1). Sex ratios favored women who represented 62% of all patients (sporadic, 61%; familial, 62%).

Genotype and allele frequencies for patients were compared with those of 278 unrelated, cognitively intact controls aged 50 to 93 years (mean ±SD, 69.2±8.6 years). Controls also had no family history of AD, and 55% were women.

To determine whether the DRD2 gene is passed on through generations along with AD, linkage analysis was performed using 60 families with AD from the National Alzheimer's Disease Center (Indiana University, Indianapolis). Each family had 2 or more sampled affected relatives meeting standard clinical criteria for AD.22 These families contained 133 genotyped, affected persons and 214 unaffected, at-risk, genotyped persons. The average (±SD) age at onset for affected relatives was 70.0±7.5 years, and 61% were women.

All subjects were white and were recruited from the National Institute on Aging's Alzheimer's Disease Centers and affiliated clinics. Patients and controls were referred to these sites by other university and community physicians and staff, the local chapter of the Alzheimer's Association, and others in the community. Those recruited for the association studies came from the University of California, Los Angeles, Alzheimer's Disease Center and Affiliates (Neuropsychiatric Institute and Hospital and West Los Angeles Veterans Affairs Medical Center), 19 sporadic cases of AD, 38 familial cases of AD, and 16 controls, the Joseph and Kathleen Bryan Alzheimer's Disease Research Center Memory Disorders Clinic at Duke University, Durham, NC (274 sporadic cases of AD, 188 controls), the Massachusetts General Hospital Memory Disorders Clinic, Charlestown (193 sporadic cases of AD, 74 controls), and the National Alzheimer's Disease Center (Indiana University 30 familial cases of AD). Mean subject ages, sex ratios, and allele frequencies did not differ significantly among recruitment sites (data not shown). All clinical diagnoses were made with investigators blinded to genetic data. Prior to study enrollment, informed consent was obtained from subjects or, if necessary, their legal guardians. All study protocols were approved by the human subjects protection committees at each participating site.

DNA was obtained from blood samples using standard techniques either from direct extraction or from lymphoblast cultures. The APOE and DRD2 genotypes were determined as previously described.10,19-26 Resulting gels and autorads were visually scored, and data entered into computerized database systems.

Allele frequencies were compared with Pearson χ² tests. A conditional logistic regression model was constructed to assess whether the risk of AD was associated with the presence of the DRD2 A1 allele and the number of APOE*4 alleles, while controlling for age at examination and sex. A logistic regression model also was used to determine the effect of age on A1 allele presence controlling for disease status, number of APOE*4 alleles, and sex. Because of the small number of subjects homozygous for the A1 allele, the homozygous and heterozygous categories were collapsed.

To determine whether the DRD2 marker is linked to AD, 2-point lod scores (logarithm to base 10 of the odds in favor of linkage) were calculated as previously described.7 By convention, a lod score of 3 (odds of 1000:1 in favor) indicates proof of linkage, while a lod score of -2 (100:1 against) is accepted as proof that linkage is not present. Afeferred relative pair linkage analysis was calculated using software (SimIBD) described by Davis et al.27 A computer program (VITESSE)28 was used in the lod score analysis. Both autosomal-dominant and autosomal-recessive models of inheritance were tested. Because of the late and variable age at onset in AD, a low-penetrance analysis was performed for each of the models examined. In a low-penetrance model, phenotypic information on disease status is included for affected persons only, while genotypic (marker) data are included in all participating family members.

Table 1. Genetic Discoveries in Alzheimer Disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Onset</th>
<th>% of Cases</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>21</td>
<td>Early</td>
<td>&lt;1</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>APOE</td>
<td>19</td>
<td>Late and early</td>
<td>50</td>
<td>Familial and sporadic</td>
</tr>
<tr>
<td>Presenilin 1</td>
<td>14</td>
<td>Early</td>
<td>&lt;5</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Presenilin 2</td>
<td>1</td>
<td>Early</td>
<td>&lt;1</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Other</td>
<td>Unknown</td>
<td>Late</td>
<td>50</td>
<td>Unknown number of genes</td>
</tr>
</tbody>
</table>

*Percentage of cases indicates estimates of actual percentages; APP, amyloid precursor protein; APOE, apolipoprotein E; early, younger than 60 years; and late, 60 years of age or older.

There was no significant difference in A1 allele frequencies between the 486 sporadic AD cases and the 68 unrelated familial cases with AD (0.21 vs 0.14; χ², 3.7; df, 2; P = 0.05), so all other association analyses included both groups as 1 diagnostic group. The DRD2 A1 allele frequencies were similar for the 554 patients with AD and 278 controls (0.21 vs 0.20) but higher for patients when groups were stratified according to APOE*4 carrier status (non-APOE*4 carriers, 0.24 vs 0.21; APOE*4 carriers, 0.19 vs 0.17) (Table 2). The A1 allele frequencies were similar for the 494 patients (0.20) and 201 controls (0.20) who were aged 65 years or older.

As expected, the APOE*4 allele was overrepresented in the patients with AD compared with the controls (0.42

cause allele frequencies may vary with age,22 we also assessed the influence of age on A1 allele frequency.

Results

There was no significant difference in A1 allele frequencies between the 486 sporadic AD cases and the 68 unrelated familial cases with AD (0.21 vs 0.14; χ², 3.7; df, 2; P = 0.05), so all other association analyses included both groups as 1 diagnostic group. The DRD2 A1 allele frequencies were similar for the 554 patients with AD and 278 controls (0.21 vs 0.20) but higher for patients when groups were stratified according to APOE*4 carrier status (non-APOE*4 carriers, 0.24 vs 0.21; APOE*4 carriers, 0.19 vs 0.17) (Table 2). The A1 allele frequencies were similar for the 494 patients (0.20) and 201 controls (0.20) who were aged 65 years or older.

As expected, the APOE*4 allele was overrepresented in the patients with AD compared with the controls (0.42
vs 0.13; χ², 132.2; df, 2; P < .001), and the control frequency for APOE*4 was not significantly different from those reported elsewhere. The distributions of the APOE and DRD2 genotypes were in Hardy-Weinberg equilibrium for the patients and the controls (data not shown).

The logistic regression examining the main effects of the APOE*4 allele and the presence of the DRD2 A1 allele confirmed the dose-dependent effect of the APOE*4 allele (P < .001) and found no effect of the DRD2 A1 allele (Table 3). No significant interaction was found between the 2 genes on the risk of AD using a likelihood ratio test (χ², 0.34; df, 2; P = .84) (data not shown).

For the linkage analysis on the 60 families with late-onset AD, the 2-point lod scores are given in Table 4. Tight linkage was excluded with the DRD2 locus for both age-adjusted and the low-penetrance analyses. In addition, results of the linkage analysis (SimIBD) provided no evidence of linkage or association between AD and DRD2 (P = .68).

To assess the influence of the presence of the A1 allele on age, we used a logistic regression, controlling for diagnostic status, sex, and number of APOE*4 alleles, and found a significant negative association between the presence of the A1 allele and age (β = −.022; SE, 0.009; P = .01). The conditional odds ratio for a 10-year increase in age is 0.800 (95% confidence interval, 0.668-0.958). For both patients and controls, A1 allele frequencies decreased at the age of 80 years or older (Figure).

### Table 2. DRD2 Distribution in APOE*4 Carriers and Non-APOE4 Carriers for AD*

<table>
<thead>
<tr>
<th>DRD2 alleles</th>
<th>Non–APOE*4, No. %</th>
<th>APOE*4, No. (%)</th>
<th>Total, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AD</td>
<td>Control</td>
<td>AD</td>
</tr>
<tr>
<td>A1</td>
<td>87 (0.24)</td>
<td>86 (0.21)</td>
<td>140 (0.19)</td>
</tr>
<tr>
<td>A2</td>
<td>277 (0.76)</td>
<td>316 (0.79)</td>
<td>604 (0.81)</td>
</tr>
<tr>
<td>Total</td>
<td>364</td>
<td>402</td>
<td>744</td>
</tr>
</tbody>
</table>

### Table 3. Odds Ratios for Developing Alzheimer Disease for the APOE and DRD2 Genotypes*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Odds Ratio (95% Confidence Interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XX</td>
<td>1.00 (Referent)</td>
<td></td>
</tr>
<tr>
<td>4/X</td>
<td>4.81 (3.25-6.34)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4/4</td>
<td>32.05 (11.23-89.75)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>DRD2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/2</td>
<td>1.00 (Referent)</td>
<td></td>
</tr>
<tr>
<td>1/1 or 1/2</td>
<td>1.36 (0.95-1.89)</td>
<td>.10</td>
</tr>
</tbody>
</table>

*Because of the small sample size, the A1/A1 and A1/A2 genotypes were collapsed (ie, 1/1 or 1/2). X refers to either the apolipoprotein 2 (APOE*2) or APOE*3 allele. Odds ratios are controlled for age at examination and sex. DRD2 indicates D2 dopamine receptor; ellipses, not applicable.

### Table 4. Lod Scores for 60 Families With Late-Onset Alzheimer Disease for DRD2*

<table>
<thead>
<tr>
<th>Model</th>
<th>0.00</th>
<th>0.05</th>
<th>0.10</th>
<th>0.15</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dom</td>
<td>−3.73</td>
<td>−0.49</td>
<td>0.12</td>
<td>0.34</td>
<td>0.40</td>
<td>0.37</td>
<td>0.09</td>
</tr>
<tr>
<td>Rec</td>
<td>−12.23</td>
<td>−2.34</td>
<td>−0.73</td>
<td>−0.03</td>
<td>0.28</td>
<td>0.33</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Low-penetrance analyses, wherein phenotypic information on disease status was included for affected persons only. Theta values indicate distance from D2 dopamine receptor (DRD2) marker. Dom, assumes dominant mode of inheritance for disease gene; and Rec, assumes recessive mode of inheritance for disease gene.

### RISK FOR AD

These data are consistent with previously reported data sets, indicating a dose-dependent effect for APOE*4. Because the APOE*4 allele is neither necessary nor sufficient for the occurrence of AD, searches for other genetic risk sources are reasonable. Although the patients with AD and controls had similar A1 allele frequencies (0.21 vs 0.20, Table 2), when subjects were stratified according to APOE*4 carrier status, patients had higher A1 allele frequencies than controls. The logistic regression, however, failed to demonstrate a significantly increased risk for AD from the A1 allele. Moreover, there was no evidence of linkage between DRD2 and AD. Together, these findings argue against an A1 allele effect in AD.

### AGE EFFECTS

We found a significant negative association between the presence of the A1 allele and age using a logistic regression model. Moreover, the lack of a DRD2 and APOE interaction indicates that the A1 allele effect on age is independent of previously reported age effects for APOE*4. The observation that A1 allele frequency decreases with age suggests that the allele is...
associated with reduced survival both in patients with AD and controls. Several hypotheses could explain such a result. The DRD2 gene may be in genetic linkage disequilibrium with the actual longevity-reducing mutation. When 2 loci are close enough together on the genome so that recombination rarely occurs, such linkage disequilibrium can exist so that alleles are passed through generations in cis orientation. This leads to an increase in the linked cis allele, although it has no biological role in the disease of interest.

An alternate explanation is that the DRD2 gene has a direct biological role in reducing survival. The precise mechanism is unknown, but the association of the A1 allele with diminished DRD2 binding sites\(^1\) suggests binding site availability as a possible mediating factor. Another potentially relevant factor is the observation in some studies that the A1 allele is associated with alcoholism\(^3\) and other substance dependencies\(^4,5\) and DRD2s appear to mediate response to such drugs of abuse as cocaine.\(^6\) Physical illnesses (eg, cirrhosis) associated with alcoholism and other forms of substance abuse could be the critical mediating factor resulting in premature death and thus explaining the lower A1 allele frequency in older subjects in the present study.

Regardless of the mechanism explaining these findings, such age-related changes in A1 allele frequency could contribute to inconsistencies of previous disease association studies. For example, some studies of A1 allele frequency in alcoholism have compared patients and controls of significantly different ages,\(^3\) or else have not reported the ages of controls\(^4\) or of both patients and controls.\(^5\) One report\(^6\) showing considerable variability in A1 allele frequency in different ethnic groups did not report subject ages. Although the contribution of age to allele frequency may be minimal in some of these studies, failure to consider age could result in skewed findings and explain some of the inconsistencies from study to study.

In summary, these findings argue for controlling age in disease association studies involving the DRD2 A1 allele. They also suggest several hypotheses requiring additional study. Investigations of other genetic polymorphisms within the DRD2 gene, particularly those closer to the promoter region, might reveal allelic associations that more accurately reflect DRD2 binding site concentrations in the brain. Such studies also could provide more direct evidence for the possibility that DRD2 concentrations mediate the decrease in A1 allele frequency associated with old age.

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Reprints: Gary W. Small, MD, UCLA Neuropsychiatric Institute, 760 Westwood Plaza, Los Angeles, CA 90024.


