Loss of Apolipoprotein E Receptor LR11 in Alzheimer Disease

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Background: Genetic, epidemiologic, and biochemical evidence suggests that apolipoprotein E, low-density lipoprotein receptors, and lipid metabolism play important roles in sporadic Alzheimer disease (AD).

Objective: To identify novel candidate genes associated with sporadic AD.

Design: We performed an unbiased microarray screen for genes differentially expressed in lymphoblasts of patients with sporadic AD and prioritized 1 gene product for further characterization in AD brain.

Setting: Emory University, Atlanta, Ga.

Subjects: Cell lines were used from 14 patients with AD and 9 normal human control subjects.

Results: Six genes were differentially expressed in lymphoblasts of 2 independent groups of patients with probable AD and autopsy-proven AD. We hypothesized that 1 of the genes, termed low-density lipoprotein receptor relative with 11 binding repeats (LR11) (reduced 1.8- and 2.5-fold in AD lymphoblasts vs controls), might be associated with sporadic AD on the basis of its function as neuronal apolipoprotein E receptor. We found dramatic and consistent loss of immunocytochemical staining for LR11 in histologically normal-appearing neurons in AD brains. This reduction of LR11 protein was confirmed by quantitative Western blotting (P=.01).

Conclusions: There is loss of the microarray-derived candidate, LR11, in neurons of AD brains. This study shows that microarray analysis of widely available lymphoblasts derived from patients with AD holds promise as a primary screen for candidate genes associated with AD.

Arch Neurol. 2004;61:1200-1205

For editorial comment see page 1178

ALZHEIMER DISEASE (AD) is the most important cause of aging-related dementia, and its prevalence has risen dramatically in the oldest segments of the population. While the mechanisms underlying the disease remain poorly understood, deposition of senile plaques composed of fibrillar aggregates of amyloid-β (Aβ) peptide is believed to play an important pathogenic role. Converging lines of evidence also implicate apolipoprotein E (ApoE), low-density lipoprotein (LDL) receptors, and lipid metabolism in AD pathogenesis. Epidemiologic data link serum cholesterol level, dietary fatty acids, and exposure to certain lipid-lowering drugs to AD risk. Apolipoprotein E is the most abundant apolipoprotein expressed in brain, and a common polymorphism in the APOE gene represents the major genetic risk factor for sporadic, late-onset cases of AD. Other genetic association studies implicate members of the LDL receptor family, which bind ApoE, in AD pathogenesis. The mechanism underlying these associations is unclear. Apolipoprotein E binds Aβ peptide and may modulate Aβ fibrillization and amyloid deposition, or promote internalization of Aβ through the LDL receptor–related protein (see reviews). Previous studies have shown abnormal biochemical responses in extraneuronal tissues in sporadic AD, including platelets and lymphoblasts. In addition, most genes implicated in AD are ubiquitously expressed, and skin fibroblasts from individuals carrying a familial AD mutation secrete excessive amounts of Aβ peptide. Therefore, physiologically relevant alterations in AD might be reflected in shared gene expression changes in neural and extraneural tis-
This novel strategy identified changes in 6 transcripts, including the lipoprotein receptor LR11 (LDL receptor relative with 11 binding repeats). On the basis of its function as a neuronal ApoE receptor and its expression in the brain, we hypothesized that LR11 might play a role in sporadic AD. To test this hypothesis, we examined LR11 protein expression in AD brains.

**SUBJECTS**

Lymphoblast lines were obtained from healthy elderly control subjects and patients with AD who were all well characterized via annual assessments in the Alzheimer’s Disease Center at Emory University, Atlanta, Ga. Informed consent was obtained in accordance with the regulations of the institutional review board at Emory University. The diagnosis of probable AD was made according to criteria of the National Institute of Neurological Disorders and Stroke and consensus of 2 experienced clinicians. (Multiple clinicians, including A.I.L. and J.J.L., participated in establishing the consensus diagnosis.) The pathology diagnosis of definite AD was made by a neuropathologist according to criteria of the Consortium to Establish a Registry for Alzheimer’s Disease. (Multiple neuropathologists, including M.G., were involved.) Cell lines were used from a total of 14 patients with AD and 9 normal human control subjects (Table 1).

**LYMOBASTS**

Patient lymphocytes were immortalized by the Neitzel method in the Emory General Clinical Research Center. In brief, the lymphocytes were removed in the buffy coat layer after gradient separation (Histopaque-1077; Sigma-Aldrich Corp, St Louis, Mo). The lymphocytes were then incubated with Epstein-Barr virus (B95-8, American Type Culture Collection, Manassas, Va) in transforming medium consisting of RPMI-1640 (Gibco-BRL, Gaithersburg, Md), 20% heat-inactivated fetal bovine serum (Gibco-BRL), 2-µg/mL cyclosporine (Sandoz, Inc, Princeton, NJ), 2mM l-glutamine, and penicillin-streptomycin. After 7 days, the lymphocytes were cultured in RPMI-1640 medium supplemented with 15% fetal bovine serum and 110-µg/mL sodium pyruvate, and stocks of cell lines were stored in liquid nitrogen. The mRNA was isolated according to a batch protocol (Oligotex; Qiagen) and quantified by spectrophotometry. Complementary DNA probe synthesis, hybridization with human UniGEM V complementary DNA microarrays, and signal analysis were conducted by Incyte Genomics (St Louis, Mo) as described. Transcript abundance for 7270 genes was assessed in experiment 1 and for 9374 genes in experiment 2. Because false-positive results are particularly high for low-intensity genes, a selective intensity filter (absolute fluorescence intensities ≥800) was applied to exclude genes with low hybridization signal intensities. Then, genes with an AD-to-control fluorescence intensity ratio (fold change) of 1.8 or greater were considered significant according to standard recommendations based on reproducibility data generated by Incyte Genomics, indicating that the level of detectable differential expression is 1.8-fold for UniGEM arrays.

**NORTHERN ANALYSIS**

In experiment 1, quantification of mRNA was additionally confirmed by Northern blot hybridization with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Northern hybridization analysis was performed with the use of formaldehyde to denature 500 ng of polyA RNA, followed by electrophoresis and transfer to a hybridization transfer membrane (GeneScreen; NEN Research Products, Boston, Mass). An in vitro transcription kit.

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**Table 1. Characteristics of Subjects for Lymphoblast Cell Lines**

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>Diagnosis†</th>
<th>APOE Genotype</th>
</tr>
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<tbody>
<tr>
<td>Experimental Group 1 (G1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/F/76</td>
<td>Probable AD</td>
<td>4/4</td>
</tr>
<tr>
<td>2/F/77</td>
<td>Probable AD</td>
<td>3/4</td>
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<tr>
<td>3/M/71</td>
<td>Probable AD</td>
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<tr>
<td>4/F/79</td>
<td>Probable AD</td>
<td>3/4</td>
</tr>
<tr>
<td>5/F/76</td>
<td>Probable AD</td>
<td>4/4</td>
</tr>
<tr>
<td>6/F/77</td>
<td>Probable AD</td>
<td>3/4</td>
</tr>
<tr>
<td>7/M/82</td>
<td>Definite AD</td>
<td>NA</td>
</tr>
<tr>
<td>8/F/NA</td>
<td>Probable AD</td>
<td>NA</td>
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**Experimental Group 2 (G2)**

<table>
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<tr>
<td>3/M/68</td>
<td>3/3</td>
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<tr>
<td>4/M/85</td>
<td>3/4</td>
</tr>
<tr>
<td>5/F/62</td>
<td>4/4</td>
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<tr>
<td>6/F/83</td>
<td>3/4</td>
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**Control Group**

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<td>G1, G2</td>
</tr>
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<td>2/F/80</td>
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<td>3/M/76</td>
<td>3/4</td>
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<tr>
<td>4/M/91</td>
<td>3/3</td>
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<tr>
<td>5/M/57</td>
<td>G1, G2</td>
</tr>
<tr>
<td>6/F/52</td>
<td>G1</td>
</tr>
<tr>
<td>7/M/63</td>
<td>G2</td>
</tr>
<tr>
<td>8/F/65</td>
<td>3/4</td>
</tr>
<tr>
<td>9/F/72</td>
<td>G1, G2</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; APOE, apolipoprotein E gene; NA, data were not available or missing.

*Mean ± SD age was 76.9 ± 3.3 years in group 1, 74.5 ± 8.8 years in group 2, 71.8 ± 13.1 years in control group G1, and 73.3 ± 12.3 years in control group G2. There were no significant differences in mean age between patient groups and their respective controls by 2-tailed t test (G1 patients vs controls, P = .33; G2 patients vs controls, P = .85).

†Definite AD was confirmed neuropathologically.
western blotting

Tissue samples from frontal cortex of 5 human controls and 6 patients with AD were thawed and homogenized in Tris-EDTA, pH 7.4, plus protease inhibitors (Complete Protease Inhibitors; Hoffman-La Roche, Inc, Nutley, NJ). Protein concentrations were measured with a protein assay kit (BCA; Pierce, Rockford, Ill). Samples were separated across a 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel and transferred overnight to polyvinylidene fluoride membranes (Immobilon-P; Millipore, Billerica, Mass). Blots were blocked in 5% nonfat milk–Tris-buffered saline at room temperature for 30 minutes, then probed overnight at 4°C with a polyclonal antibody to 14-3-3 (LR11, is an ApoE receptor mRNA was decreased, while hybridization signal for IgG3 mRNA was increased (Figure 1). These results were consistent with the fold changes observed for each of these genes by microarray hybridization (−2.1, −2.4, and +3.3, respectively). Hybridization signal for the housekeeping gene GAPDH was unchanged.

Secondary microarray screen in lymphoblasts of patients with definite AD

We sought to validate our initial results and narrow the list of candidate genes by analyzing samples from an independent group of patients with autopsy-confirmed diagnosis of AD. In this second experiment, lymphoblast mRNA from 6 patients with definite AD was compared with lymphoblast mRNA from 6 age-, sex-, and race-matched normal controls (Table 1). In the definite AD group, of 9374 genes analyzed, mRNA expression of 108 was decreased by 1.8-fold or greater, expression of 7 was increased by 1.8-fold or greater, and 9259 mRNAs were below significance threshold (a complete list of differentially expressed genes is available from the authors). Down-regulation of 5 genes and up-regulation of 1 gene was confirmed in both microarray experiments (Table 2). One of the consistently down-regulated genes, LR11, is an ApoE recep-
tor that is predominantly expressed in brain and possesses structural and functional homologies to LDL receptor–related protein, a receptor etiologically linked to AD. On the basis of these considerations, we selected LR11 for further examination in control and AD brains.

**LR11 PROTEIN EXPRESSION IN HUMAN BRAIN**

To establish the potential biological relevance of changes in LR11 gene expression for AD, we examined LR11 in control and AD brains at the level of protein expression. Immunohistochemistry of 13 AD brains and 7 controls showed a remarkable reduction in LR11 expression in AD (**Figure 2A and B**). In control brains, pyramidal neurons in the frontal cortex showed strongly labeled small cytoplasmic puncta throughout the cell body and the proximal dendrites (**Figure 2C**). In striking contrast, there was dramatic loss of LR11 staining in pyramidal neurons in AD frontal cortex (**Figure 2D**). The difference between control and AD brains was remarkably consistent, and marked loss of LR11 staining in pyramidal neurons was found in each of the AD cases examined. In addition to neurons, punctate LR11 staining was also found in glial cells. However, unlike pyramidal neurons, glial staining in frontal cortex was preserved in AD brains (**Figure 2E and F**). Similar glial staining was present in control (E) and AD (F) brains. Hematoxylin-counterstained hippocampal dentate granule neurons showed strong LR11 immunoreactivity in controls (G), but very little staining in AD brain (H). Scale: in A and B, bars indicate 100 µm; in C-H, 10 µm.

<table>
<thead>
<tr>
<th>Probable AD</th>
<th>Definite AD</th>
<th>Gene Name</th>
<th>Function</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1.8</td>
<td>−2.5</td>
<td>LDL receptor relative with 11 repeats (LR11)</td>
<td>LDL receptor</td>
<td>Y08110</td>
</tr>
<tr>
<td>−2.4</td>
<td>−2.3</td>
<td>Interferon-γ receptor 1</td>
<td>Interferon receptor</td>
<td>J03143</td>
</tr>
<tr>
<td>−2.1</td>
<td>−2.2</td>
<td>Stimulated trans-acting factor (Staf-50)</td>
<td>Transcription regulation</td>
<td>AA853455</td>
</tr>
<tr>
<td>−1.8</td>
<td>−2.1</td>
<td>Pleckstrin</td>
<td>PKC substrate</td>
<td>X07743</td>
</tr>
<tr>
<td>−2.3</td>
<td>−1.9</td>
<td>Amylo-(1,4-1,6)-transglycosylase 1</td>
<td>Glycogen branching enzyme</td>
<td>L07956</td>
</tr>
<tr>
<td>+1.9</td>
<td>+9.8</td>
<td>Homo sapiens SNC73 mRNA</td>
<td>Immunoglobulin heavy chain</td>
<td>AF067420</td>
</tr>
</tbody>
</table>

**Table 2. Identities of Consistently Altered Transcripts in Microarray Screens**

<table>
<thead>
<tr>
<th>Fold Change*</th>
<th>Gene Name</th>
<th>Function</th>
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<tr>
<td>−1.8</td>
<td>LDL receptor relative with 11 repeats (LR11)</td>
<td>LDL receptor</td>
<td>Y08110</td>
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<tr>
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<td>Interferon-γ receptor 1</td>
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<tr>
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<td>Stimulated trans-acting factor (Staf-50)</td>
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<td>Glycogen branching enzyme</td>
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</tr>
<tr>
<td>+1.9</td>
<td>Homo sapiens SNC73 mRNA</td>
<td>Immunoglobulin heavy chain</td>
<td>AF067420</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; LDL, low-density lipoprotein; mRNA, messenger RNA; PKC, protein kinase C substrate.

*Change in transcript abundance compared with reference control mRNA.
in neuronal LR11 staining by immunocytochemistry, Western blotting indicated relatively modest reduction in LR11 band intensity in total cortical homogenates. This difference may reflect the contribution of glial LR11, which is retained in AD cortex (Figure 2E and F).

**COMMENT**

Our findings suggest a novel link between LR11 and AD; to our knowledge, this report is the first to identify a candidate disease-associated gene in an unbiased microarray screen of blood from patients with AD. Peripheral cells express genes associated with AD and model some processes involved in pathological changes of AD brains.27,28 The structure and function of LR11 as a mosaic ApoE receptor lends biological plausibility to the microarray results, and examination of LR11 in brain strongly supports the hypothesis that it plays a role in AD. In agreement with previous studies,29 we detected LR11 in widespread populations of neurons in neocortex, limbic cortex, and cerebellum. In AD brains, LR11 immunoreactivity was lost with remarkable consistency. Moreover, LR11 staining was decreased specifically in neurons, but staining was preserved in glia. The loss of immunoreactivity was not simply due to cell loss, as hematoxylin counterstaining showed otherwise healthy-appearing neurons (Figure 2). Most of the AD brains in this study were from patients with late-stage disease. Additional studies of patients with mild AD and mild cognitive impairment will be helpful in determining whether LR11 plays a role in early stages of disease development.

The unique multidomain structure of LR11 suggests potential roles as a cell-surface lipoprotein receptor and as an intracellular sorting receptor. There is a cluster of extracellular ligand binding repeats and a cytoplasmic internalization sequence that are present in all endocytosis competent lipoprotein receptors.30 In addition, LR11 contains a VPS10 homology domain near the amino terminus and a Golgi-localized, gamma-ear-homology domain, adenosine diphosphate–ribosylation (ARF)–binding protein (GGA) binding domain in the cytoplasmic tail.19,20,31 The VPS10 domains are involved in trafficking from the Golgi to the vacuole in yeast,32 and GGAs have been shown to mediate trafficking between Golgi and the endosomal-lysosomal system.33 Given its structural features, LR11 seems to be ideally positioned to interact with AD-associated proteins, and additional studies suggest that LR11 expression may influence levels of Aβ (K.O., unpublished data, 2003).

This exploratory study suggests that gene expression analysis of widely available lymphoblasts derived from patients with AD holds promise as a primary screen for candidate genes associated with AD. Our current studies, using this approach, identified the brain ApoE receptor, LR11, as an intriguing candidate molecule for sporadic AD. Further studies using larger sample sizes and refined microarray and bioinformatics procedures coupled with mechanistic validation of candidates are warranted.

**Accepted for Publication:** November 19, 2003.

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**Author Contributions:** Study concept and design: Scherzer, Levey, Lah, and Offe. Acquisition of data: Scherzer, Gearing, Rees, Bujo, Lah, Offe, Fang, and Heilman. Analysis and interpretation of data: Scherzer, Gearing, Schaller, Levey, Lah, and Offe. Drafting of the manuscript: Scherzer, Bujo, Levey, Lah, and Fang. Critical revision of the manuscript for important intellectual content: Scherzer, Gearing, Rees, Schaller, Levey, Lah, Offe, and Heilman. Obtained funding: Levey. Administrative, technical, and material support: Gearing, Rees, Bujo, Levey, Lah, Fang, and Heilman. Study supervision: Levey and Lah.

**Funding/Support:** This study was supported in part by National Institutes of Health grants P30AG10130 from the National Institute on Aging and M01RR000039 from the National Center for Research Resources, and by the Rotary CART (Coins for Alzheimer's Research Trust) fund (to Dr Levey).
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Correction

Omission in Acknowledgments. In the Original Contribution by Scherzer et al titled “Loss of Apolipoprotein E Receptor LR11 in Alzheimer Disease,” published in the August 2004 issue of the ARCHIVES (2004;61:1200-1205), the acknowledgment of funding and support for the study that should have appeared on page 1204 was inadvertently deleted during prepublication processing. That acknowledgment, which would have followed the Author Contributions, should have read as follows: “Funding/Support: This study was supported by National Institutes of Health grants P30AG10130 from the National Institute on Aging and M01RR000039 from the National Center for Research Resources, and by the Rotary CART (Coins for Alzheimer’s Research Trust) fund (to Dr Levey).” Online versions of this article on the Archives of Neurology Web site were corrected on February 6, 2007.