Increased Brain β-Amyloid Load, Phosphorylated Tau, and Risk of Alzheimer Disease Associated With an Intrinsic CYP46 Polymorphism

Andreas Papassotiropoulos, MD; Johannes R. Streffer, MD; Magdalini Tsolaki, MD; Simon Schmid, MD; Dietmar Thal, MD; Francesca Nicosia, PhD; Vassiliki Iakovidou, MD; Alessia Maddalena, MD; Dieter Lütjohann, PhD; Estifanos Gebremedhin, MD; Thomas Hegi, MD; Thomas Pasch, MD; Muriel Träxler, BSc; Annette Bruhl, MD; Luisa Benussi, PhD; Giuliano Binetti, MD; Heiko Braak, MD; Roger M. Nitsch, MD; Christoph Hock, MD

Background: CYP46, the gene encoding cholesterol 24-hydroxylase, plays a key role in the hydroxylation of cholesterol and thereby mediates its removal from brain.

Objective: To study the association of polymorphic sites on CYP46 with Alzheimer disease (AD) traits and with the risk of the development of AD.

Design: Alzheimer disease traits (β-amyloid load, β-amyloid peptides, hyperphosphorylated tau protein) were assessed in brain tissues and in the cerebrospinal fluid of patients with AD and control subjects. Genetic associations were studied in 2 independent populations.

Setting: Specialized centers for memory disorders in Switzerland, Greece, and Italy.

Participants: Fifty-five brain tissues from nondemented elderly patients for the histopathological studies; 38 patients with AD and 25 control subjects for the cerebrospinal fluid studies; 201 patients with AD and 248 control subjects for the genetic association studies.

Results: A polymorphism of CYP46 was associated with increased β-amyloid load in brain tissues as well as with increased cerebrospinal fluid levels of β-amyloid peptides and phosphorylated tau protein. Moreover, this CYP46 polymorphism was associated with higher risk of late-onset sporadic AD in 2 independent populations (odds ratio, 2.16; 95% confidence interval [CI], 1.41-3.32; \(P<.001\)). The additional presence of 1 or 2 apolipoprotein E\(\epsilon4\) alleles synergistically increased the risk of AD to an odds ratio of 9.6 (95% CI, 4.9-18.9; \(P<.001\)) as compared with 4.4 for apolipoprotein E\(\epsilon4\) alone (95% CI, 2.8-6.8; \(P<.001\)).

Conclusion: CYP46 influences brain β-amyloid load, cerebrospinal fluid levels of β-amyloid peptides and phosphorylated tau, and the genetic risk of late-onset sporadic AD.

Arch Neurol. 2003;60:29-35

T he CYP46 gene encodes cholesterol 24-hydroxylase, the rate-limiting enzyme for cholesterol removal from the brain.1-3 Cholesterol 24-hydroxylase mediates the hydroxylation of brain cholesterol to 24-hydroxycholesterol that can be transported readily through the blood-brain barrier.4 Because depletion of brain cholesterol levels reduces the generation of β-amyloid peptides (A\(\beta\)),5,6 and because cholesterol-lowering drugs may reduce the risk of dementia,7 we tested whether polymorphisms of CYP46 are associated with brain β-amyloid load in humans by measuring the sequential pattern of β-amyloid deposition in the medial temporal lobe. Brain β-amyloid deposits contain large amounts of A\(\beta_{32}\). Consequently, we next tested whether CYP46 genotypes affected levels of A\(\beta_{32}\) in cerebrospinal fluid (CSF) obtained from patients with mild to moderate Alzheimer disease (AD).

Because A\(\beta_{32}\) fibrils can cause the formation of neurofibrillary tangles9,10 composed of phosphorylated tau protein, and because such cholesterol-related brain diseases as Niemann-Pick type C are paralleled by hyperphosphorylation of tau,11,12 we measured CSF levels of tau phosphorylated at threonine 181 (phospho-tau 181) and stratified the sample according to the CYP46 genotypes.

For editorial comment see page 16

Given the reported importance of brain cholesterol metabolism on A\(\beta\) production and phosphorylation of tau, CYP46 is a candidate gene for AD. In agreement with accepted recommendations for genetic association studies,13,14 we performed a...
Table 1. Population Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Hypothesis Testing Sample (Southern Europe)</th>
<th>Hypothesis Confirming Sample (Zurich, Switzerland)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Subjects (n = 76)</td>
<td>Patients With AD (n = 107)</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>72.9 ± 4.8</td>
<td>72.7 ± 5.6</td>
</tr>
<tr>
<td>Sex, No. (%) female</td>
<td>50 (66)</td>
<td>68 (64)</td>
</tr>
<tr>
<td>MMSE score, mean ± SD</td>
<td>29.3 ± 1.2</td>
<td>19.6 ± 5.3</td>
</tr>
<tr>
<td>APOE4 allele, frequency</td>
<td>0.81</td>
<td>0.65</td>
</tr>
<tr>
<td>APOE4 allele, frequency</td>
<td>0.13</td>
<td>0.31</td>
</tr>
<tr>
<td>APOE2 allele, frequency</td>
<td>0.06</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; APOE3, apolipoprotein E ε3 allele; MMSE, Mini-Mental State Examination.

---

Figure 1. Schematic representation of single nucleotide polymorphisms (SNPs) mapping to the analyzed genomic region of chromosome 14q. The SNPs in italics were polymorphic in a sample of 50 individuals. The underlined SNPs (rs4934 and rs754203) were used in this study. SERPINA3 gene encodes α1-antichymotrypsin, and CYP46 gene encodes cholesterol 24-hydroxylase. The SNP information was derived from the database of SNPs at http://www.ncbi.nlm.nih.gov/SNP/index.html.

---

Neuropathological examinations were performed in the brains of 55 elderly individuals (mean age at death, 72.2 years; range, 60-91 years; 23 women). The DNA was extracted from the respective freshly frozen brain tissues by a standard protocol (Qiagen GmbH, Hilden, Germany). Antemortem clinical examinations showed absence of dementia signs as measured by the Clinical Dementia Rating Scale. The brain tissue of these subjects was devoid of significant neuropathological abnormalities. Pathological findings of neurofibrillary tangles were excluded by Braak staging. None of these 55 brains met the criteria of the Consortium to Establish a Registry for Alzheimer Disease (CERAD) for AD. The evolutionary phases (0-6) of β-amyloid load in the medial temporal lobes of these subjects were determined as described. According to this immunohistochemistry-based staging, the medial temporal lobe serves as a model for the changes in the anatomic distribution pattern of different types of Aβ deposits occurring in the course of AD. In the first phase, diffuse nonneuritic plaques are present in the basal temporal neocortex, followed by fleecy amyloid deposits in the internal entorhinal layers and in the corpus amonita 1 region of the hippocampus (phase 2). Phase 3 is characterized by Aβ deposits in the molecular layer of the fascia.
dentata, by bandlike Aβ deposits in the subpial portion of the molecular layer of both the entorhinal region and the temporal neocortex, and by confluent lacelike Aβ deposits in the paraventricular layer of the presubiculum region. Diffuse and core-only plaques in the corpus ammonis 4 region are features of the fourth phase.

In addition to the 55 brains from elderly individuals without AD pathological findings, amyloid staging was also done in 21 brains meeting CERAD criteria for definite AD (mean age at death, 81.4 years; range, 69-90 years; 13 women). All neuropathological evaluations were done in a blinded manner with respect to genotype.

GENETIC ASSOCIATION STUDIES

Genetic studies were conducted on 2 independent European populations: a hypothesis testing sample (n=183) and a hypothesis confirming sample (n=266). The clinical diagnoses of AD were made according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association and were based on medical interview and results of physical examination, neuropsychological testing, brain magnetic resonance imaging or computed tomography, and blood and CSF tests. The control group (n=248) consisted of elderly individuals who were either the spouses of patients with AD or subjects recruited from the outpatient clinics of the participating institutions. Dementia and memory deficits in control subjects were excluded by neuropsychological testing, consisting of the CERAD neuropsychological test battery and the Mini-Mental State Examination. Only participants with a Mini-Mental State Examination score equal to or greater than 27 were included in the control group.

CSF MEASUREMENTS

The CSF was obtained by lumbar puncture in a subset of the participants of the genetic studies in Zurich, Switzerland. All participants gave informed written consent. Thirty-eight patients with AD (mean age, 70.1 years; 16 women) and 25 control subjects were compared by Pearson χ² tests. Forward and backward unconditional logistic regression analyses were performed. Nonparametric tests were used when testing for association in subsets of patients with and without AD.

STATISTICS

Genotype and allelic frequencies between patients with AD and control subjects were compared by Pearson χ² tests. Forward and backward unconditional logistic regression analyses were done for the simultaneous assessment of the influence of age, sex, APOE, and CYP46 genotypes on the risk of AD. The estimate haplotype frequencies program was used to test for LD between SNPs. It computes the maximum-likelihood estimates for the haplotype frequencies assuming no association (H0) and allelic association (H1) and calculates the χ² statistic as the 2-fold difference between the log likelihoods. Stages

RESULTS

BRAIN β-AMYLOID LOAD AND CSF Aβ42 CONCENTRATIONS

Brain β-amyloid load (ie, evolutionary phases [0-4] of β-amyloid load) in nondemented elderly subjects with the CYP46*TT genotype (the CYP46 gene encodes cholesterol 24-hydroxylase) vs CYP46*TT-negative subjects (asterisk indicates P=.005, Mann-Whitney test). Bars represent mean±SEM. B, Higher brain β-amyloid load in nondemented elderly subjects with both CYP46*TT and apolipoprotein E e4 (APOE4) alleles (asterisk indicates P=.008, Kruskal-Wallis test). Statistical differences (Mann-Whitney tests) were observed between CYP46*TT-positive/APOE4-positive subjects and CYP46*TT-negative/ APOE4–negative subjects (P=.049), between CYP46*TT-positive/APOE4–positive subjects and CYP46*TT-negative/APOE4–negative subjects (P=.001), and between CYP46*TT-negative/APOE4–positive subjects and CYP46*TT-negative/APOE4–negative subjects (P=.045). Bars represent mean±SEM. Minus sign indicates negative; plus sign, positive.

Figure 2. A, Higher brain β-amyloid load (ie, evolutionary phase of β-amyloid load) in nondemented elderly subjects with the CYP46*TT genotype (the CYP46 gene encodes cholesterol 24-hydroxylase) vs CYP46*TT-negative subjects (asterisk indicates P=.005, Mann-Whitney test). Bars represent mean±SEM. B, Higher brain β-amyloid load in nondemented elderly subjects with both CYP46*TT and apolipoprotein E e4 (APOE4) alleles (asterisk indicates P=.008, Kruskal-Wallis test). Significant differences (Mann-Whitney tests) were observed between CYP46*TT-positive/APOE4-positive subjects and CYP46*TT-negative/ APOE4–negative subjects (P=.049), between CYP46*TT-positive/APOE4–positive subjects and CYP46*TT-negative/APOE4–negative subjects (P=.001), and between CYP46*TT-negative/APOE4–positive subjects and CYP46*TT-negative/APOE4–negative subjects (P=.045). Bars represent mean±SEM. Minus sign indicates negative; plus sign, positive.

of neurofibrillary tangles and phases of β-amyloidosis between groups were compared with the Mann-Whitney (Wilcoxon rank sum) test. Statistical significance was assumed at P<.05.
CYP46 gene encodes cholesterol 24-hydroxylase and patients with Alzheimer disease (AD) with the apolipoprotein E CYP46*TT genotype. Bars represent mean±SEM. Minus sign indicates negative; plus sign, positive.

**PHOSPHO-TAU 181 LEVELS IN THE CSF**

The CSF levels of phospho-tau 181 were markedly higher in patients with AD (n=38) than in controls (n=26) (t test, P<.001). Carriers of the CYP46*TT genotype had higher CSF levels of phospho-tau 181 both in AD and in the control groups (F=9.0, P=.004, multifactorial analysis of variance controlled for age, sex, and APOE4 effects). We observed the highest CSF levels of phospho-tau 181 in patients with AD with CYP46*TT (20±2 pg/mL; n=19), followed by CYP46*TT-negative patients with AD (12±2 pg/mL; n=19), followed by control subjects with CYP46*TT (6±2 pg/mL; n=10), and followed by CYP46*TT-negative control subjects (3±2 pg/mL; n=15) (Figure 3A). Forward and backward linear regression analysis controlled for age, sex, and APOE4 effects showed that this gradual difference was highly significant (r standardzied = 0.62; n = 63; P<.001). The additional presence of 1 or 2 APOE4 alleles resulted in the highest CSF levels of phospho-tau 181 in patients with AD (0.024±0.003 ng/mL; n=11), intermediate levels in those carrying either the APOE4 allele (0.017±0.003 ng/mL; n=12) or CYP46*TT genotype (0.017±0.003 ng/mL; n=8), and lowest levels in those negative for both APOE4 and CYP46*TT (0.006±0.004 ng/mL) (F=6.2, P=.002; age- and sex-corrected analysis of variance) (Figure 3B). Post hoc least significant difference tests for pairwise comparisons confirmed statistically significant differences between carriers of both APOE4 and CYP46*TT vs APOE4 carriers only (P=.04), patients carrying neither APOE4 nor CYP46*TT vs those carrying APOE4 only (P=.01), and patients carrying neither APOE4 nor CYP46*TT vs those carrying CYP46*TT only (P=.02). Thus, the CYP46*TT and APOE4 alleles were associated with high CSF levels of phospho-tau 181 both in patients with AD and in control subjects.

**24S-HYDROXYCHOLESTEROL AND CHOLESTEROL LEVELS IN THE CSF**

Cholesterol-corrected CSF levels of 24S-hydroxycholesterol were significantly higher in patients with AD (0.56±0.19 ng/μg; n=24) than in controls (0.44±0.42 ng/μg; n=22) (P=.001, Mann-Whitney test). There were no differences in the CSF levels of 24S-hydroxycholesterol between CYP46*TT-positive and -negative patients with AD or control subjects. CYP46*TT-positive patients with AD had higher CSF cholesterol concentrations (0.54±0.20 mg/dL [0.0140±0.0052 mmol/L]; n=15) than CYP46*TT-negative patients with AD (0.42±0.12 mg/dL [0.0109±0.0031 mmol/L]; n=16). However, this difference failed to reach statistical significance (P=.07; t test).

**GENETIC ASSOCIATION STUDIES**

CYP46 genotype distribution in both samples was as expected under Hardy-Weinberg equilibrium conditions both in patients with AD and in control subjects (P≥.45 for each comparison). The frequencies of CYP46*TT were higher in patients with AD as compared with control subjects in both samples (60.7% vs 46.1%; P=.049; 58.5% vs 43.0%; P=.02; respectively) (Table 2). The distributions of APOE and CYP46 genotypes were similar in both samples (P>.2). We therefore combined the samples and confirmed significant associations of APOE and CYP46 genotypes with AD (both P<.001) (Table 3). The age-sex-adjusted odds ratio (OR) for the risk of AD in homozygous carriers of the CYP46*TT allele was 2.16 (95% confidence interval [CI], 1.41-3.32). The OR for APOE4 allele carriers was 4.38 (95% CI, 2.83-6.77). Separate analysis of the 2 independent samples resulted in similar ORs (Table 3). The OR for the presence of both the CYP46*TT and the APOE4 genotypes was 9.63 as compared with the absence of these genotypes (95% CI, 4.89-18.96; P<.001). The OR for APOE4 carriers without the CYP46*TT genotype was 4.06 (95% CI, 2.22-7.44; P<.001). The OR for CYP46*TT in APOE4-negative subjects was 2.03 (95% CI, 1.17-3.53; P=.01). These data suggest synergistic interactions between APOE and CYP46 on the risk of AD.

In a sample of 334 participants, the frequency of the SERPINA3*A allele was 55.0% in 181 control subjects and 55.2% in 153 patients with AD (P=.95). Neither a significant interaction between SERPINA3 and CYP46...
There is growing experimental evidence that elevated cholesterol levels may increase amyloid production, possibly by modulating α- and β-secretase activities in the endoproteolytic processing of amyloid precursor protein. In humans, statins may lower the lifetime risk of dementia. Among all human tissues, the relative concentrations of cholesterol are highest in the central nervous system, possibly because of the large surface-to-volume ratios of brain cells along with their high density. Despite these high concentrations, the mechanisms that regulate central nervous system levels of cholesterol are limited to hydroxylation to 24-hydroxycholesterol by cholesterol 24-hydroxylase. In humans, CYP46*TT resulted in elevated levels of CSF Aβ42 in patients with AD. This is therefore intriguing to speculate that functional alterations of cholesterol 24-hydroxylase may modulate cholesterol concentrations in vulnerable neurons, thereby leading to altered membrane composition and associated changes in amyloid precursor protein processing and in Aβ production.

We observed that brain β-amyloid load in subjects with the CYP46*T T genotype was significantly higher than in CYP46*TT-negative subjects. Moreover, the genetic combination of APOE4 and CYP46*T T was associated with the highest levels of brain β-amyloid load. In addition, CYP46*T T resulted in elevated levels of CSF Aβ42 in patients with AD. These observations underscore a possible relationship between cholesterol and brain amyloid formation. Further in vitro experiments (e.g., neuronal transfections) should elucidate the role of CYP46 in Aβ generation.

We also observed that CYP46*T T and APOE4 were associated with high CSF levels of phospho-tau 181 in both patients with AD and control subjects. Because threonine 181 of tau is hyperphosphorylated in neurofibrillary tangles in AD and because hyperphosphorylation may precede tangle formation, our findings possibly relate CYP46 and APOE to neurofibrillary tangle formation. Interestingly, Niemann-Pick type C disease, which is caused by disturbances of cholesterol distribution and cholesterol accumulation in neurons, is characterized by hyperphosphorylation of tau and the development of brain tautopathy. Measurements of 24S-hydroxycholesterol in CSF failed to demonstrate significantly different levels among CYP46 genotype groups. Neuronal levels of cholesterol 24-hydroxylase reportedly are decreased in AD and are paralleled by increased levels of cholesterol 24-hydroxylase in reactive astrocytes. In addition, levels of 24S-hydroxycholesterol vary across different severity.

Table 2. Significantly Different CYP46 Genotype Distribution Between Control Subjects and Patients With AD

<table>
<thead>
<tr>
<th>CYP46 Genotype</th>
<th>Hypothesis-Testing Sample, No. (%)</th>
<th>Hypothesis-Confirming Sample, No. (%)</th>
<th>Combined Sample, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Subjects (n = 76)</td>
<td>Patients With AD (n = 107)</td>
<td>Control Subjects (n = 172)</td>
</tr>
<tr>
<td>CC</td>
<td>6 (7.9) 5 (4.7)</td>
<td>19 (11.0) 7 (7.4)</td>
<td>25 (10.1) 12 (6.0)</td>
</tr>
<tr>
<td>CT</td>
<td>35 (46.1) 37 (34.6)</td>
<td>79 (45.9) 32 (34.0)</td>
<td>114 (46.0) 69 (34.3)</td>
</tr>
<tr>
<td>TT</td>
<td>35 (46.1) 65 (60.7)</td>
<td>74 (43.0) 55 (58.5)</td>
<td>114 (46.0) 69 (34.3)</td>
</tr>
</tbody>
</table>

Statistics:

Hypothesis-Testing Sample:

- Pearson χ² = 4.01
- P = .14

Hypothesis-Confirming Sample:

- Pearson χ² = 5.87
- P = .05

Combined Sample:

- Pearson χ² = 11.37
- P = .003

Table 3. Association of the APOE4 Allele and CYP46*TT Genotype With AD

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothesis-Testing Sample (107 Patients With AD, 76 Control Subjects)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE4 allele</td>
<td>3.30</td>
<td>1.68-6.47</td>
<td>.001</td>
</tr>
<tr>
<td>CYP46*TT genotype</td>
<td>1.90</td>
<td>1.02-3.55</td>
<td>.04</td>
</tr>
<tr>
<td>Age</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Sex</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Hypothesis-Confirming Sample (94 Patients With AD, 172 Control Subjects)

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE4 allele</td>
<td>6.16</td>
<td>3.35-11.34</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CYP46*TT genotype</td>
<td>2.38</td>
<td>1.25-4.53</td>
<td>.009</td>
</tr>
<tr>
<td>Age</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Sex</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Combined Sample (201 Patients With AD, 248 Control Subjects)

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE4 allele</td>
<td>4.38</td>
<td>2.83-6.77</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CYP46*TT genotype</td>
<td>2.16</td>
<td>1.41-3.32</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Sex</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; CYP46, gene encoding cholesterol 24-hydroxylase.

*By forward and backward unconditional logistic regression analysis.
stages of AD. Therefore, measurements of 24S-hydroxycholesterol in CSF may not be sufficient to determine changes in cholesterol metabolites in neurons. We also observed slightly elevated (yet not significantly different) levels of CSF total cholesterol in demented CYP46*TT carriers. This elevation might mirror CYP46 genotype–dependent differences of brain cholesterol homeostasis in AD and should be further examined in larger samples.

Finally, we demonstrated that the CYP46*TT genotype is associated with the risk of late-onset, sporadic AD in 2 independent populations and observed a genetic synergism between APOE4 and CYP46*TT on the risk of AD. Heterozygous individuals or CYP46*C allele homozygotes were at decreased risk of AD. The SNP rs754203 is located 5′ to exon 3 of CYP46. Intronic SNPs have been shown to modulate genetic risk of AD, possibly through alternative splicing or altered RNA stability, or through LD with other causal loci. Sequencing of all 15 exons of CYP46 in 40 chromosomes derived from subjects homozygous for either T (n = 20) or C (n = 20) alleles failed to identify additional linked exonic SNPs. CYP46 maps to chromosome 14q32.1, 6.4 cM 3′ of SERPINA3, which encodes α1-antichymotrypsin. Because SERPINA3 was previously associated with AD, we determined whether SERPINA3 and CYP46 are in LD and whether SERPINA3 is associated with AD in our sample. The frequency of the SERPINA3*A allele was similar in control subjects and in patients with AD. In addition, neither a significant interaction between SERPINA3 and CYP46 nor significant LD between SERPINA3 and CYP46 was observed. The possibility that CYP46 is a risk gene for AD warrants replication in independent populations from independent groups.

In conclusion, our findings indicate that CYP46 polymorphisms are associated with brain β-amyloid load, CSF levels of both AB42 and phospho-tau 181, and the risk of late-onset sporadic AD. Our findings also suggest a synergistic interaction of CYP46 with APOE4 on the risk of AD. Therefore, these data are consistent with the possibility that CYP46 is a novel susceptibility gene for AD.

Accepted for publication September 24, 2002.

From the Division of Psychiatry Research, University of Zurich, Zurich, Switzerland (Drs Papassotriopoulos, Streffer, Schmid, Maddalena, Bruhl, Nitsch, and Hock and Ms Traxler); Third Department of Neurology, Aristotle University of Thessaloniki, Thessaloniki, Greece (Drs Tsolaki and Iakovidou); Institute of Anatomy, Johann Wolfgang Goethe-University, Frankfurt/Main, Germany (Drs Thal, Ghebremedhin, and Braak); Neurobiology Laboratory, Istituto di Ricovero e Cura a Carattere Scientifico Centro S. Giovanni di Dio-Fatebenefratelli, Brescia, Italy (Drs Nicoia, Benussi, and Benetti); Institute for Clinical Pharmacology, University of Bonn, Bonn, Germany (Dr Lütjohann); and Institute for Anesthesiology, University of Zurich, Zurich, Switzerland (Drs Hegi and Pasch).

Author contributions: Study concept and design (Drs Papassotriopoulos, Streffer, Schmid, Thal, Bruhl, Nitsch, and Hock); acquisition of data (Drs Papassotriopoulos, Streffer, Tsolaki, Schmid, Thal, Nicosia, Iakovidou, Maddalena, Lütjohann, Ghebremedhin, Hegi, Pasch, Benussi, Binetti, Braak, Nitsch, and Hock and Ms Traxler); analysis and interpretation of data (Drs Papassotriopoulos, Streffer, Schmid, Thal, Lütjohann, Nitsch, and Hock and Ms Traxler); drafting of the manuscript (Drs Papassotriopoulos, Schmid, Bruhl, and Nitsch); critical revision of the manuscript for important intellectual content (Drs Papassotriopoulos, Streffer, Tsolaki, Schmid, Thal, Nicosia, Iakovidou, Maddalena, Lütjohann, Ghebremedhin, Hegi, Pasch, Benussi, Binetti, Braak, Nitsch, and Hock and Ms Traxler); statistical expertise (Drs Papassotriopoulos and Nitsch); obtaining funding (Drs Papassotriopoulos, Nitsch, and Hock); administrative, technical, or material support (Drs Papassotriopoulos, Streffer, Tsolaki, Schmid, Thal, Nicosia, Iakovidou, Maddalena, Lütjohann, Ghebremedhin, Hegi, Pasch, Bruhl, Benussi, Binetti, Braak, Nitsch, and Hock and Ms Traxler); study supervision (Drs Papassotriopoulos, Nitsch, and Hock).

This study was supported in part by grant 32-65869.01 from the Science National Foundation, Bern, Switzerland (Dr Papassotriopoulos); by grant 22-2001 from the Roche Research Foundation, Basel, Switzerland (Dr Papassotriopoulos); and by the National Center for Competence in Research, “Neuronal Plasticity and Repair,” Zurich.

We thank Esmeralda Garcia, Christina Wilde, Andrea Hauer, and Estelle Obrist for patient care and sampling.

Corresponding authors and reprints: Andreas Papassotriopoulos, MD, Division of Psychiatry Research, University of Zurich, Lenggstrasse 31, 8029 Zurich, Switzerland (e-mail: papas@bli.unizh.ch); Roger M. Nitsch, MD, Division of Psychiatry Research, University of Zurich, Augustus-Forell-Str 1, 8008 Zurich, Switzerland (e-mail: nitsch@bli.unizh.ch).

REFERENCES


Call for Papers

**ARCHIVES Express**

The Archives launched a new ARCHIVES Express section in the September 2000 issue. This section will enable the editors to publish highly selected papers within approximately 2 months of acceptance. We will consider only the most significant research, the top 1% of accepted papers, on new important insights into the pathogenesis of disease, brain function, and therapy. We encourage authors to send their most exceptional clinical or basic research, designating in the cover letter a request for expedited ARCHIVES Express review. We look forward to publishing your important new research in this accelerated manner.

Roger N. Rosenberg, MD
Editor