Relationship Between Cyclophilin A Levels and Matrix Metalloproteinase 9 Activity in Cerebrospinal Fluid of Cognitively Normal Apolipoprotein E4 Carriers and Blood-Brain Barrier Breakdown

In humans, apolipoprotein E (apoE) has 3 isoforms: apoE2, apoE3, and apoE4. APOE4 is a major genetic risk factor for Alzheimer disease (AD). Apolipoprotein E4 has direct effects on the cerebrovascular system, resulting in microvascular lesions and blood-brain barrier (BBB) damage, as recently reviewed. Neurovascular dysfunction is also present in cognitively normal APOE4 carriers and individuals with APOE4-associated disorders including AD. Moreover, postmortem brain tissue analysis has indicated that BBB breakdown in patients with AD is more pronounced in APOE4 carriers compared with APOE3 or APOE2. Our recent studies in transgenic mice have demonstrated that apoE4 leads to BBB breakdown by activating the proinflammatory cyclophilin A (CypA)-matrix metalloproteinase 9 (MMP-9) pathway in brain pericytes, which in turn results in degradation of the BBB tight junctions and basement membrane proteins. It has also been shown that apoE4-mediated BBB breakdown leads to secondary neuronal injury and cognitive decline in transgenic mice. Apolipoprotein E2 and apoE3 maintained normal BBB integrity in transgenic mice by suppressing the CypA-MMP-9 pathway. Here, we studied the cerebrospinal fluid (CSF)/plasma albumin quotient (QAlb), an established marker of BBB breakdown, in young and older cognitively normal individuals. Enzyme-linked immunosorbent assays were used to determine levels of CypA (catalog no. sE90979Hu; USCN Life Science), active MMP-9 (catalog no. 72017; AnaSpec), and albumin (catalog no. E-80AL; Immunology Consultant Laboratories). Data were analyzed by multifactorial analysis of variance with 2 factors (age and APOE4 genotype), with Bonferroni post hoc tests to adjust for multiple comparisons, and Pearson correlation analysis using Graphpad Prism version 5.0. Analyses were performed by an investigator blinded to the experimental conditions. A P value of less than .05 was considered statistically significant.

Results | Older cognitively normal individuals carrying 1 APOE4 allele compared with younger cognitively normal APOE4 carriers or age-matched APOE4 noncarriers had increased QAlb by approximately 77% and 67%, respectively (P < .01; Figure, A). No age-dependent increase in QAlb was associated with APOE2 or APOE3 alleles. Compared with cognitively normal younger APOE4 carriers or age-matched APOE4 noncarriers, older cognitively normal APOE4 carriers had increased CSF levels of CypA by approximately 190% and 95%, respectively.

Table. Demographic Data

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<tbody>
<tr>
<td>No.</td>
<td>5</td>
<td>6</td>
<td>18</td>
<td>10</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Female, %</td>
<td>40.0</td>
<td>16.7</td>
<td>55.6</td>
<td>50.0</td>
<td>80.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Age at lumbar puncture, mean (SD), y</td>
<td>53.0 (10.6)</td>
<td>73.3 (4.7)</td>
<td>59.2 (7.3)</td>
<td>70.8 (4.8)</td>
<td>56.2 (7.8)</td>
<td>75.2 (4.4)</td>
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<tr>
<td>Mini-Mental State Examination score, mean (SD)</td>
<td>29.6 (0.5)</td>
<td>29.2 (1.2)</td>
<td>29.7 (0.7)</td>
<td>29.4 (0.8)</td>
<td>30.0 (0)</td>
<td>29.4 (0.5)</td>
</tr>
<tr>
<td>Clinical Dementia Rating score</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Education, mean (SD), y</td>
<td>14.6 (1.7)</td>
<td>16.2 (3.1)</td>
<td>15.6 (2.5)</td>
<td>15.2 (2.4)</td>
<td>15.6 (0.9)</td>
<td>19.2 (2.7)</td>
</tr>
</tbody>
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Abbreviation: APOE, apolipoprotein E.
(P < .01; Figure, B) and active MMP-9 by 167% and 110%, respectively (P < .05; Figure, C). No age-dependent changes in CypA or MMP-9 CSF levels were associated with APOE2 or APOE3 alleles. Importantly, increases in QAb values correlated positively with both CypA and active MMP-9 CSF levels in all studied individuals (r = 0.37, P < .01; and r = 0.45, P < .01, respectively) (Figure, D and E), indicating the greater the increase in CypA and active MMP-9 levels, the greater the magnitude of BBB breakdown assayed by QAb.

Discussion | This study showed that APOE4 carriers may be susceptible to an age-dependent BBB breakdown prior to onset of clinical decline as determined by Clinical Dementia Rating and Mini-Mental State Examination scores. Furthermore, these findings are consistent with experimental studies suggesting that apoe4 leads to BBB damage in transgenic mice via activation of the CypA-MMP-9 pathway.7 These findings warrant future longitudinal studies to investigate QAb and CSF levels of CypA and active MMP-9 in cognitively normal APOE4 carriers as they progress to mild cognitive impairment and eventually AD. With current diagnostic markers, by the time the earliest detectable clinical signs of disease appear, significant brain injury has likely already occurred. Therefore, studying markers of BBB damage along with commonly used β-amyloid 42 and tau CSF levels may contribute to early detection of vascular dysfunction of those at risk for cognitive decline and AD.

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Author Contributions: Dr Zlokovic had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Analysis and interpretation of data: Halliday, Mack, Zlokovic.

Drafting of the manuscript: Halliday, Zlokovic.

Critical revision of the manuscript for important intellectual content: Pomara, Sagare, Mack, Frangione, Zlokovic.

Statistical analysis: Halliday, Mack.

Obtained funding: Pomara, Zlokovic.

Administrative, technical, or material support: Pomara

Study supervision: Pomara, Zlokovic.

Conflict of Interest Disclosures: None reported.

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COMMENT & RESPONSE

Should CLIPPERS Be Considered a Prelymphoma State or a New Inflammatory Disease? To the Editor We read with interest the clinical observation titled “Fatal B-cell lymphoma following chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids [CLIPPERS].” The patient presented with steroid-responsive brainstem attacks compatible with CLIPPERS. Biopsy findings showed characteristic perivascular lympho-histiocytic infiltrates with T-lymphocyte predominance. During the third attack, magnetic resonance imaging revealed an atypical enhancing pontine lesion. Treatment with steroids and rituximab led to slight transient clinical improvement, followed by rapidly progressive worsening with brainstem herniation and death. Also during the third attack, new biopsy findings disclosed large atypical B lymphocytes positive for Epstein-Barr virus (EBV)-encoded RNA, leading to a final diagnosis of lymphomatoid granulomatosis (LYG).

Lymphomatoid granulomatosis involves most typically the lung and, less frequently, the skin and brain. Central nervous system (CNS) LYG histology is scored on a scale of grade I to III. In the described case, diagnosis was compatible with an initial grade I with progression to grade III CNS LYG. However, histological LYG characteristics, including angiocentric/angiodestructive lymphoreticular proliferation, were not described. Therefore, a diagnosis of EBV-positive type B primary CNS lymphoma (PCNSL) could also be considered. Epstein-Barr virus expression concerns 6.1% of PCNSL and is associated with a median survival time of less than 1 year. In the reported case, the first biopsy might have been a sentinel lesion of PCNSL. Sentinel lesions, considered as host immunity fighting against the tumor, have been initially described as enhancing lesions receding spontaneously or after corticosteroids preceding type B PCNSL within 12 months.

Another reported case of steroid-responsive brainstem attack compatible with CLIPPERS with rapidly fatal outcome revealed CNS lymphoma. In that case, the biopsy of a supratentorial-enhancing lesion was consistent with type B PCNSL. Since EBV hybridization was not performed, a diagnosis of LYG (progressing to LYG III) could not be ruled out.

Central nervous system perivascular T-lymphocyte infiltrates are seen in PCNSL sentinel lesions, grade I LYG, and CLIPPERS. Therefore, in some cases with initial clinical, radiological, and histological features compatible with CLIPPERS, lesions might correspond to grade I CNS LYG or PCNSL sentinel lesions. In contrast to CLIPPERS, LYG and PCNSL are frequently characterized by rapid disease progression (often after initial but transient improvement owing to steroid treatment) and death. Patients with an initial diagnosis compatible with CLIPPERS should be monitored closely especially during the first year. If a biopsy is performed, a part of the sample should be frozen to allow genetic molecular analysis to assess whether CLIPPERS is a new inflammatory disease or a prelymphoma state.

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