Carrying the ε4 allele of the APOE gene encoding apolipoprotein E (APOE4) markedly increases the risk for late-onset Alzheimer’s disease (AD), in which APOE4 exacerbates the brain accumulation and subsequent deposition of amyloid-β (Aβ) peptides. While the LDL receptor–related protein 1 (LRP1) is a major apoE receptor in the brain, we found that its levels are associated with those of insoluble Aβ depending on APOE genotype status in postmortem AD brains. Thus, to determine the functional interaction of apoE4 and LRP1 in brain Aβ metabolism, we crossed neuronal LRP1-knockout mice with amyloid model APP/PS1 mice and APOE3–targeted replacement (APO3-TR) or APOE4-TR mice. Consistent with previous findings, mice expressing apoE4 had increased Aβ deposition and insoluble amounts of Aβ40 and Aβ42 in the hippocampus of APP/PS1 mice compared with those expressing apoE3. Intriguingly, such effects were reversed in the absence of neuronal LRP1. Neuronal LRP1 deficiency also increased detergent-soluble apoE4 levels, which may contribute to the inhibition of Aβ deposition. Together, our results suggest that apoE4 exacerbates Aβ pathology through a mechanism that depends on neuronal LRP1. A better understanding of apoE isoform–specific interaction with their metabolic receptor LRP1 on Aβ metabolism is crucial for defining APOE4-related risk for AD.
APOE4-mediated amyloid-β pathology depends on its neuronal receptor LRP1

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Introduction

Dementia is a devastating disorder in the aging population, progressively compromising cognitive performance. Alzheimer’s disease (AD) is responsible for the development of 60% to 80% of all dementia cases (1). AD is histologically diagnosed by the presence of senile plaques and neurofibrillary tangles (2, 3), while neuropathological studies have revealed that pathologic features of other dementias also coexist in postmortem AD brains (1). Although the pathogenesis of AD has not been fully defined, accumulating evidence supports the amyloid cascade hypothesis (4): accelerated generation of amyloid-β (Aβ) peptides and/or disturbed Aβ metabolism, we crossed neuronal LRP1-knockout mice with amyloid model APP/PS1 mice and APOE3–targeted replacement (APO3-TR) or APOE4-TR mice. Consistent with previous findings, mice expressing apoE4 had increased Aβ deposition and insoluble amounts of Aβ40 and Aβ42 in the hippocampus of APP/PS1 mice compared with those expressing apoE3. Intriguingly, such effects were reversed in the absence of neuronal LRP1. Neuronal LRP1 deficiency also increased detergent-soluble apoE4 levels, which may contribute to the inhibition of Aβ deposition. Together, our results suggest that apoE4 exacerbates Aβ pathology through a mechanism that depends on neuronal LRP1. A better understanding of apoE isoform–specific interaction with their metabolic receptor LRP1 on Aβ metabolism is crucial for defining APOE4-related risk for AD.

Results and Discussion

Reduced brain LRP1 levels have been reported during aging and AD development (11). Positive regional associations between LRP1 and insoluble Aβ42 levels have also been detected in individuals without dementia (12) and in familial AD cases (13). To further address the contribution of LRP1 to Aβ metabolism depending on APOE in AD, we first examined the temporal cortices from pathologically con-
Figure 1. Impact of APOE4 on the association between levels of LRP1 and insoluble Aβ in AD brains. (A–D) The concentrations of Aβ40 (A) and Aβ42 (B) in GDN-HCl fractions, and LRP1 (C) and LDLR (D) in TBS-X fractions of the temporal cortex samples are plotted as they relate to APOE genotype. Horizontal lines, boxes, and whiskers correspond to median, interquartile range (IQR), and the furthest points within ×1.5 IQR from the box, respectively (GDN-HCl fractions, and LRP1 (C) and LDLR (D)). (E–H) The regression plots for concentrations between LRP1-Aβ40 (E), LRP1-Aβ42 (F), LDLR-Aβ40 (G), and LDLR-Aβ42 (H) are presented. Their interactions were assessed by ANCOVA by adjusting for age, sex, Braak stage, Thal phase, and averaged CAA scores. R squares in each APOE genotype are as follows: (E) ε3/ε3, R² = 0.1165, ε3/ε4, R² = 0.1657; ε3/ε4, R² = 0.1622, ε4/ε4, R² = 0.0843, ε6 = 0.1593; (F) ε3/ε3, R² = 0.0725, ε3/ε4, R² = 0.3237, ε4/ε4, R² = 0.0111, ε4/ε4, R² = 0.1610; (G) ε3/ε3, R² = 0.0386, ε3/ε4, R² = 0.4500; ε3/ε4, R² = 0.0228, ε4/ε4, R² = 0.1583, ε4/ε4, R² = 0.0489; and (H) ε3/ε3, R² = 0.0005, P = 0.9340; ε3/ε4, R² = 0.0160, ε4/ε4, R² = 0.1857; P = 0.0315. White circles, data from female patients; black circles, data from male patients.

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confirmed late-onset AD patients with APOE ε3/ε3 (n = 18), ε3/ε4 (n = 17), or ε4/ε4 (n = 25) genotype through biochemical analyses (Supplemental Table 1; supplemental material available online with this article; https://doi.org/10.1172/JCI124853DS1). Brain tissues were lysed through a sequential extraction method based on differential solubility in TBS, TBS plus 1% Triton X-100 (TBS-X), and guanidine hydrochloride (GDN-HCl) (12–14). ELISA measurements of insoluble Aβ40 and Aβ42 in the GDN-HCl fraction, and LRP1 and LDL receptor (LDLR) in the TBS-X fraction, showed no significant differences among patients with different APOE genotypes after adjustment for age, sex, Braak stage (15), Thal phase (16), and cerebral amyloid angiopathy (CAA) severity (Figure 1, A–D). Furthermore, association of insoluble Aβ levels with LRP1 and LDLR levels were evaluated using linear regression models depending on APOE genotype (Figure 1, E–H). Although negative correlations of the amounts of insoluble Aβ40 or Aβ42 with LRP1 levels were detected in AD patients with APOE ε3/ε3, they were positively associated in AD patients with APOE ε3/ε4 or APOE ε4/ε4 genotype. Analysis of covariance revealed a significant interaction effect of APOE genotype and LRP1 levels on insoluble Aβ40 (P = 0.0233) or Aβ42 level (P = 0.0043) after adjustment for age, sex, Braak stage, Thal phase, and CAA severity. There was no significant interaction between APOE4 and LRPI levels for soluble Aβ (Supplemental Figure 1). While LDLR is another major apoE receptor in the brain, the APOE genotype did not influence the association between LDLR and insoluble Aβ levels. These results imply that LRPI is possibly involved in the molecular mechanisms of APOE4 exacerbation of brain Aβ deposition in AD. Although CAA is often detected in AD brains, APOE genotype and/or LRPI levels did not influence CAA severity in this cohort (Supplemental Figure 2).

Therefore, to investigate whether LRPI mediates deleterious effects of APOE4 on Aβ pathology, we generated neuron-specific LRPI-knockout mice (Lrp1fl/fl, CaMKII-Cre−/−; hereafter referred to as nLrp1−/− mice) (17,18) in the background of APP/PS1 amyloid model mice (APPSwe/PSEN1ΔE9) (19), crossed with either APOE3–targeted
detergent-soluble TBS-X fractions, or detergent-insoluble GDN-HCl fractions in the hippocampus from these mice were measured by ELISA at 9 months of age. Consistent with the results from immunostaining, higher concentrations of insoluble Aβ40 (Figure 3C) and Aβ42 (Figure 3F) in the GDN-HCl fractions were detected in mice in the APP/PS1; APOE4 control group than in the APP/PS1; APOE3 control or APP/PS1; APOE3; nLrp1–/– groups. Importantly, the amounts of insoluble Aβ in APP/PS1; APOE4; nLrp1–/– mice were reduced to levels comparable to those of mice in the APP/PS1 group with apoE3. We did not detect significant effects of apoE4 and/or neuronal LRP1 deficiency on the levels of Aβ40 (Figure 3, A and B) or Aβ42 (Figure 3, D and E) in the TBS or TBS-X fraction. Together, these results indicate that apoE4 predominantly affects Aβ aggregation and deposition in mouse brains, which depend on a mechanism mediated by neuronal LRP1 deficiency. Similarly, CAA formation was less in APP/PS1; APOE4; nLrp1–/– mice compared with APP/PS1; APOE4 control mice (Figure 2H). Brain Aβ immunoreactivity did not differ between mice in the APP/PS1; APOE3 control group and the APP/PS1; APOE3; nLrp1–/– group. In addition, the amounts of Aβ40 (Figure 3, A–C) and Aβ42 (Figure 3, D–F) in TBS-soluble fractions, detergent-soluble TBS-X fractions, or detergent-insoluble GDN-HCl fractions in the hippocampus from these mice were measured by ELISA at 9 months of age. Consistent with the results from immunostaining, higher concentrations of insoluble Aβ40 (Figure 3C) and Aβ42 (Figure 3F) in the GDN-HCl fractions were detected in mice in the APP/PS1; APOE4 control group than in the APP/PS1; APOE3 control or APP/PS1; APOE3; nLrp1–/– groups. Importantly, the amounts of insoluble Aβ in APP/PS1; APOE4; nLrp1–/– mice were reduced to levels comparable to those of mice in the APP/PS1 group with apoE3. We did not detect significant effects of apoE4 and/or neuronal LRP1 deficiency on the levels of Aβ40 (Figure 3, A and B) or Aβ42 (Figure 3, D and E) in the TBS or TBS-X fraction. Together, these results indicate that apoE4 predominantly affects Aβ aggregation and deposition in mouse brains, which depend on a mechanism mediated by neu-
When hippocampal amounts of apoE were analyzed by ELISA in these mice (Figure 3G), TBS-soluble apoE levels were lower in the APP/PS1 mice with apoE4 than in those with apoE3, regardless of neuronal LRP1 deficiency. In the TBS-X fraction, which would be predicted to mainly contain membrane-associated proteins (22), APP/PS1; APOE4 control mice had lower APOE levels than APP/PS1; APOE3 control mice. However, APP/PS1; APOE4; nLrp1–/– mice possessed higher amounts of apoE in the TBS-X fraction than did APP/PS1; APOE4 control mice as well as APP/PS1 mice with apoE3 (Figure 3H). Insoluble apoE amounts in the GDN-HCl fraction were higher in APP/PS1; APOE4 control mice than in APP/PS1; APOE3 control mice, which was not affected by neuronal LRP1 deficit (Figure 3I). These results imply that APOE4 is associated with reduced soluble apoE levels and increased insoluble apoE aggregates, where

Figure 3. ApoE4 increases insoluble $\alpha$β levels in the hippocampus of APP/PS1 mice depending on neuronal LRP1. The concentrations of $\alpha$β40 (A–C), $\alpha$β42 (D–F), and apoE (G–I) in the hippocampus extracted in TBS, TBS-X, and GDN-HCl from APP/PS1; APOE3 control, APP/PS1; APOE3; nLrp1–/–, APP/PS1; APOE4 control, and APP/PS1; APOE4; nLrp1–/– mice were measured by ELISA at 9 months of age ($n = 18–23$/group). Horizontal lines, boxes, and whiskers correspond to median, IQR, and the furthest points within ×1.5 IQR from the box, respectively. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$, Tukey-Kramer post hoc analysis of 2-way ANOVA. White circles, data from female mice; black circles, data from male mice.

When hippocampal amounts of apoE were analyzed by ELISA in these mice (Figure 3G), TBS-soluble apoE levels were lower in the APP/PS1 mice with apoE4 than in those with apoE3, regardless of neuronal LRP1 deficiency. In the TBS-X fraction, which would be predicted to mainly contain membrane-associated proteins (22), APP/PS1; APOE4 control mice had lower APOE levels than APP/PS1; APOE3 control mice. However, APP/PS1; APOE4; nLrp1–/– mice possessed higher amounts of apoE in the TBS-X fraction than did APP/PS1; APOE4 control mice as well as APP/PS1 mice with apoE3 (Figure 3H). Insoluble apoE amounts in the GDN-HCl fraction were higher in APP/PS1; APOE4 control mice than in APP/PS1; APOE3 control mice, which was not affected by neuronal LRP1 deficit (Figure 3I). These results imply that APOE4 is associated with reduced soluble apoE levels and increased insoluble apoE aggregates, where
LRP1 may mediate the trafficking of membrane-associated apoE4 in neurons. In addition, we also analyzed the correlations among hippocampal Aβ40, Aβ42, and apoE levels in TBS, TBS-X, and GDN-HCl fractions among the 4 groups of mice (Figure 4, A–D). While insoluble levels of Aβ40, Aβ42, and apoE were strongly correlated with each other in both APP/PS1; APOE3 control and APP/PS1; apoE4 control mice, the interaction between insoluble Aβ40 and Aβ42 was weakened in the absence of neuronal LRP1. Furthermore, apoE levels in the TBS-X fraction displayed significant inverse correlations with insoluble Aβ42 and apoE levels in the GDN-HCl fraction in APP/PS1; APOE3 control and APP/PS1; apoE4 mice, these results suggest that neuronal LRP1 deficiency preserves more apoE4 in the detergent soluble fraction, which is important to consider potential effects of these factors whenever investigating roles of apoE-related molecules in Aβ metabolism and AD.

Whereas clearance of soluble Aβ in the interstitial fluid (ISF) was directly measured by in vivo microdialysis in the hippocampus of mice in the APP/PS1; APOE3, APP/PS1; APOE4; nLrp1–/–, APP/PS1; APOE4 control, and APP/PS1; APOE4; nLrp1–/– groups at the age of 12–14 months, we did not observe an effect of neuronal LRP1 deficiency on ISF Aβ clearance in the mice with apoE3 or apoE4, although the presence of amyloid pathology at this age may complicate the determination of clearance rate due to buffering of soluble Aβ through incorporation into plaques (Supplemental Figure 6). We previously demonstrated that cortical Aβ clearance was disturbed in APP/PS1; nLrp1–/– mice with a murine Apoe background compared with control APP/PS1 mice, thereby exacerbating Aβ pathology (18). Since murine apoE is shown to coaggregate with Aβ more robustly than human apoE (25), neuronal LRP1 may mediate endocytosis of the complex of murine apoE and Aβ in mouse brains, rather than free Aβ. Furthermore, as human apoE likely antagonizes LRP1 for cellular Aβ uptake (26), the effect of neuronal LRP1 deficiency on ISF Aβ clearance may not be evident in the presence of apoE3 or apoE4 compared with the condition with murine apoE alone. Although further studies are needed, it is also possible that neuronal LRP1 predominantly contributes to the apoE4-related Aβ aggregation process rather than soluble Aβ clearance. In addition, unlike the results of those from the hippocampus, the depletion of neuronal LRP1 did not influence the concentrations of Aβ and apoE in the cortex of APP/PS1 mice expressing apoE3 or apoE4 (Supplemental Figure 7). Thus, our findings indicate that neuronal LRP1 function in Aβ metabolism differs depending on the presence of murine and human apoE or brain regions. It may be important to consider potential effects of these factors whenever investigating roles of apoE-related molecules in Aβ metabolism using mouse models. In summary, our study has shown that apoE4 aggravates Aβ pathology by interacting with neuronal LRP1 in mouse models, which supports our observation that APOE4 facilitates the positive association between LRP1 and insoluble Aβ levels in postmortem AD brains. Recent findings have demonstrated that increasing apoE4 during the Aβ-seeding phase (0–6 months of age) in APP/PS1 mice specifically leads to exacerbated amyloid
pathology (9). Thus, it is predicted that neuronal LRPI deficiency also prevents apoE4-related Aβ aggregation at an early stage. One limitation of our study is that we could not analyze Aβ pathology in older mice due to a reduced survival rate at the age of 12 months, for unknown reasons. At older ages, neuronal LRPI deficiency may accelerate Aβ deposition independently of apoE4. In this regard, suppressing LRPI levels may not be a suitable approach as potential AD therapy because LRPI plays a critical role in maintaining brain homeostasis (5, 10). Nonetheless, increasing apoE4 amounts in the TBS–X-soluble fraction may be an alternative therapeutic intervention for AD with APOE4. Modifying apoE4 solubility and/or retaining more apoE4 onto the cell surface at an early stage of AD could potentially be beneficial, in addition to lowering the amount of apoE4 aggregates through treatment with specific antisense oligonucleotides (27) or antibodies (28). Taken together, our results indicate that exploring interactive roles of apoE and apoE receptors in Aβ metabolism would help us to better understand the mechanisms underlying the contribution of APOE4 to the risk of AD development and progression.

Methods

Study approval. The Mayo Clinic Institutional Review Board approved all protocols for human study in which experimental procedures were conducted. All subjects gave informed consent. The Mayo Clinic Institutional Animal Care and Use Committee approved all animal procedures, which were conducted in accordance with the regulations of the American Association for the Accreditation of Laboratory Animal Care.

Details about experimental procedures are provided in the Supplemental Methods.

Author contributions

MT, MLH, GB, and TK designed the research studies. MT, MLH, CCL, MS, TA, HO, YY, YAM, MEM, PM, KS, GW, SG, and DWD conducted experiments and acquired data. MT, MLH, CCL, MS, TA, HO, YY, YAM, MEM, PM, KS, GW, DWD, GB, and TK analyzed the data. MT, MLH, GB, and TK wrote the first draft. All authors contributed to writing the final manuscript.

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