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LRP1 expression in cerebral cortex, choroid plexus and meningeal blood vessels: Relationship to cerebral amyloid angiopathy and *APOE* status

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HIGHLIGHTS

- LRP1 mRNA is elevated in meningeal vessels in association with *APOE* ε4.
- LRP1 mRNA is elevated in choroid plexus and meningeal vessels with AD.
- *APOE* ε2 is associated with decreased LRP1 protein in meningeal vessels with CAA.
- *APOE* ε3 is associated with increased LRP1 protein in choroid plexus with CAA.
- Association of *APOE* and CAA may be partly mediated through LRP1 in the vasculature and choroid plexus.

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ABSTRACT

APOE genotype is a risk factor for Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). The risk and severity of CAA increase with possession of *APOE* ε4, whereas *APOE* ε2 increases the risk of vessel rupture. Uptake of Aβ by cerebrovascular smooth muscle cells (CVSMCs) is mediated by low-density lipoprotein receptor-related protein-1 (LRP1). To determine whether *APOE* influences CAA by altering LRP1 expression, particularly by CVSMCs, we analysed *APOE* genotype, CAA severity, and LRP1 levels in post-mortem cerebral cortex, choroid plexus and meningeal vessels. LRP1 mRNA and protein were not related to CAA severity and presence. LRP1 mRNA was increased in meningeal vessels, but not cortex or choroid plexus, in AD and in association with *APOE* ε4, and was decreased in association with *APOE* ε3. In brains with CAA, *APOE* ε2 was associated with decreased LRP1 protein in meningeal vessels, and ε3 with increased LRP1 in choroid plexus. These findings suggest that *APOE* may influence the severity of CAA through altered expression of LRP1.

1. Introduction

Apolipoprotein E (apoE) is a lipid-binding protein, important for cholesterol transport [2]. ApoE modulates fibrillization of amyloid β (Aβ), which forms the amyloid deposits in AD and CAA [14,18,19], and is involved in Aβ clearance. It forms a complex with Aβ that binds to LRP1, initiating uptake from the extracellular space [8,44].

LRP1 belongs to a family of receptors involved in the metabolism of cholesterol, endocytosis and cell signalling [7,16]. It is present in AD plaques, associated with Aβ or apoE-Aβ [34,17,4,33] and facilitates Aβ-precursor protein endocytic trafficking and processing [9,40,31]. LRP1 is also involved in Aβ uptake by endothelium and CVSMCs [42] and facilitates Aβ clearance across the BBB [38,36].

In CAA, the tunica media of leptomeningeal and cortical arterioles is progressively replaced by Aβ, causing degeneration of CVSMCs, and occasionally vessel rupture and brain haemorrhage. *APOE* ε4 is associated with increased likelihood and severity of CAA [11,23,3], and ε2 with increased risk of CAA-associated haemorrhage [25,29,28] and with vasculopathic complications of CAA such as vessel necrosis [26].

LRP1 contributes to the uptake of Aβ by CVSMCs, resulting in their degeneration and death in vitro [42,35]. We have now investigated the relationships of *APOE* and CAA to the expression of LRP1 mRNA and protein in cerebral cortex, choroid plexus and leptomeningeal blood vessels from AD and control brains.

2. Materials and methods

2.1. Brain tissue and vessel preparations

We studied 23 AD and 19 control brains, from the South West Dementia Brain Bank, Bristol. The brains had been removed within 72 h of death, and the left cerebral hemisphere frozen at −80 °C. *APOE* genotypes had been determined [11], and the

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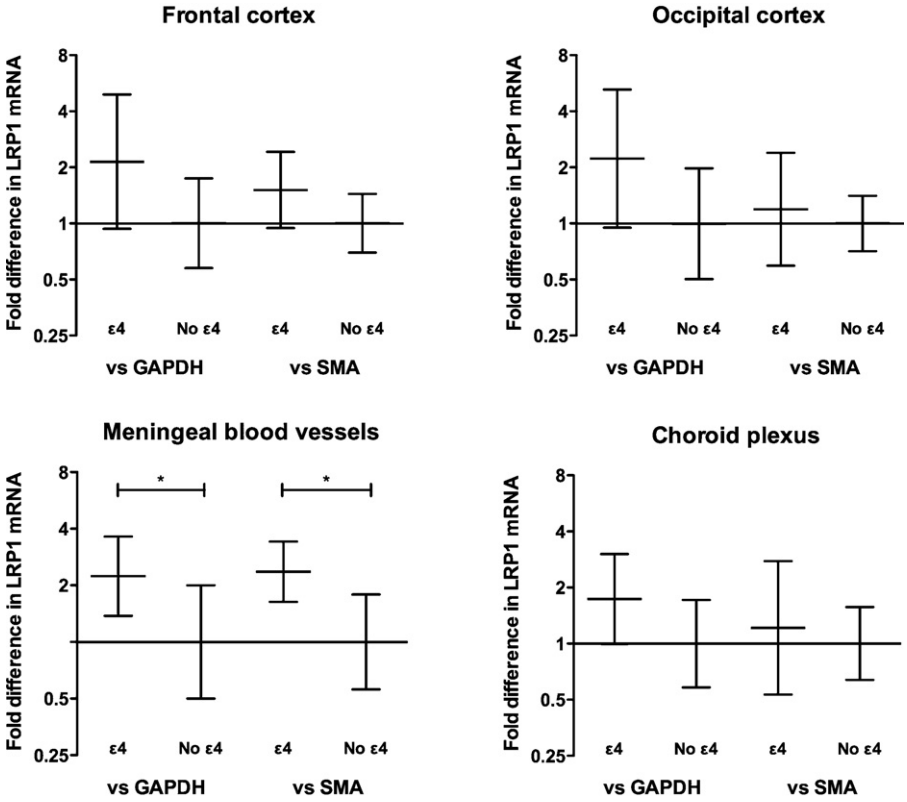

Fig. 1. Fold difference in LRP1 mRNA in $\varepsilon 4$-positive relative to $\varepsilon 4$-negative frontal and occipital cortex, meningeal vessels and choroid plexus. The levels were calibrated against GAPDH and SMA mRNAs, and have been plotted on a logarithmic scale to the base 2. The horizontal lines indicate the geometric mean and 95% confidence intervals. There was a trend towards elevated LRP1 mRNA in $\varepsilon 4$-positive cases but this was significant only in meningeal vessels (asterisks).

right cerebral hemisphere fixed in 10% formalin for neuropathological assessment. The AD cases had a CERAD diagnosis of 'definite AD' [27] and a Braak tangle stage of IV–VI [39], and 20 fulfilled NIA-Reagan criteria of a high likelihood that the dementia was due to AD [39]. CAA severity was scored semi-quantitatively as described [11]: 27 cases had none/mild CAA (score < 1.5) and 15 moderate/severe CAA ($\geq$1.5). 3–5 mm^3 samples of frontal (BA6) and occipital cortex (BA17/18) and 100 mg samples of occipital and frontal leptomeningeal vessels were analysed.

2.2. LRP1 mRNA measurement

Tissue samples were homogenized in TRIzol reagent (Invitrogen, CA), incubated for 3 min in chloroform then centrifuged at 12,000 × *g* for 15 min at 4 °C. The aqueous phase was mixed with an equal volume of isopropyl alcohol and 30 μg of glycogen, incubated for 10 min and centrifuged at 12,000× *g* for 10 min at 4 °C to pellet the RNA. The pellet was washed with 70% ethanol, resuspended in water and treated with DNase-I (40U, Roche, UK). cDNA was produced using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, UK) and quantified by Quant-iT PicoGreen dsDNA assay (Invitrogen, UK). RT-PCR was performed in triplicate using the ViiA 7 Real-Time PCR System (ABI Prism, Applied Biosystems, UK) with Assay-on-demand gene expression products for LRP1 (Hs00233856_m1, accession number NM_002332.2), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Hs99999905_m1, NM_002046.3) and smooth muscle actin (SMA) (Hs00426835_g1, NM_001141945.1 and NM_001613.2), TaqMan Gene Expression Master Mix and 10 ng of cDNA in 5 μl: 50 °C for 2 min; 95 °C for 10 min; 40 cycles of 95 °C for 15 min and 60 °C for 1 min. LRP1 mRNA level was calibrated against GAPDH and SMA mRNAs; the fold difference between groups was calculated by the $2^{-\Delta\Delta Ct}$ method [22] and analysed as described [30].

2.3. LRP1 protein measurement

Meningeal vessels were homogenized in TM buffer (Millipore, UK) and cortex and choroid plexus in SDS lysis buffer. Protein concentrations were measured using Total Protein Kit (Sigma–Aldrich, UK). Homogenates were diluted in Tris-buffered saline from 1:12.5 to 1:1000 and loaded into a dot blot apparatus (Bio-Rad, UK) for 1 h. A standard curve was prepared from serial dilutions of a reference homogenate. The membrane was blocked in 10% milk overnight at 4 °C and incubated with mouse anti-LRP1 (ab28320, Abcam, UK, 1:2000, 4 h) after confirmation of the specificity of the antibody by western blot. The membrane was washed, incubated with peroxidase anti-mouse antibody (Vector Laboratories, UK) at 1:5000 for 1 h, washed, incubated with chemiluminescent HRP substrate (Millipore, UK) and developed. Dot-blots were scanned, and integrated density measurements interpolated against the standard curve.

3. Results

3.1. LRP1 mRNA level

LRP1 mRNA level was higher in $\varepsilon 4$-positive brains in all regions examined but the increase was statistically significant only in meningeal blood vessels, in relation to GAPDH ($P = 0.046$, Mann Whitney test) and SMA ($P = 0.043$) (Fig. 1). *APOE* $\varepsilon 3$ was associated with lower LRP1 in relation to GAPDH mRNA in meningeal vessels ($P = 0.022$) and choroid plexus ($P = 0.029$) (Fig. 2), and with lower LRP1 mRNA in brains with CAA