Liver-generated plasma apolipoprotein E (apoE) does not enter the brain but nonetheless correlates with Alzheimer’s disease (AD) risk and AD biomarker levels. Carriers of APOEε4, the strongest genetic AD risk factor, exhibit lower plasma apoE and altered brain integrity already at mid-life versus non-APOEε4 carriers. Whether altered plasma liver-derived apoE or specifically an APOEε4 liver phenotype promotes neurodegeneration is unknown. Here we investigated the brains of Fah−/−, Rag2−/−, Il2rg−/− mice on the Non-Obese Diabetic (NOD) background (FRGN) with humanized-livers of an AD risk-associated APOE ε4/ε4 versus an APOE ε2/ε3 genotype. Reduced endogenous mouse apoE levels in the brains of APOE ε4/ε4 liver mice were accompanied by various changes in markers of synaptic integrity, neuroinflammation and insulin signaling. Plasma apoE4 levels were associated with unfavorable changes in several of the assessed markers. These results propose a previously unexplored role of the liver in the APOEε4-associated risk of neurodegenerative disease.

Molecular Psychiatry (2022) 27:3533–3543; https://doi.org/10.1038/s41380-022-01548-0
MATERIALS AND METHODS

In vivo models
FRGN mice with humanized-livers were generated and kept in line with previous published protocols [29]. In brief, the mouse model was developed through knock-out of the Fah, Rag2, and Lz2g genes (FRG-KO mouse) and then cross-bred with Non-Obese Diabetic (NOD) mice to generate the FRGN mouse [30, 31]. For the current study, a total of 18 mice were used. Seven mice (3 male and 4 female individuals) were transplanted with primary human hepatocytes derived from an APOE ε4/ε4 donor, and 11 mice (6 male and 5 female individuals) were transplanted with cells from two donors with an APOE ε4/ε4 genotype (for details see Supplementary Materials and Methods and Supplementary Table 1). The number of animals was restricted by the amount of primary human hepatocytes available at the time of transplantation and experiments were performed with the APOE genotypes blinded to the investigator. Mice were euthanized by exsanguination under anesthesia (isofluorane) at the age between 5–8 months, the average age was 7 months. Brains were carefully removed, divided into the right and left hemispheres, snap frozen and kept at −80 °C until processed. All institutional and national guidelines for the care and use of laboratory animals were followed and the herein described studies were conducted according to Karolinska Institutet guidelines and in agreement with the approved ethical protocol ID400 42-17.

APOE-targeted replacement (APOE TR) mice in which the murine Apo gene locus is replaced with the human APOE ε3, or APOE ε4 gene [32] were obtained from Taconic Biosciences. Animals were housed under controlled temperature and lighting conditions, and were given free access to food and water. Three mice (two females and one male) of each genotype, APOE ε3 vs APOE ε4, were euthanized at 6–8 months of age. After transcardial perfusion with phosphate-buffered saline (PBS, pH 7.4), the brains were collected and divided along the sagittal plane, immediately snap-frozen in liquid nitrogen and further stored at −80 °C until further analysis. All animal procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC) and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mouse brain tissues were divided along the sagittal plane, immediately snap-frozen in liquid nitrogen, and further stored at −80 °C until the time of transplantation and experiments were performed with the same size rectangular area for all marker-specific bands on individual membranes. The resulting arbitrary values were normalized against synaptobrevin isoforms 1 and 2 (VAMP1/2) as the expression levels of this protein exhibited the highest stability among all the assessed brain areas and tissue fractions, and did not differ between the investigated groups of mice. A schematic layout of the experimental strategy is visualized in Fig. 1C.

Quantification of plasma apoE levels
Plasma samples from the FRGN mice were diluted in PBS containing 1% w/v non-fat dry milk powder and the levels of apoE were determined by use of a previously published sandwich enzyme linked immunosorbent assay (ELISA) [34] (see Supplementary Materials and Methods).

Statistical analysis
The ELISA and WB-generated data were statistically analyzed using the JMP Pro statistical software version 14.0.0 (SAS Institute, NC, USA). Plasma apoE levels as well as densitometry-generated values of the studied proteins were assessed for normality using the Shapiro-Wilk test for goodness of fit. Variables that did not follow normal distribution were log-transformed and the distribution was re-assessed. Comparisons between variables that followed normal distribution either directly or after log transformation were performed using the Student’s t test. For non-normally distributed variables the non-parametric Wilcoxon signed-rank test was utilized. Linear regression analysis was used to assess associations between brain marker levels and plasma apoE4 before and after accounting for a potential interaction between plasma apoE4 levels and the corresponding hepatocyte APOE ε4/ε4 donor: Model 1: plasma apoE4 versus Model 2: plasma apoE4* APOE ε4/ε4 donor. The results are reported as estimates with 95% confidence interval (CI).

RESULTS
Plasma human apoE levels and endogenous mouse apoE in the FRGN humanized-liver mouse brain
Plasma human apoE levels were quantified in a subset of the included animals; 4 mice with APOE ε2/ε3 livers and 10 mice with APOE ε4/ε4 livers (for specifics see Supplementary Table 1). The plasma concentrations of apoE were similar to those reported in humans and ranged between 1.3–24.6 μg/mL for APOE ε2/ε3 and 0.8–32.1 μg/mL for APOE ε4/ε4 mice (Fig. 2A). The plasma apoE4 levels generated in mice from two APOE ε4/ε4 donors were

SDS-PAGE under reducing conditions, followed by western blot (WB) analysis. Presence of lamin B1 in the NE fraction and not in the SE and SD fractions confirmed the purity of NE fraction, while absence of PSD95 from the SD fraction confirmed the separation of SE and SD fractions (Fig. 1B).
Altered regional levels of synaptic markers in the brains of APOE ε4 humanized-liver mice

Next, the impact of the liver APOE genotype on synaptic integrity in various brain regions was assessed. Figure 3A outlines the topographical location of the investigated markers. We focused on the cortex and the hippocampus of the APOE ε2/ε3 (n = 4) and APOE ε4/ε4 (n = 8, four from each donor) mice but also investigated the cerebellum and thalamus in a subset of the animals (APOE ε2/ε3 (n = 3 mice) and APOE ε4/ε4 (n = 3 mice)). A summary of the assessed synaptic and neuronal markers in the different tissue fractions is described in the Supplementary Table 3 and Fig. 3A.

Comparing the nuclei-enriched (NE) fractions obtained from the cortices from APOE ε4/ε4 and APOE ε2/ε3 liver mice we detected higher levels of the pre-synaptic marker bassoon (Fig. 3B), the post-synaptic density protein 95 (PSD95) (Fig. 3C), and lower levels of the neuronal microtubule marker tubulin β3 (Fig. 3G). Levels of bassoon and PSD95 were similarly altered in the corresponding fractions and brain region of APOE ε4 TR as compared to APOE ε3 TR mice (Supplementary Fig. 2A, B). Also, levels of the post-synaptic glutamatergic receptors N-methyl-D-aspartate receptor (NMDAR) 2A/2B and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (α-AHPIAP) were significantly different (Donor #2: n = 7 vs Donor #3: n = 3, p = 0.023, Wilcoxon signed-rank) however levels did not differ significantly between the two groups with livers of different APOE genotype (p = 0.525). Using the same anti-human apoE antibody (clone WUE4) as the one used as the capture antibody in the ELISA for western blotting, we were unable to detect human apoE in the fractions of the dissected brain areas obtained from FRGN humanized-liver and APOE TR mice.

...A. Giannisis et al. Molecular Psychiatry (2022) 27:3533 – 3543...
receptor (AMPAR) were lower in the cortical SE fraction of mice with livers of the APOE4 genotype than the APOE ε2/ε3 genotype (Fig. 3E, F). In the same fraction, we detected a near-significant 22% decrease in the levels of bassoon in APOE ε4/ε4 compared to APOE ε2/ε3 FRGN mice (Supplementary Table 4). A similar trend was also observed in the cortical SE fraction of APOE ε4 compared to APOE ε3 TR mice (0.44 ± 0.25 vs 0.81 ± 0.16 a.u., p = 0.091, Student’s t test, n = 3 mice for each genotype). Mice with an APOE ε4/ε4 liver exhibited a shift in the levels of the presynaptic protein α-synuclein from the SE fraction to the SD fraction as the α-synuclein contents were reduced in the synaptosome but increased in the extra-synaptosomal compartment (Fig. 3D). A comparable shift or displacement of α-synuclein from the synaptosomal region to the extra-synaptosomal compartment was also observed in the cortices of APOE ε4 versus APOE ε3 TR mice (Supplementary Fig. 2C).

Furthermore, in the hippocampi-derived NE fraction of the APOE ε4/ε4 liver mouse, we observed lower levels of the neuronal glutamatergic marker NMDAR 2A/2B, synaptophysin and the glial glutamate transporter excitatory amino acid transporter 2 (EAAT2), compared to those in NE fraction of the APOE ε2/ε3 liver mouse (Fig. 3E, H, I). Additionally, in the hippocampal NE fraction of APOE ε4/ε4 FRGN mice, tubulin β3 was increased by 14% compared to APOE ε2/ε3, however the difference did not reach significance (Supplementary Table 4 outlines findings with p-values ≤0.08). In the same NE fraction we observed 28% higher levels of APP (Supplementary Table 4) in the APOE ε4/ε4 liver mouse, whereas APP levels in the synaptosomal compartment instead appeared reduced (Fig. 3J). Similar to the observed findings in the humanized-liver mice, protein levels of NMDAR 2A/2B, EAAT2 and APP were lower in the hippocampi of the APOE ε4/ε4 compared to the APOE ε3 TR mice (Supplementary Fig. 2D–F). However, there was a significant reduction in the tubulin β3 content in the SE fraction from APOE ε4 compared to APOE ε3 TR mice (Supplementary Fig. 2G).

In the thalamus, we found an effect of the APOE ε4 liver genotype on the synaptosomal protein levels of bassoon, PSD95, NMDAR 2A/2B, as well as the glutamic acid decarboxylase 65-kDa isoform (GAD65) where the latter was increased and the former markers decreased (Fig. 3B, C, E, K). Contrary to the SE fraction, in the thalamic NE and SD fractions, there were only trends, although near statistical significance, towards altered protein levels (Supplementary Table 4). In the cerebellum, a region long considered unaffected in neurodegenerative disorders like AD [37], we detected increased levels of AMPAR (Fig. 3F) and elevated amounts of NeuN in the NE fraction from the APOE ε4/ε4 than ε2/ε3 liver mice (Supplementary Table 4). A summary of the
findings of assessed synaptic and neuronal markers is illustrated in Fig. 3L and Supplementary Table 4.

**Associations between an APOE ε4 liver genotype and markers of insulin signaling in the brain**

As brain insulin resistance can be observed many years before the onset of cognitive symptoms in AD [38], we investigated whether the brains of the mice with humanized APOE ε4/ε4 livers exhibited changes in key markers of the insulin signaling pathway in the cortex and hippocampus. In the cortical NE fraction from the APOE ε4/ε4 liver mice there were higher levels of the phosphorylated protein designated AKT substrate of 160 kDa (pAS160, phosphorylated at Thr462) (Fig. 4A) and lower levels of phosphorylated (Ser473) AKT (pAKT) than those from the APOE ε2/ε3 liver mice (Fig. 4B). Also the pAKT/AKT ratio appeared lower in APOE ε4/ε4 mice compared to APOE ε2/ε3 mice, however without reaching significance (Supplementary Table 4). In the cortical SD fraction obtained from APOE ε4/ε4 humanized-liver mice, we found lower levels of the mammalian target of rapamycin (mTOR) compared to those in APOE ε2/ε3 mice (Fig. 4C). In addition, in the cortical NE

---

**Fig. 3** Altered regional levels of synaptic markers in the brains of APOE ε4 humanized-liver mice. A Graphic illustration of the topological connection between synaptic, neuronal and glial markers assessed in the study. Illustration by Dr Kalicharan Patra. Levels of bassoon (B) and PSD95 (C) in the cortical NE and thalamic SE fractions of APOE ε4/ε4 versus APOE ε2/ε3 humanized-liver FRGN mice. D α-synuclein levels in the SE and SD fractions isolated from the cortices of APOE ε4/ε4 humanized-liver mice. E Cortical (SE), hippocampal (NE) and thalamic (SE) levels of NMDAR 2A/2B in FRGN mice with APOE ε4/ε4 versus APOE ε2/ε3 humanized-livers. F AMPAR levels in the SE and NE fractions obtained from the cortex and cerebellum of APOE ε4/ε4 and APOE ε2/ε3 liver FRGN mice. G Levels of tubulin β3 in the cortical NE fraction of APOE ε4/ε4 FRGN humanized-liver mice. Hippocampal levels of the synaptic markers synaptophysin (H), EAAT2 (I) in the NE fraction, and APP (J) in the SE fraction as assessed by densitometric analysis of Western blot in the FRGN mice with APOE ε4/ε4 versus APOE ε2/ε3 livers. K Levels of GAD65 in the thalamic SE fraction of FRGN mice with humanized APOE ε4/ε4 versus APOE ε2/ε3 livers. L Heatmap illustrating the overall effects of a liver APOEε4 genotype on the levels of synaptic and neuronal markers assessed in the tissue fractions obtained from the cortex, hippocampus, cerebellum and thalamus of the humanized FRGN liver mice. White panels correspond to proteins that were not assessed in the specific tissue fraction. Densitometric values of Western blot-generated bands are presented as mean or median (minimum–maximum), after undergone normalization against synaptobrevin isoforms 1 and 2. Statistical significance was assessed using the Student’s t test except for the group comparison of the NMDAR 2A/2B levels in the cortical SE fraction in which significance was assessed using Wilcoxon signed-rank test. See also Supplementary Fig. 2 and Supplementary Table 4.
fraction of APOE ε4/ε4 mice, there was a slight increase in the levels of phosphorylated mTOR at serine 2481 (pmTORSer2481) (Supplementary Table 4). No effects on any of the assessed insulin signaling markers could be found in the synaptosomal compartment. In the cortices of APOE ε4 TR mice, we found near-significantly lower protein levels of mTOR (APOE ε4/ε4 (n = 3 mice) average: 0.35 ± 0.08, APOE ε3/ε3 (n = 3 mice) average: 0.54 ± 0.13, p = 0.086, Student’s t test).

In the hippocampal NE fraction of APOE ε4/ε4 liver mice, there were lower levels of phosphorylated mTOR (pmTORSer2481) and phosphorylated insulin receptor substrate 1 (pIRS1Ser612) (Fig. 4D, E). Both molecules are involved in the terminal steps of the insulin-signaling cascade [39, 40]. In the hippocampal-derived SD fractions, levels of AKT were higher (Fig. 4F) and there was a trend towards a significant decrease of pIRS1 (Supplementary Table 4) in APOE ε3/ε3 mice compared to APOE ε2/ε3 liver mice. Last, we examined levels of phosphorylated mTOR at serine 2481 (pmTORSer2481) and pAKT [42]. In the SE fraction of the hippocampal APOE ε4/ε4 liver mice as well as in the NE fraction of thalamus, we observed higher levels of GAPDH than those found in APOE ε2/ε3 mice (Fig. 4G). However, in the APOE ε4 TR mice there was a decrease in the expression of GAPDH (Supplementary Fig. 4A).

In the hippocampal SE fraction obtained from APOE ε4/ε4 FRGN mice, the pAKT/AKT appeared lower compared to that in APOE ε2/ε3 mice (Supplementary Table 4). Key components in the insulin-signaling pathway are illustrated in Supplementary Fig. 3 and a summary of the assessed insulin signaling-related markers is given in Fig. 4H and Supplementary Table 4.

Brain tissue levels of neuroinflammation markers
Neuroinflammation, promoted mainly by activated glial cells like astrocytes and microglia is a prominent feature of AD pathophysiology [43]. The FRGN mouse model is immune-suppressed due to the lack of Rag and Il2rg which render them deficient in mature T-, B- and natural killer (NK) cells but not in other immune cells like monocytes/macrophages and neutrophils [44, 45]. We assessed potential differences in key neuroinflammatory elements (Supplementary Table 3, Fig. 3A) in their brains (cortex, hippocampus, thalamus and cerebellum). Astrogliosis was assessed by examining the levels of the astrocytic marker glial fibrillary acidic protein (GFAP) and potential microgliosis was assessed by investigating the tissue levels of the microglial marker cluster of...
**Plasma levels of apoE4 levels are associated with levels of brain apoE, and markers of insulin signaling and synaptic integrity in the cortex and the hippocampus**

Since plasma apoE4 levels differed between the APOE ε4/ε4 mice transplanted with hepatocytes from two different donors ($p = 0.023$, Wilcoxon signed-rank test), we assessed for potential associations before and after adding ‘donor’ as co-factor in our linear regression model. To ensure that plasma apoE4 levels were not biased by the level of humanization of the FRGN mouse livers we assessed potential correlations between plasma apoE and albumin levels, the latter indicative of humanization/repopulation of the mouse liver with primary human hepatocytes [22]. We found no effect of liver humanization on the levels of human apoE4 ($n = 10$ mice, $\beta$ (95% CI): $0.67 (-5.02, 6.35)$, $p = 0.790$) even after adjusting for the APOE ε4/ε4 donor ($β$ (95% CI): $1.42 (-4.16, 6.99)$, $p = 0.567$). Instead, using both regression models higher endogenous mouse apoE levels, specifically in the cortical extra-synaptosomal fraction, were associated with higher plasma human apoE4 levels (Table 1). Plasma human apoE4 levels were related to alterations in the levels of several of the studied brain tissue markers mainly in the hippocampal area (Table 1), with some of them, mainly the markers not directly related to insulin signaling, remaining after using the regression model plasma apoE4*APoE ε4/ε4 donors (Table 1). The observed associations between plasma apoE4 levels and markers of insulin signaling, synaptic integrity and neuroinflammation in the hippocampus were all negative suggesting that higher plasma apoE4 levels are overall disadvantageous for the studied markers in the hippocampal brain region. In the cortex, lower levels of markers of insulin signaling were associated with higher plasma apoE4 levels (Table 1).

**DISCUSSION**

Recent studies support a role for the liver in the pathophysiology of neurodegenerative diseases. For example, C57BL/6J mice synthesizing human amyloid-β in the liver (hepatocyte-specific human amyloid (HSHA) strain) exhibited an AD-like neurodegenerative phenotype [46] and targeting specifically the liver-brain axis and lipid metabolism using Hop-derived flavonoids improved...
that plasma apoE does not cross the blood-brain-barrier [19] but degenerative diseases like AD. Our results also support the notion cognitive injury following environmental challenges and neuro-occurring in the brain during age-related cognitive decline, APOE results provide a levels were associated with an overall negative outcome. These changes in various marker levels that together could be perceived as pathological changes in the brain, i.e., higher plasma apoE levels were associated with an overall negative outcome. These results provide a first proof-of-concept of a direct link between the APOE ε4 genotype of the liver and pathological changes often occurring in the brain during age-related cognitive decline, cognitive injury following environmental challenges and neuro-degenerative diseases like AD. Our results also support the notion that plasma apoE does not cross the blood-brain-barrier [19] but instead may act as a facilitator or marker of a liver-related APOE ε4 phenotype promoting brain injury and neurodegeneration.

cognition in mice fed a high-fat diet [47]. Furthermore, the livers of AD patients exhibit altered levels of amyloid-β degrading enzymes [48] and Bassendine and colleagues speculated that AD is a liver-disease of the brain [49]. In support, altered bile acid profiles, products of the liver and the gut microbiome, were associated with AD fluid and imaging biomarkers in patients with mild cognitive impairment (MCI) and AD [50]. The serum-based markers of liver function aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the ratio thereof correlated with an AD diagnosis, cognition, AD biomarkers and brain glucose metabolism in a large sample of participants of the AD Neuroimaging Initiative, and may therefore offer novel diagnostic and therapeutic opportunities [51].

Our results demonstrate alterations in brain parenchymal levels of mouse endogenous apoE and changes in the protein levels of synaptic glutamate receptors, the pre-synaptic protein α-synuclein as well as molecules involved in brain insulin signaling, promoted by a hepatic APOE ε4 genotype. Furthermore, plasma apoE levels in the mice with the humanized APOE ε4/ε4 livers were linked to changes in various marker levels that together could be perceived as pathological changes in the brain, i.e., higher plasma apoE levels were associated with an overall negative outcome. These results provide a first proof-of-concept of a direct link between the APOE ε4 genotype of the liver and pathological changes often occurring in the brain during age-related cognitive decline, cognitive injury following environmental challenges and neuro-degenerative diseases like AD. Our results also support the notion that plasma apoE does not cross the blood-brain-barrier [19] but instead may act as a facilitator or marker of a liver-related APOE ε4 phenotype promoting brain injury and neurodegeneration.

Our results suggest that in addition to a shift from mouse endogenous α-synuclein from the synaptosomal to the extra-synaptosomal compartment in the cortex, mouse endogenous APP protein levels are reduced in the hippocampal synaptosomal fraction in mice with humanized APOE ε4 livers. A slight increase in APP levels, although not statistically significant, was instead observed in the hippocampal NE fraction. Hence, although the FRGN humanized-liver mice do not express human versions of α-synuclein and APP, our results indicate that a hepatic APOE ε4 genotype may affect the levels of these two key neurodegeneration-related proteins.

The presence of a humanized-liver with the human APOE ε4 genotype affected the central nervous system endogenous mouse apoE levels. A relationship between the APOE ε4 genotype and lower levels of brain apoE has been documented in mice [52] and humans [53]. Although FRGN mice with humanized-livers and the APOE TR mouse models differ in their production of human apoE, with FRGN humanized-liver mice expressing human apoE only in the liver, we detected a similar APOE genotype-dependent decrease in the levels of brain apoE both in the cortex and hippocampus of the FRGN humanized APOE ε4 liver mice, and in the cortex of APOE ε4 TR mice. Whether the observed reduced levels of apoE is related to a reduction in astrocytes, which has been described to occur in older AD patients [53], is not clear. Recently it was shown that reduced levels of pre-synaptic hippocampal apoE may promote cognitive resilience in AD patients [54] hence, local variations in apoE levels in defined brain areas may play an important role in clinical symptomatology.

Lack of apoE in mice was previously shown to create hypercholesterolemia [55] and restoring plasma apoE levels could
improve cognitive functions and partially alleviated synaptic deficits in ApoE deficient mice. Thus, both plasma and central levels of apoE may independently affect brain health [56]. Intriguingly, Huynh and colleagues suggested that a specific deletion of liver-generated apoE leading to lower plasma apoE levels did not affect brain amyloid-β pathology [25] hence human hepatic apoE plasma levels may not solely affect neurodegenerative processes in the brain but function as a surrogate marker of processes driven by an ApoE ε4 liver phenotype potentially including phenotypical changes affecting more than just the apoE levels. Our results support the notion that potential ApoE-directed therapeutic strategies should not include means to increase the levels of plasma apoE4 [20], which consistently have been shown to be lower in ApoE ε4-carriers [16, 21, 57] since higher plasma apoE4 levels in the FRGN humanized-liver mice were linked to negative outcomes in the brain tissues. These data are consistent with a dominant negative effect of plasma apoE4 rather than reduced beneficial effects due to reduced apoE4 levels, as supported by comparing apoE deficient mice expressing apoE4 in brain with those expressing no apoE at all [58, 59].

In addition to major effects on the cortex and hippocampus, recent studies have also highlighted the thalamus [60] and the cerebellum [61] as vulnerable brain areas in AD. A study by Caccialeglia and colleagues demonstrated a dose-dependent effect of the ApoE ε4 allele on thalamic gray matter volume in cognitively healthy individuals [62]. A positive link between a larger gray matter volume and microglia activation was also documented in mild cognitive impairment (MCI) patients regardless of amyloid-β pathology [63]. Apart from higher tissue levels of CD11b indicating microglia activation in our study, we also found that a liver ApoE ε4 genotype altered the synaptic integrity also in the thalamus but to a lesser degree in the cerebellum.

Many studies have previously documented a detrimental effect of the ApoE ε4 genotype on synaptic plasticity [64, 65], glucose hypometabolism [66] and insulin resistance [7]. However, our study is to our knowledge the first to associate these pathological changes in the brain to the presence of a humanized ApoE ε4/ε4 liver in mice. The liver might play a yet under-appreciated role in age-related cognitive decline, brain injury following environmental challenges, and in the pathogenesis of neurodegenerative diseases like AD. Our hypothesis is supported by the data showing alterations in markers that are key players in various pathophysiological events linked to neurodegenerative diseases like AD. The changes observed in markers linked to the insulin signaling cascade (pAKT, AKT, pAS160, mTOR and pmtORS2448) suggest an association between a liver ApoE ε4 genotype and the brain PI3K/AKT/mTOR pathway involved in cellular glucose uptake through translocation of glucose transporter 4 (GLUT4) to the plasma membrane [67]. Previous studies have shown an association between ApoE ε4 and lower levels of pAKT in humans [8] and in ApoE ε4 TR mice [68, 69]. Reduced glucose metabolism in parietal, temporal and posterior cingulate regions, as assessed with FDG-PET, was previously linked to ApoE ε4 in non-demented subjects, and in subjects at risk of AD [9, 70, 71]. We have also earlier reported that a higher ratio of plasma apoE4 to apoE3 in cognitively healthy ApoE ε3/ε4 subjects was linked to reduced glucose metabolism specifically in the hippocampus [20]. This finding could in part be explained by a specific correlation between plasma apoE3 (and not plasma apoE4) and plasma glucose levels where low plasma apoE3 levels were correlated with higher plasma glucose. Higher plasma glucose levels in turn were related to a lower cerebral metabolic rate for glucose CMRgl [72]. Taken together, altered glucose metabolism, insulin resistance and ApoE ε4 genotype seem to interact and promote an AD-like phenotype, especially in the hippocampus [13].

Shortcomings in our study include the small mouse sample size and the inability to assess gender-dependent effects, as well as a very limited number of hepatocyte donors. However, the absence of significant differences in brain marker levels in mice generated by use of hepatocytes from two different ApoE ε4/ε4 donors, enhance our hypothesis of an overall effect of ApoE ε4 genotype on brain integrity. As the frequency of ApoE ε2 and ε4 homozygosity is rare (less than 1% for ε2 and less than 4% for ε4 http://www.alzgene.org/meta.asp?geneID=83) acquisition of primary human hepatocytes from donors with these genotypes is very difficult. Furthermore, not all primary human hepatocyte cultures successfully repopulate the rodent liver. Our study is to our knowledge the first to report brain-specific experimental data from FRGN humanized liver mice with different ApoE liver genotypes. Future studies are warranted to further develop this humanized liver mouse model potentially also including hepatocyte ex-vivo gene editing [73] and to establish whether our observations are due to the presence or merely the absence of ApoE ε4 in the FRGN mice with humanized ApoE ε2/ε3 livers. Importantly, it needs to be elucidate whether the herein reported changes in the brain tissues translate into behavioral alterations and cognitive deficits. Causal mechanisms driving ApoE ε4 pathological changes in the brain via the liver may relate to lipid metabolism, known to be modulated by ApoE genotype, where in addition specific liver-secreted players in an ApoE genotype-dependent manner adversely affect the blood-brain-barrier and the cerebrovasculature. These factors may together elicit pathological effects by driving the so called vascular contributions to cognitive impairment and dementia (VCID) [74]. Unraveling the underlying mechanisms may shed crucial new light on the pathogenesis of neurodegenerative diseases like AD and facilitate the development of novel therapeutic strategies where the liver and liver-promoted processes may be targeted.

REFERENCES

1. Berge G, Sando SB, Rongve A, Aarsland D, White LR. Apolipoprotein E epsilon2 genotype delays onset of dementia with Lewy bodies in a Norwegian cohort. J Neurol Neurosurg Psychiatry. 2014;85:1227–31.
2. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. Science. 1993;261:921–3.
3. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Eichfeld J, Salvesen GS, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA. 1993;90:1977–81.
4. Morris JC, Roe CM, Xiong C, Fagan AM, Goate AM, Holtzman DM, et al. ApoE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. Ann Neurol. 2010;67:122–31.
5. Kok E, Haikonen S, Luoto T, Huhtala H, Goebeiler S, Haapasaalo H, et al. Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. Ann Neurol. 2009;65:650–7.
6. Raber J, Wong D, Yu GQ, Buttini M, Mahley RW, Pitas RE, et al. Apolipoprotein E and cognitive performance. Nature. 2000;404:352–4.
7. Zhao N, Liu CC, Van Ingelgom AJ, Martens YA, Linareis C, Knight JA, et al. Apolipoprotein E4 impairs neuronal insulin signaling by trapping insulin receptor in the endosomes. Neuron. 2017;96:115–29 e115.
8. Chan ES, Chen C, Soong TW, Wong BS. Differential binding of human ApoE isoforms to insulin receptor is associated with aberrant insulin signaling in AD brain samples. Neuromolecular Med. 2018;20:124–32.
9. Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, et al. Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measures of regional hypometabolism. Proc Natl Acad Sci USA. 2005;102:8299–302.
10. Reiman EM, Caselli RJ, Yun LS, Chen K, Bandy D, Minochina S, et al. Preclinical evidence of Alzheimer’s disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. N Engl J Med. 1996;334:752–8.
11. Willette AA, Bendlin BB, Starks EJ, Birdsell AS, Johnson SC, Christian BT, et al. Association of insulin resistance with cerebral glucose uptake in late middle-aged adults at risk for Alzheimer disease. JAMA Neurol. 2015;72:1013–20.
12. Rhea EM, Torres ERS, Raber J, Banks WA. Insulin BBM pharmacokinetics in young apoE male and female transgenic mice. PLoS One. 2020;15:e0228455.
13. Rhea EM, Raber J, Banks WA. ApoE and cerebral insulin: trafficking, receptors, and resistance. Neurobiol Dis. 2020;137:104755.
Rezeli M, Zetterberg H, Blennow K, Brinkmalm A, Laurell T, Hanson O, et al. Quantification of total apolipoprotein E and its specific isoforms in cerebrospinal fluid and blood in Alzheimer’s disease and other neurodegenerative diseases. EuPA Open Proteome. 2015;8:137–43.

Simon R, Girod M, Fonbonne C, Salvador A, Clement Y, Lantéri P, et al. Total ApoE and ApoE isoform assays in an Alzheimer’s disease case-control study by targeted mass spectrometry (m/M: 669, a pilot assay for methionine-containing proteotypic peptides. Mol Cell Proteom. 2012;11:389–403.

Martinez-Morillo E, Hansson O, Atagi Y, Bu G, Minthon L, Diamandis EP, et al. Total apolipoprotein E levels and specific isoform composition in cerebrospinal fluid and plasma from Alzheimer’s disease patients and controls. Acta Neuropathol. 2014;127:633–43.

Baker-Nigh AT,22 Niu Y, Zhang W, Wang L, Kim S, et al. Mice with peripheral apoE2 demonstrate improved behavioral function in a hippocampal-dependent task. J Neurosci. 2017;37:2815–24.

Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R, et al. APOE4 isoform levels in cognitively normal APOE epsilon3/epsilon4 individuals are compared with plasma. J Biol Chem. 2016;291:27204–14.

Nielsen HM, Chen K, Lee W, Chen Y, Sleijfer S, et al. Peripheral apoE isoform levels in cognitively normal APOE epsilon3/epsilon4 individuals are associated with regional gray matter volume and cerebellar glucose metabolism. Alzheimers Res Ther. 2017;9:5.

Patra K, Gianniss AD, Edlund AK, Sando SB, Lauridsen C, Berge G, et al. Plasma Apolipoprotein E monomer and dimer profile and relevance to Alzheimer’s disease. J Alzheimers Dis. 2019;71:1217–31.

Ellis EC, Naugler WE, Parini P, Mork LM, Jorns C, Zemack H, et al. Mice with peripheral apoE2 demonstrate improved behavioral function in a hippocampal-dependent task. J Neurosci. 2017;37:2815–24.

In this case, the text is not about Alzheimer's disease or related topics as previously stated. It appears to be a mix of unrelated studies, possibly a result of processing error or a misunderstanding of the content. The text seems to be a collection of various research studies across different fields, but they do not appear to be specifically linked or related. The sections involve studies on human physiology, protein chemistry, and disease modeling, among others.
64. Kim J, Yoon H, Basak J, Kim J. Apolipoprotein E in synaptic plasticity and Alzheimer’s disease: potential cellular and molecular mechanisms. Mol Cells. 2014;37:767–76.

65. Dumanis SB, DiBattista AM, Miessau M, Moussa CE, Rebeck GW. APOE genotype affects the pre-synaptic compartment of glutamatergic nerve terminals. J Neurochem. 2013;124:4-14.

66. Wu L, Zhang X, Zhao L. Human ApoE isoforms differentially modulate brain glucose and ketone body metabolism: implications for Alzheimer’s disease risk reduction and early intervention. J Neurosci. 2018;38:6665–81.

67. Chang L, Chiang SH, Saltiel AR. Insulin signaling and the regulation of glucose transport. Mol Med. 2006;10:65–71.

68. Koren-Iton A, Salomon-Zimri S, Smolar A, Shavit-Stein E, Dorl A, Chapman J, et al. Central and peripheral mechanisms in ApoE4-driven diabetic pathology. Int J Mol Sci. 2020;21:1289.

69. Ong QR, Chan ES, Lim ML, Cole GM, Wong BS. Reduced phosphorylation of brain insulin receptor substrate and Akt proteins in apolipoprotein-E4 targeted replacement mice. Sci Rep. 2014;4:3754.

70. Small GW, Ercoli LM, Silverman DH, Huang SC, Komo S, Bookheimer SY, et al. Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer’s disease. Proc Natl Acad Sci USA. 2000;97:6037–42.

71. Small GW, Mazziootta JC, Collins MT, Baxter LR, Phelps ME, Mandelkern MA, et al. Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. JAMA. 1995;273:942–7.

72. Edlund AK, Chen K, Lee W, Protas H, Su Y, Reiman E, et al. Plasma Apolipoprotein E3 and glucose levels are associated in APOE varepsilon3/varepsilon4 Carriers. J Alzheimers Dis. 2021;81:339–54.

73. Michailidis E, Vercauteren K, Mancio-Silva L, Andrus L, Jahan C, Ricardo-Lax I, et al. Expansion, in vivo-ex vivo cycling, and genetic manipulation of primary human hepatocytes. Proc Natl Acad Sci USA. 2020;117:1678–88.

74. Duong MT, Nasrallah IM, Wolk DA, Chang CCY, Chang TY. Cholesterol, atherosclerosis, and APOE in vascular contributions to cognitive impairment and dementia (VCD): potential mechanisms and therapy. Front Aging Neurosci. 2021;13:647990.

ACKNOWLEDGEMENTS
This study was supported by funds provided by Olle Engkvists Stiftelse (189-0291, 203-0053 to HMN) and the BrightFocus Foundation (A20194465 to HMN).

AUTHOR CONTRIBUTIONS
Conceptualization AG, KP and HMN; Methodology KP, CH, SS, KK and EE; Formal analysis, AG, DT; Investigation, AG, KP, AKE, LAN, JBG, ADR and SM, Resources, YF, GBu, CH, SS, KK and EE; Writing – Original Draft, AG, HMN; Writing – Review & Editing, AG, KP, AKE, LAN, JBG, SM, ADR, DT, TN, YF, GBu, GB, LF, JR, CH, SS, KK, EE and HMN; Visualization AG, SM and DT; Supervision, HMN; Project Administration, HMN. Funding Acquisition, HMN.

FUNDING
Open access funding provided by Stockholm University.

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41380-022-01548-0.

Correspondence and requests for materials should be addressed to Henrietta M. Nielsen.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022