Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector

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Efforts to develop drugs for Alzheimer’s disease (AD) have shown promise in animal studies, only to fail in human trials, suggesting a pressing need to study AD in human model systems. Using human neurons derived from induced pluripotent stem cells that expressed apolipoprotein E4 (ApoE4), a variant of the APOE gene product and the major genetic risk factor for AD, we demonstrated that ApoE4-expressing neurons had higher levels of tau phosphorylation, unrelated to their increased production of amyloid-β (Aβ) peptides, and that they displayed GABAergic neuron degeneration. ApoE4 increased Aβ production in human, but not in mouse, neurons. Converting ApoE4 to ApoE3 by gene editing rescued these phenotypes, indicating the specific effects of ApoE4. Neurons that lacked APOE behaved similarly to those expressing ApoE3, and the introduction of ApoE4 expression recapitulated the pathological phenotypes, suggesting a gain of toxic effects from ApoE4. Treatment of ApoE4-expressing neurons with a small-molecule structure corrector ameliorated the detrimental effects, thus showing that correcting the pathogenic conformation of ApoE4 is a viable therapeutic approach for ApoE4-related AD.

The complexity and multifactorial etiology of AD pose unique challenges for studying its pathogenesis and developing therapies. Efforts to target AD-related pathways have shown promise in animal studies, only to fail in human trials1,2. There is a pressing need to identify novel mechanisms and therapeutic targets for AD using human model systems, such as human neurons. AD is pathologically characterized by the formation of intracellular neurofibrillary tangles (NFTs), comprised of hyperphosphorylated tau protein, and extracellular amyloid plaques, comprised of Aβ peptides. ApoE4, the presence of which is the major genetic risk factor for AD1,4, is found to be associated with increases in both pathologies. In general, ApoE4 increases AD risk and lowers the age of onset in a gene-dose-dependent manner5. Of note, the lifetime risk estimate of developing AD by age 85 is ~65% in people with two copies of the ε4 allele, which encodes apoE4, but only ~10% in people with two copies of the APoE-ε3 allele, which encodes ApoE36. This notable difference highlights the importance of ApoE4 in the pathogenesis of AD.

Human ApoE3 and ApoE4 differ from each another only at one amino acid residue at position 112. ApoE3, the common isoform, has Cys112, whereas ApoE4 has Arg112.2,3. Structurally, APOE has two domains: the amino-terminal domain and the carboxy-terminal domain, which contain the receptor-binding region and the lipid-binding region, respectively. The two domains are linked by a structurally flexible hinge region. Interaction between the carboxy- and amino-terminal domains, called domain interaction, is a unique biophysical property of ApoE42,4. In ApoE4, domain interaction occurs as a result of the formation of a salt bridge between Arg61 and Glu255, owing to the effect of Arg112. This interaction occurs to a much less extent in ApoE3, because the side chain of Arg61 adopts a different conformation owing to Cys112 that results in a less accessible side chain conformation for formation of a salt bridge with Glu2552,4. Domain interaction has been suggested to be a molecular basis for ApoE4’s detrimental effects in AD pathogenesis; consequently, it has been pursued as a drug target to identify small-molecule structure correctors capable of converting ApoE4 to ApoE3 both structurally and functionally7–9.

Studies in animal models and postmortem human tissues have provided key insights into the pathogenesis of AD1,2,10. However, mouse models of AD do not recapitulate many AD features, and postmortem human brain tissues have characteristics of end-stage disease that may not be present at earlier stages1,2,10. Until recently, studies of the cellular and molecular mechanisms of AD have been hindered by the lack of access to live human neurons. Now, induced pluripotent stem cells (iPSCs) derived from human somatic cells with AD-linked mutations or polymorphisms, together with gene-editing techniques, are promising in vitro models for studying disease pathogenesis in relevant cell types, including human neurons11–13.

Here we analyzed AD-related phenotypes of cultured neurons derived from human iPSC (hiPSC) lines of different APOE genotypes, including gene-edited isogenic and APOE-deficient lines. We also tested the effects of gene editing to convert APOE-ε4 into APOE-ε3 and of a small-molecule structure corrector to render ApoE4 ApoE3-like. Our data demonstrate that ApoE4 induced AD-related pathological phenotypes, due to a gain of toxic effects, specifically in human neurons, which could be dramatically ameliorated by treatment with a small-molecule ApoE4-structure corrector.
Results

Generating hiPSC lines from APOE-e3 and APOE-e4 homozygotes. hiPSC lines were generated from subjects who were homozygous for the APOE-e3 or APOE-e4 alleles (hereafter referred to as ApoE3/3 and ApoE4/4 hiPSC lines, respectively) (Supplementary Table 1) as described\(^{47,48}\). All of the hiPSC lines were morphologically similar to embryonic stem (ES) cells (Supplementary Fig. 1b) and expressed ES cell markers, such as NANOG, SOX2, TRA-1-60 and TRA-1-81 (Supplementary Fig. 1c–e). DNA sequencing confirmed the APOE genotypes of all of the hiPSC lines, and chromosomal analysis revealed normal karyotypes (Supplementary Fig. 1f). After injection into immunodeficient mice, all of the hiPSC lines formed teratomas, confirming their pluripotency\(^{16,17}\). Three ApoE3/3 hiPSC lines (E3/3-A, E3/3-B and E3/3-C) and three ApoE4/4 hiPSC lines (E4/4-A, E4/4-B and E4/4-C), each of which was derived from a subject with the corresponding APOE genotype, were fully characterized and used in this study (Supplementary Table 1). All six of these hiPSC lines developed well into neural stem cells, which expressed SOX2, nestin, PAX6 and FOXG1 (Supplementary Fig. 1g–i), and then into mature neurons that had neuronal morphology (Supplementary Fig. 1j) and expressed the neuronal markers TUJ1 and MAP2 (Supplementary Fig. 1k,l). Quantification showed that 90 ± 1.5% (mean ± s.e.m., \( n=12 \) randomly collected images from three independent experiments with total of 326 cells counted) of the cells were positive for neuronal marker, MAP2, indicating the high purity of neuronal culture.

Human ApoE4/4 neurons produce less full-length APOE protein and more APOE fragments than human ApoE3/3 neurons. Western blot analyses of neuronal lysates and culture medium revealed that human ApoE4/4 neurons produced ~35% less full-length intracellular APOE protein and secreted ~60% less full-length APOE protein into the medium than human ApoE3/3 neurons, both in individual lines (Supplementary Fig. 2a) and as shown by mean values (Fig. 1a–c). Consequently, the ratio of intracellular APOE to secreted APOE was ~20% higher for ApoE4 than for ApoE3, suggesting that ApoE4 tended to be retained inside neurons. The ratio of APOE fragments to full-length APOE in neuronal lysates was also significantly higher for ApoE4/4 neurons (Fig. 1d,e and Supplementary Fig. 2b). The major APOE fragments were ~12–20kDa (Fig. 1d), which were similar in size to those in the brains of transgenic mice that expressed ApoE4 specifically in neurons\(^{19}\) and in patients with AD who had an APOE-e4 genotype\(^{18}\). ApoE4 fragments were undetectable in the culture medium, suggesting that the fragments tended to accumulate inside neurons.

Levels of phosphorylated tau are higher in human ApoE4/4 neurons than in human ApoE3/3 neurons. Western blot analysis with antibodies specific for phosphorylated tau (p-tau) (AT8, AT180, PHF1 and AT270) revealed significantly higher p-tau levels in ApoE4/4 neurons than in ApoE3/3 neurons, both in individual lines (Supplementary Fig. 2c–f) and as shown by mean values (Fig. 1f–i). Double immunostaining with anti-p-tau (AT8 or PHF1) and anti-MAP2 consistently showed that significantly higher percentages of ApoE4/4 neurons were also positive for p-tau (Fig. 1j–m). Furthermore, p-tau accumulated mostly in the soma and dendrites of ApoE4/4 neurons, suggesting that ApoE4 induced both tau phosphorylation and mislocalization of p-tau in human neurons, a pathological hallmark of p-tau in brains from individuals with AD\(^{20,21}\).

Human ApoE4/4 neurons produce more Aβ than human ApoE3/3 neurons. The levels of Aβ peptides, including Aβ\(_{40}\) and Aβ\(_{42}\), in the neuronal culture medium were measured by an ELISA\(^{10,22}\). ApoE4/4 neurons secreted >2-fold more Aβ\(_{40}\) and Aβ\(_{42}\) into the culture medium than ApoE3/3 neurons, both in individual lines (Supplementary Fig. 2g,h) and as shown by mean values (Fig. 1n,o). The ApoE4/4 neurons also secreted greater amounts of soluble amyloid-β precursor protein (sAPP) into the medium (Fig. 1p,q), suggesting increased processing of APP. Of note, the levels of full-length APP protein (Fig. 1r,s) and of the APP mRNA (Fig. 1t) were similar in ApoE4/4 and ApoE3/3 neurons, as determined by western blot and qRT–PCR analysis, respectively, suggesting that ApoE4 did not alter APP expression in human neurons. Notably, the ratios of TUJ1 to actin were similar in ApoE3/3 and ApoE4/4 neuronal cultures (Fig. 1r,a), which was indicative of comparable neuronal populations in amount and purity.

Because it has been reported that in APOE-e3- and APOE-e4-knock-in mice expressing human APP with mutations that cause familial AD, ApoE4 seems to reduce Aβ clearance and stimulate Aβ deposition in the brain without affecting Aβ production\(^23\), we wondered whether the difference in ApoE4’s effect on Aβ production between our human neuron study and the previously reported mouse data reflected species difference in APOE regulation of Aβ metabolism. To address this question, we also generated mouse iPSC (miPSC) lines from fibroblasts of the APOE-e3 KI mice and the APOE-e4 KI mice, differentiated them into neurons in culture for 7–8 weeks and measured Aβ secretion from the miPSC-derived neurons in culture. Clearly, there were no significant differences in both Aβ\(_{40}\) and Aβ\(_{42}\) levels in the culture medium of neurons derived from the ApoE3/3 miPSC and ApoE4/4 miPSC lines (Supplementary Fig. 3). These data indicate the species difference in APOE isoform regulation of Aβ metabolism, i.e., ApoE4 stimulates Aβ production in human neurons but not in mouse neurons.

Increased p-tau levels in ApoE4/4 neurons are independent of high levels of Aβ. It has been suggested that Aβ accumulation could increase phosphorylation and accumulation of tau\(^{46,47}\). To assess the potential causal relationship between increased Aβ production and increased p-tau levels, we treated ApoE4/4 neurons with an inhibitor of β-secretase (OM99-2) or of γ-secretase (compound E) for 1 week to inhibit Aβ production\(^{10}\). Aβ\(_{40}\) and Aβ\(_{42}\) levels in the culture medium decreased by 60–75% (OM99-2) or by >95% (compound E) (Fig. 2a,b). Notably, despite these drastic reductions of Aβ levels, p-tau levels were unaffected in ApoE4/4 neurons, as shown by western blot analysis with monoclonal antibodies AT8 and AT180 (Fig. 2c–e).

ApoE4 causes GABAAergic neuron degeneration or loss in hiPSC-derived neuronal culture. Immunofluorescence staining revealed significantly fewer GABA+ neurons derived from ApoE4/4 hiPSCs than from ApoE3/3 hiPSCs, both in individual lines (Supplementary Fig. 2i) and as shown by mean values (Fig. 3a–c). Levels of GAD2 and GAD1 (GABAAergic neuron markers; also known as GAD65 and GAD67, respectively) were lower in neuronal cultures derived from ApoE4/4/hiPSCs than in those derived from ApoE3/3 hiPSCs, both in individual lines (Supplementary Fig. 2i) and as shown by mean values (Fig. 3d,e). Immunostaining revealed no significant difference in the numbers of GABA+ neurons at early time points (within 4 weeks) in culture between ApoE4/4 hiPSCs and ApoE3/3 hiPSCs (relative to the ApoE3/3 hiPSCs: ApoE3/3: 3 ± 0.11 (mean ± s.e.m.), \( n=5 \) fields with total of 570 GABA+ neurons counted; ApoE4/4: 0.98 ± 0.14 (mean ± s.e.m.), \( n=8 \) fields with total of 894 GABA+ neurons counted), suggesting that the detrimental effect of ApoE4 on GABAergic neurons in culture was not a developmental effect. The decrease in GABAergic neuron numbers occurred during the late stage of neuronal differentiation of ApoE4/4 hiPSCs, suggesting that ApoE4 induced GABAergic neurodegeneration or loss. Of note, ApoE3/3 hiPSCs and ApoE4/4 hiPSCs had similar efficiencies in generating TBR1+ glutamatergic neurons and TH+ dopaminergic neurons in culture (Supplementary Fig. 4), suggesting a specific detrimental effect of ApoE4 on GABAergic neurons. Furthermore, ~60% of GABAergic neurons derived from ApoE4/4 hiPSCs, but only ~30% of those derived from ApoE3/3 hiPSCs, were immunopositive for p-tau (Fig. 3f–h).
Fig. 1 | Human ApoE4/4 neurons generate more APOE fragments, have higher p-tau levels and produce more Aβ than human ApoE3/3 neurons. a–c, Representative western blot analysis (a) and quantification of full-length APOE in lysates (intracellular) (b) or the medium (secreted) (c) from neurons that were derived from ApoE3/3 hiPSCs (E3/3) or ApoE4/4 hiPSCs (E4/4). Actin was used as a loading control. In b, values were normalized to those of E3/3 (E3/3, n = 23 biologically independent samples (n = 9 from ApoE3/3-A; n = 8 from ApoE3/3-B; n = 6 from ApoE3/3-C); E4/4, n = 20 biologically independent samples (n = 6 from ApoE4/4-A; n = 6 from ApoE4/4-B; n = 8 from ApoE4/4-C)). In c, values were normalized to those of E3/3 (E3/3, n = 15 biologically independent samples (n = 6 from ApoE3/3-A; n = 6 from ApoE3/3-B; n = 3 from ApoE3/3-C); E4/4, n = 9 biologically independent samples (n = 3 from ApoE4/4-A; n = 3 from ApoE4/4-B; n = 3 from ApoE4/4-C)).

In d, e, Representative western blot analysis (d) and quantification (e) of full-length APOE and of APOE fragments in lysates from E3/3 and E4/4 neurons. Values were normalized to those of E3/3 (E3/3, n = 13 biologically independent samples (n = 3 from ApoE3/3-A; n = 5 from ApoE3/3-B; n = 5 from ApoE3/3-C); E4/4, n = 14 biologically independent samples (n = 3 from ApoE4/4-A; n = 5 from ApoE4/4-B; n = 6 from ApoE4/4-C)). f–j, Western blot analysis (f) and quantification of p-tau in lysates of E3/3 and E4/4 neurons with the p-tau-specific monoclonal antibodies AT8 (g), AT180 (h), PHF1 (i) and AT270 (j). In f, Tau5 and TUJ1 were used for loading control and normalization. In g, values were normalized to those of E3/3 (E3/3, n = 31 biologically independent samples; E4/4, n = 25 biologically independent samples). In h, values were normalized to those of E3/3 (E3/3, n = 22 biologically independent samples (n = 7 from ApoE3/3-A; n = 7 from ApoE3/3-B; n = 8 from ApoE3/3-C); E4/4, n = 18 biologically independent samples (n = 6 from ApoE4/4-A; n = 4 from ApoE4/4-B; n = 8 from ApoE4/4-C)). In i, values were normalized to those of E3/3 (E3/3, n = 17 biologically independent samples (n = 4 from ApoE3/3-A; n = 6 from ApoE3/3-B; n = 7 from ApoE3/3-C); E4/4, n = 25 biologically independent samples (n = 8 from ApoE4/4-A; n = 8 from ApoE4/4-B; n = 9 from ApoE4/4-C)). In j, values were normalized to those of E3/3 (E3/3, n = 23 biologically independent samples (n = 10 from ApoE3/3-A; n = 10 from ApoE3/3-B; n = 3 from ApoE3/3-C); E4/4, n = 17 biologically independent samples (n = 10 from ApoE4/4-A; n = 4 from ApoE4/4-B; n = 3 from ApoE4/4-C)). k–m, Representative images showing immunostaining of MAP2 and p-tau (using AT8 and PHF1) in E3/3 and E4/4 neuronal cultures (k) and quantification of the percentage of MAP2+ neurons that were also positive for p-tau, as detected by using AT8 (l) or PHF1 (m), in E3/3 and E4/4 neuronal cultures (E3/3, n = 12 (n = 12 fields with total of 594 MAP2+ neurons counted for AT8; n = 12 fields with total of 945 MAP2+ neurons counted for PHF1); E4/4, n = 12 (n = 12 fields with total of 526 MAP2+ neurons counted for AT8; n = 12 fields with total of 1,030 MAP2+ neurons counted for PHF1)). Scale bar, 50 μm. n, o, Apj1 (n) and Apj2 (o) levels in the medium from E3/3 and E4/4 neuronal cultures (E3/3, n = 23 biologically independent samples (n = 4 from ApoE3/3-A; n = 7 from ApoE3/3-B; n = 12 from ApoE3/3-C); E4/4, n = 21 biologically independent samples (n = 5 from ApoE4/4-A; n = 4 from ApoE4/4-B; n = 12 from ApoE4/4-C)). p–q, Representative western blot analysis (p) and quantification (q) of sAPP in the culture medium of E3/3 and E4/4 neurons. Values were normalized to those of E3/3 (E3/3, n = 15 biologically independent samples (n = 6 from ApoE3/3-A; n = 6 from ApoE3/3-B; n = 3 from ApoE3/3-C); E4/4, n = 6 biologically independent samples (n = 2 from ApoE4/4-A; n = 2 from ApoE4/4-B; n = 2 from ApoE4/4-C). r–s, Representative western blot analysis (r) and quantification (s) of APP with the 22C11 monoclonal antibody in lysates of E3/3 and E4/4 neurons. TUJ1 was used for normalization. Values were normalized to those of E3/3 (E3/3, n = 11 biologically independent samples (n = 4 from ApoE3/3-A; n = 4 from ApoE3/3-B; n = 3 from ApoE3/3-C); E4/4, n = 19 biologically independent samples (n = 3 from ApoE4/4-A; n = 8 from ApoE4/4-B; n = 8 from ApoE4/4-C)). t, Quantification of APP mRNA levels by qRT-PCR in E3/3 and E4/4 neurons. Values were normalized to those of E3/3 (E3/3, n = 12 biologically independent samples (n = 4 from ApoE3/3-A; n = 4 from ApoE3/3-B; n = 4 from ApoE3/3-C); E4/4, n = 12 biologically independent samples (n = 4 from ApoE4/4-A; n = 4 from ApoE4/4-B; n = 4 from ApoE4/4-C)). u, TUJ1/actin ratios in lysates of E3/3 and E4/4 neurons. Values were normalized to those of E3/3 (E3/3, n = 11 biologically independent samples (n = 4 from ApoE3/3-A; n = 4 from ApoE3/3-B; n = 3 from ApoE3/3-C); E4/4, n = 19 biologically independent samples (n = 3 from ApoE4/4-A; n = 8 from ApoE4/4-B; n = 8 from ApoE4/4-C)). Throughout, data are expressed as mean ± s.e.m. Differences between groups were determined with the unpaired two-sided t-test.
interneurons were 96.03% and PHF1) also revealed that 70–100% more GABAergic neurons were decreased (Fig. 4e–g), and there were fewer p-tau and of axonal degeneration, as compared to that in ApoE4/4 GABAergic neurons (Supplementary Fig. 5g). Taken together, these data demonstrate clearly that ApoE4 induces human GABAergic neuron degeneration or death in culture.

Gene editing reveals the specificity of ApoE4 in eliciting AD-related pathologies in human ApoE4/4 neurons. To determine whether the AD-related pathologies in ApoE4/4 neurons were specifically induced by ApoE4, we used gene editing to generate an isogenic ApoE3/3 hiPSC line from the ApoE4/4 hiPSC-A line (Supplementary Fig. 6a). As in the parental line, the isogenic ApoE3/3 hiPSC line (iE3/3) had normal expression of genes involved in pluripotency (Supplementary Fig. 6b,c), and it generated normal neural stem cells, which expressed SOX2, nestin and FOXG1 (Supplementary Fig. 6e,f), and developed into mature neurons (Supplementary Fig. 6g). Of note, DNA sequencing analyses revealed no off-target effects of the gene-editing process, at least on some major AD-related genes (Supplementary Table 2).

Next we assessed AD-related pathologies in neurons derived from the iE3/3 hiPSC line. The conversion from ApoE4 to ApoE3 had marked phenotypic effects. It increased the levels of full-length APOE (Fig. 4a) and decreased the levels of APOE fragments in neuronal lysates (Fig. 4b) and the secretion of Aβ40 and Aβ42 into the culture medium (Fig. 4c,d). The levels of p-tau were decreased (Fig. 4e–g), and there were fewer p-tau+ neurons (Fig. 4i–k and Supplementary Fig. 7a–c). The conversion also rescued the degeneration or loss of GABAergic neurons in ApoE4/4 hiPSC neuronal cultures, as shown by significant increases in GAD67 levels (Fig. 4c,h) and in the number of GABA+ neurons (Fig. 4l–n). The iE3/3 hiPSC cultures also had significantly fewer p-tau+ GABAergic neurons (Supplementary Fig. 7d–i) than the parental ApoE4/4 hiPSC cultures. Likewise, GABAergic neurons generated from the iE3/3-hiPSC-derived MGE cells also had decreased levels of p-tau and of axonal degeneration, as compared to the GABAergic neurons derived from the parental ApoE4/4-hiPSC-derived MGE cells (Supplementary Fig. 8). In sum, conversion of ApoE4 to ApoE3 by gene editing abolished the detrimental effects of ApoE4 and resulted in cellular phenotypes similar to those of ApoE3-expressing cells, strongly suggesting that the AD-related pathologies in ApoE4/4 neurons were induced specifically by ApoE4.

Reintroduction of different APOE isoforms into APOE-deficient human neurons reveals a gain of toxic effects from ApoE4. To further determine the detrimental effects of ApoE4 on GABAergic interneurons, we adapted a protocol of differentiating hiPSCs to medialganglionic eminence (MGE) cells, which are GABAergic progenitors, and then to mature GABAergic interneurons in culture26–28. The purity of MGE cells and of GABAergic interneurons were 96.03 ± 1.25% and 93.15 ± 0.99% (mean ± s.e.m.), respectively, as determined by anti-NKX2.1 (a MGE cell marker) and anti-GABA immunostaining and flow cytometry analysis (Supplementary Fig. 5a–d). Western blot analysis with anti-p-tau (AT8 and PHF1) revealed significantly increased p-tau levels in ApoE4/4 GABAergic neurons, as compared to that in ApoE3/3 GABAergic neurons, after 5 weeks of culture, whereas there were no significant differences after 1 and 2 weeks of culture (Fig. 3i–k). Furthermore, immunostaining with p-tau-specific antibodies (AT8 and PHF1) also revealed that 70–100% more GABAergic neurons derived from ApoE4/4 MGE cells than from ApoE3/3 MGE cells were immunopositive for p-tau (Supplementary Fig. 5e,f). Notably, p-tau accumulated in degenerating (heading) axons, as determined by anti-p-tau (PHF1) and anti-total-tau double immunostaining, in ApoE4/4 GABAergic neurons, but not in ApoE3/3 GABAergic neurons, at 5 weeks of culture (Fig. 3l,m). Similar to that observed in brains from individuals with AD, p-tau also mislocalized to dendrites of ApoE4/4 GABAergic neurons, which did not seem to happen as much in ApoE3/3 GABAergic neurons, as revealed by anti-p-tau (PHF1) and anti-MAP2 double immunostaining (Supplementary Fig. 5h,i). Quantification of cells positive for expression of both caspase-3 and GABA suggested that there was significantly (P < 0.05) more apoptotic cell death of ApoE4/4 GABAergic neurons than of ApoE3/3 GABAergic neurons (Supplementary Fig. 5g). To determine whether ApoE4 induced a loss of function or a gain of toxic effects in human neurons reveals a gain of toxic effects from ApoE4. To determine whether ApoE4 induced a loss of function or a gain of toxic effects in human neurons, we also generated a hiPSC line from a subject with an APOE deficiency (APOE−/–). The APOE−/– hiPSC line had ES-cell-like morphology (Supplementary Fig. 9a,b), expressed pluripotency marker genes (Supplementary Fig. 9c–f), and generated neural stem cells (Supplementary Fig. 9g,h) and neurons (Supplementary Fig. 9i). Of note, the levels of p-tau (Fig. 5a,b and Supplementary Fig. 10a) and the pattern of p-tau immunostaining (Supplementary Fig. 10b–d) in these neurons were similar to those in neurons derived from the iE3/3 hiPSCs. Neurons derived from the APOE−/– hiPSCs and the iE3/3 hiPSCs secreted similar levels of Aβ40 and Aβ42 into the medium (Fig. 5c,d). Furthermore, APOE−/– hiPSCs and the iE3/3 hiPSCs generated sim-
Fig. 3 | ApoE4 causes GABAergic neuron degeneration and/or loss in hiPSC-derived neuronal cultures. a,b. Double immunostaining for GABA and MAP2 in E3/3 (a) and E4/4 (b) neuronal cultures. Scale bars, 100 μm. c. Quantification of GABA+ cells per field in E3/3 and E4/4 neuronal cultures. Values were normalized to those of E3/3 (E3/3, n = 31 fields; n = 14 from ApoE3/3-A; n = 14 from ApoE3/3-B; n = 8 from ApoE3/3-C; with a total of 9,325 GABA+ neurons counted); E4/4, n = 32 fields (n = 14 from ApoE4/4-A; n = 12 from ApoE4/4-B; n = 8 from ApoE4/4-C; with a total of 3,433 GABA+ neurons counted). d,e. Representative western blot analysis (d) and quantification, relative to TUJ1 expression, (e) of GAD65 and GAD67 in lysates of E3/3 and E4/4 neurons. Values were normalized to those of E3/3 (E3/3, n = 54 biologically independent samples; E4/4, n = 35 biologically independent samples (n = 14 from ApoE4/4-A; n = 12 from ApoE4/4-B; n = 9 from ApoE4/4-C)). f–i. Images showing immunostaining for both GABA and p-tau (using AT8) in lysates of pure E3/3 and E4/4 GABAergic neurons (relative to E3/3). j,k. Quantification of the percentage of p-tau+ GABA+ cells (j) (E3/3, n = 12 fields (n = 4 from ApoE3/3-A; n = 4 from ApoE3/3-B; n = 4 from ApoE3/3-C); E4/4, n = 12 fields (n = 4 from ApoE4/4-A; n = 4 from ApoE4/4-B; n = 4 from ApoE4/4-C; with total of 144 GABA+ neurons counted). Scale bars, 25 μm. l–m. Representative western blot analysis of p-tau (using AT8 and PHF1) in lysates of pure E3/3 and E4/4 GABAergic neurons (l) and quantification of p-tau by using AT8 (j) or PHF1 (k). Values were normalized to those of E3/3 (AT8: E3/3, n = 10 biologically independent samples (n = 5 from ApoE3/3-A; n = 5 from ApoE3/3-B); E4/4, n = 10 biologically independent samples (n = 5 from ApoE4/4-A; n = 5 from ApoE4/4-B); PHF1: E3/3, n = 10 biologically independent samples (n = 5 from ApoE3/3-A; n = 5 from ApoE3/3-B); E4/4, n = 10 biologically independent samples (n = 5 from ApoE4/4-A; n = 5 from ApoE4/4-B)). l,m. Representative images showing immunostaining for p-tau (using PHF1 green) and total tau (red) in E4/4 (l) and E3/3 (m) GABAergic neurons. The experiments were repeated independently three times with similar results. Scale bars, 25 μm. Throughout, data are expressed as mean ± s.e.m. Differences between groups were determined by an unpaired two-tailed t-test (c,e,h) or a two-way ANOVA followed by Sidak’s multiple comparisons test (j,k); **P < 0.01; ***P < 0.001.

Similar numbers of GABA+ neurons (Fig. 5e–g). Thus, the phenotypes of APOE-null neurons were similar to those of ApoE3/3 neurons.

To further test the potential gain of toxicity conferred by ApoE4, we transfected neurons derived from the APOE3/3 hiPSCs with lentiviral cDNA constructs encoding ApoE3 or ApoE4. The transfected neurons expressed similar levels of ApoE3 and ApoE4 protein (Fig. 5h,i); however, the cells expressing ApoE4 had higher p-tau levels (Fig. 5h,j) and fewer GABA+ neurons (Fig. 5m,n), and they produced more Aβ42 and Aβ40 (Fig. 5o,p), than the neurons expressing ApoE3 or the controls (Fig. 5i–l,n–p). Notably, treatment of the APOE3/3–hiPSC-derived neurons with purified recombinant human ApoE3 or ApoE4 did not signifi-
The gain of toxic effects of ApoE4 is neuron specific. Although astrocytes are the major source of APOE production in brains, neurons can also produce APOE, especially when under stress or in response to injuries. We confirmed that hiPSC-derived neurons, including glutamatergic and GABAergic neurons, did not alter Aβ production (Supplementary Fig. 10e,f), suggesting that the effect of ApoE4 on Aβ production depended on the endogenous expression of APOE. Together, these findings strongly suggest that ApoE4 confers a gain of toxic effects in hiPSC-derived neurons.

Fig. 4 | AD-related pathologies in human ApoE4/4 neurons are specifically induced by ApoE4. **a**, Quantification of the full-length APOE (a) or of APOE fragments (b) in lysates of neurons derived from ApoE4/4 hiPSCs (E4/4) or from isogenic ApoE3/3 hiPSCs (iE3/3). Values were normalized to those of E4/4 (E4/4, n = 3 biologically independent samples per group; iE3/3, n = 6 biologically independent samples per group). **c, d**, Aβ42 (c) and Aβ40 (d) levels in culture medium of E4/4 or iE3/3 neurons (E4/4, n = 8 biologically independent samples per group; iE3/3, n = 6 biologically independent samples per group). **e–h**, Representative western blot analysis for p-tau (using PHF1 and AT8) and for GAD67 (e) and quantification of p-tau levels, as detected by monoclonal antibody PHF1 (f) or AT8 (g), or of GAD67 levels (h) in lysates of E4/4 and iE3/3 neurons. Values were normalized to those of E4/4 (p-tau: E4/4, n = 6 biologically independent samples per group; GAD67: E4/4, n = 6 biologically independent samples per group). **i–k**, Representative images showing immunostaining for p-tau (using AT8) and for GABA (i) and MAP2 in E4/4 (j) and iE3/3 (k) neurons and quantification of the percentage of p-tau+MAP2+ cells (E4/4, n = 8 fields, with a total of 1,533 MAP2+ neurons counted; iE3/3, n = 8 fields, with a total of 1,691 MAP2+ neurons counted) (k). Scale bars, 50 μm. **l–n**, Representative images showing immunostaining of GABA and MAP2 in E4/4 (l) and iE3/3 (m) neurons and quantification of GABA+ cells per field in the neuronal cultures (n). Scale bars, 50 μm. Throughout, data are expressed as mean ± s.e.m. Differences between groups were determined by an unpaired two-sided t-test.

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Fig. 5 | ApoE4 confers a gain of toxic effects in hiPSC-derived neurons. **a, b**, Quantification of p-tau levels, as determined by using monoclonal antibodies AT8 (**a**) and PHF1 (**b**), in lysates of neurons derived from isogenic ApoE3/3 hiPSCs (iE3/3) or ApoE4 hiPSCs (E–/–). Values were normalized to those of iE3/3 (iE3/3, n=7 biologically independent samples; E–/–, n=7 biologically independent samples). **c, d, Aβ1-42** (**c**) and **Aβ1-40** (**d**) levels in the culture medium of iE3/3 and E–/– neurons. Values were normalized to those of iE3/3 (n=7 biologically independent samples per group). **e–g**, Representative images showing immunostaining of MAP2 and GABA in iE3/3 (**e**) and E–/– (**f**) neuronal cultures and quantification of GABA+ cells per field (**g**). Values were normalized to those of iE3/3 (iE3/3, n=6 fields, with a total of 3,520 GABA+ normalized to those of iE3/3 (iE3/3, n=6 fields, with a total of 3,897 GABA+ neurons counted). Scale bar, 50 μm. **h–j**, Representative western blot analyses of APOE expression and p-tau levels (using PHF1) in lysates of APOE-null neurons that were transfected with a control lentiviral vector (+Con), an ApoE3-expressing lentiviral construct (+E3) or an ApoE4-expressing lentiviral construct (+E4) (**h**); and quantification of the full-length APOE (**i**) and quantification of the full-length APOE (**j**) in lysates from the indicated neurons. Values were normalized to those of +E3 (relative to iE3/3) or to those of +Con neurons (j) (n=3 biologically independent samples per group). **k–n**, Representative images showing immunostaining of GABA in +Con (**k**, +E3 (**l**) and +E4 (**m**) neurons and quantification of GABA+ cells per field (**n**). Values were normalized to those of +Con neurons (+Con, n=8 fields, with a total of 1,104 GABA+ neurons counted; +E3, n=8 fields, with a total of 1,234 GABA+ neurons counted; +E4, n=8 fields, with a total of 637 GABA+ neurons counted). Scale bars, 50 μm. **o, p, Aβ1-42** (**o**) and **Aβ1-40** (**p**) levels in the culture medium of +Con, +E3 and +E4 neurons. Values were normalized to those of +Con neurons (n=3 biologically independent samples per group). Throughout, data are expressed as mean±s.e.m. Differences between groups were determined by an unpaired two-sided t-test (**a–d, i**) or a one-way ANOVA followed with Tukey’s multiple-comparison test (**j–n, p**). *P<0.05; **P<0.01.*
express APOE in culture (which likely represents a ‘stress’ condition), as determined by staining for both APOE and a neuronal marker (Supplementary Fig. 11). Because our neuronal culture was highly pure, with a negligible number of astrocytes (Supplementary Fig. 12a,c,e), the neuronal APOE was unlikely taken up from the astrocyte-secreted pool.

To further determine whether human-astrocyte-derived ApoE4 also had detrimental effects in hiPSC-derived neurons, we differentiated hiPSCs with different APOE genotypes into mature astrocytes (Supplementary Fig. 12b,d,e) and collected the astrocyte-conditioned medium (ACM), which had the secreted ApoE3 or ApoE4. The neurons in culture that were derived from the APOE-hiPSCs were treated for 1 week with the ACM containing 0.35 nM (12 ng/ml) or 1.47 nM (50 ng/ml) of either ApoE3 or ApoE4 (Supplementary Fig. 13). These concentrations of APOE were similar to (0.35 nM) or 3- to 4-fold higher than (1.47 nM) those in brain interstitial fluid (ISF) of mice, which largely reflect the pool of astrocyte-secreted APOE in brains. Clearly, treatment with the ACM containing the ApoE4 at either concentrations did not significantly affect the levels of p-tau (as assessed by AT8 and PHF1) and GAD67 (GABAergic neuron marker), as well as the production and/or secretion of Aβ42 and Aβ40 in APOE-hiPSC-derived neuron culture (Supplementary Fig. 13). Thus, human-astrocyte-derived ApoE4 does not confer detrimental effects in human neurons (at least for tau phosphorylation, GABAergic neuron degeneration and Aβ production), supporting the conclusion that the gain of toxic effects of ApoE4 is neuron specific.

Detrimental effects of ApoE4 in hiPSC-derived neurons are ameliorated by a small-molecule structure corrector. We previously reported ApoE4 domain interaction (Supplementary Fig. 14a) and identified small-molecule structure correctors that render ApoE4 ApoE3-like both structurally and functionally (Supplementary Fig. 14b). In ApoE4/hiPSC-derived neurons, treatment with one of the structure correctors, PH002 (Supplementary Fig. 14b), significantly decreased ApoE4 fragment levels (Fig. 6a,b), increased GABAergic neuron numbers (Fig. 6c) and GAD67 levels (Fig. 6d,f), reduced p-tau levels (Fig. 6e), and decreased Aβ42 and Aβ40 production and/or secretion (Fig. 6g,h) in a dose-dependent manner (Fig. 6i–l). Thus, the detrimental effects of ApoE4 in hiPSC-derived neurons could be ameliorated by a small-molecule ApoE4 structure corrector. Of note, treatment of neurons could be ameliorated by a small-molecule ApoE4 structure corrector. PH002 (Supplementary Fig. 14b), PH002 (Supplementary Fig. 14c–f), indicating that the efficacy of PH002 depended on the presence of ApoE4.

Discussion

Here we assessed the phenotypes of cultured neurons that were derived from multiple hiPSC lines of different APOE genotypes, including gene-edited isogenic and APOE-deficient lines. We found that ApoE4 specifically induced AD-related pathologies, including the increase in APOE fragmentation, tau phosphorylation and Aβ production, as well as the degeneration or loss of GABAergic neurons. Notably, these AD-related pathologies were associated with a neuron-specific gain of toxic effects of ApoE4 and were ameliorated by treatment with a small-molecule structure corrector that renders ApoE4 structurally and functionally similar to ApoE3.

Lower levels of ApoE4, as compared to those of ApoE3, have been reported in the brains of mice in which the genes encoding human ApoE4 or ApoE3 were knocked in and in samples of human brain and cerebrospinal fluid. Indeed, we found that cultured human neurons have ~35% less intracellular ApoE4 than ApoE3 and secrete ~60% less ApoE4 than ApoE3. Consequently, the ratio of intracellular APOE to secreted APOE was more than 20% higher in ApoE4/4 neurons, suggesting that ApoE4 tended to be retained inside neurons. These findings might reflect a prolonged retention time of ApoE4 in the endoplasmic reticulum and the Golgi apparatus, as reported in cultured rat neuronal cells. APOE fragments accumulate in the brains of mice with neuron-specific expression of ApoE4 and in patients with AD who carried the gene encoding ApoE4. However, our study shows for the first time in cultured human neurons that ApoE4 is more susceptible than ApoE3 to proteolytic cleavage, generating more neurotoxic fragments.

In humans and transgenic mice, ApoE4 is associated with increased Aβ accumulation and amyloid plaque formation in the brain. In APOE-e3- and APOE-e4-knock-in mice expressing human APP with mutations that cause familial AD, ApoE4 seems to reduce Aβ clearance and stimulate Aβ deposition, especially during the initial seeding stage of plaque formation, in the brain without affecting Aβ production. We found that the endogenously expressed ApoE4 significantly stimulated Aβ production in cultured human neurons, likely by enhancing APP processing. Thus, ApoE4’s effects on Aβ production are species specific. Notably, conversion of ApoE4 to ApoE3 by gene editing significantly lowered Aβ production, consistent with a specific effect of ApoE4. This observation highlights the phenotypic differences between mouse and human cellular models of AD. Those differences must be recognized to understand why so many AD drugs in development failed in human clinical trials, even though they worked very well in mouse models of AD, and to develop drugs that are more likely to be effective in patients with AD.

It has recently been reported that ApoE4 enhances APP expression, which leads to increased Aβ production, in a pure excitatory human neuron culture. However, we did not observe a similar effect of ApoE4 on APP expression in a mixed human neuronal culture system. This could be due to the following differences between the two experimental systems. First, the induced excitatory neurons were generated by artificially expressing a transcription factor, NGN2, in hiPSCs, without inhibitory neurons in the culture. As reported in previous in vitro and in vivo studies, Aβ production is regulated by neuronal activity—increasing neuronal activity stimulates Aβ production and secretion. Thus, in this pure excitatory neuronal culture, in which there are no inhibitory neurons to control the activity, the Aβ production pathway and its regulation could be altered in a way that might not be physiologically relevant. In contrast, in the mixed neuronal culture the presence of inhibitory neurons balances neuronal activities, as seen under a physiological condition, making Aβ production more physiologically controlled. Second, in the pure excitatory neuron study, conditioned medium containing 294 nM (10 μg/ml) ApoE3 or ApoE4 was collected from transfected HEK293 cells and was then used to treat pure excitatory neurons in culture. This APOE concentration is >800-fold higher than the physiological concentrations of APOE (~0.35 nM) in mouse brain ISF. In addition, it has been reported that HEK293-cell-secreted APOE differs from astrocyte-secreted APOE, including their poor lipidation status, self-aggregation, and inability to bind well with the low-density-lipoprotein (LDL) receptor. In our study, however, we used astrocyte-secreted ApoE3 or ApoE4 at concentrations (0.35 or 1.47 nM) similar to those in mouse brain ISF.

ApoE4 has been associated with AD-related tau pathologies both in humans and in transgenic and gene-targeted mice. Our study shows that ApoE4, in fact, increases p-tau levels in cultured human neurons. ApoE4/4 neurons in culture were positive for multiple p-tau-specific monoclonal antibodies (e.g., AT8, AT180, AT270 and PHF1), suggesting hyperphosphorylation of tau. Furthermore, p-tau in ApoE4/4 human neurons was largely mislocalized to the soma and dendrites, as seen in brains from individuals with AD. Again, conversion of ApoE4 to ApoE3 by gene editing significantly lowered p-tau levels, consistent with a specific effect of ApoE4. It has been suggested that Aβ accumulation leads to increase in phosphorylation and accumulation of tau. However, we showed...
that treatment of ApoE4/4 human neurons with a β-secretase or a γ-secretase inhibitor drastically reduced Aβ42 and Aβ40, without altering p-tau levels at all. These findings strongly suggest that ApoE4 induces p-tau accumulation in an Aβ-independent manner in human neurons, at least in culture. In line with this conclusion, a recent study suggests that ApoE4 also has an Aβ-independent and gain-of-toxicity effect on tau-mediated neurodegeneration in a tauopathy model of mice expressing mutant human tau41.

ApoE4 also had a detrimental effect on GABAergic neurons in hiPSC-derived neuronal cultures. This was especially true in relatively pure GABAergic neuronal cultures derived from hiPSCs and was consistent with observations in brains from mice in which the gene encoding human ApoE4 was knocked in50–53. Hyperphosphorylated tau was found to be accumulated in axons of GABAergic neurons, which led to axonal degeneration, and it also mislocalized to neuronal dendrites, as seen in the brains of

Fig. 6 | The gain of toxic effects of ApoE4 in hiPSC-derived neurons can be ameliorated by a small-molecule structure corrector. a,b. Representative western blot analysis of APOE fragment levels in lysates of untreated E3/3 and E4/4 neurons, as well as in lysates of E4/4 neurons that were treated with either DMSO or the small-molecule structure corrector PH002 for 1 week. (a) and quantification of the APOE fragment levels under the different conditions (b). Values were normalized to those of untreated E4/4 neurons (E3/3, n = 5 biologically independent samples; E4/4, n = 5 biologically independent samples; E4/4 + DMSO, n = 6 biologically independent samples; E4/4 + PH002, n = 6 biologically independent samples). c. Quantification of GABA+ cells per field in E3/3, E4/4, E3/3 + DMSO, E4/4 + DMSO and E4/4 + PH002 neuronal cultures. Values were normalized to those of the E3/3 neuronal cultures (E3/3, n = 6 fields, with a total of 3,071 GABA+ neurons counted; E4/4, n = 6 fields, with a total of 1,223 GABA+ neurons counted; E3/3 + DMSO, n = 3 fields, with a total of 1,358 GABA+ neurons counted; E4/4 + DMSO, n = 3 fields, with a total of 511 GABA+ neurons counted; E4/4 + PH002, n = 3 fields, with a total of 1,077 GABA+ neurons counted). d–f. Representative western blot analyses of p-tau (using AT8) and GAD67 (d) and quantification of p-tau (e) and GAD67 (f) levels in lysates from E3/3, E4/4, E3/3 + DMSO and E4/4 + PH002 neurons. Values were normalized to those of E3/3 neurons (p-tau: E3/3, n = 6 biologically independent samples; E4/4, n = 6 biologically independent samples; E4/4 + DMSO, n = 6 biologically independent samples; E4/4 + PH002, n = 6 biologically independent samples). g,h. Aβ42 (g) and Aβ40 (h) levels in the culture medium of E3/3, E4/4, E4/4 + DMSO and E4/4 + PH002 neurons (n = 4 biologically independent samples per group for E3/3 and E4/4; n = 3 biologically independent samples per group for E4/4 + DMSO and E4/4 + PH002). i–l. Dose effects of PH002 treatment on Aβ42 (i), Aβ40 (j), p-tau (using PHF1) (k) and GAD67 (l) levels in culture medium or cell lysates from E4/4 neurons (n = 4 biologically independent samples per group). DMSO treatment was used as a control. Throughout, data are expressed as mean ± s.e.m. Differences among groups were determined by a one-way ANOVA followed by Tukey’s multiple-comparison test. *P < 0.05; **P < 0.01; ***P < 0.001; versus E4/4 or E4/4 + DMSO (b,e–f) or versus E4/4 + DMSO (i–l).
individuals with AD. These effects, too, were ApoE4 specific, as they were abolished by conversion of ApoE4 to ApoE3. GABAergic interneuron loss and neuronal hyperactivity due to loss of inhibitory tone have been observed in mice in which the gene encoding ApoE4 was knocked in. Notably, GABAergic interneuron deficits in these mice correlate with spatial learning and memory impairment, and deletion of the APOE-e4 gene in GABAergic interneurons in loxP-flanked (flxed) APOE-e4 knock-in mice restores normal learning and memory, and it rescues ApoE4-induced impairment of hippocampal network activity. Thus, ApoE4-induced GABAergic interneuron degeneration and/or dysfunction could contribute significantly to dementia in patients with AD.

A long-standing question has been whether the detrimental effects of ApoE4 in AD pathogenesis reflect a loss of normal function or a gain of toxic effects. Studies in animals suggest both possibilities, and it has been difficult to address this question in humans. Our hiPSC-derived neuron model of APOE deficiency and the complementary experiments of reintroducing ApoE4 into the APOE-null human neurons strongly argue for a gain of toxic effects of ApoE4 in AD pathogenesis, at least for its effects on tau phosphorylation, Aβ production and GABAergic neuron degeneration. This conclusion is consistent with the report of an APOE-deficient patient in which it was detailed that neurocognitive studies failed to demonstrate any deficits. This information has critical implications for developing drugs targeting ApoE4—reducing ApoE4 expression might be an attractive strategy.

Finally, our findings in hiPSC-derived neurons provide a proof of concept that correcting the pathogenic conformation of ApoE4 is a viable therapeutic approach for ApoE4-related AD. Treatment of ApoE4/4 hiPSC-derived neurons with the ApoE4 structure corrector PH002 ameliorated the detrimental effects of ApoE4 on tau phosphorylation, Aβ production and GABAergic neuron degeneration in a dose-dependent manner. These findings warrant further development of ApoE4 structure correctors and, ultimately, testing in clinical trials.

Methods
Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41591-018-0004-z.

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Author contributions

C.W. and Y.H. designed and coordinated the study; C.W. performed most of the studies and data analyses; R.N. performed all MGE studies and related data analysis; Q.X. and D.W. helped with off-target analysis of gene editing and work with APOE-expressing lentiviral vectors and performed some western blots; D.W. designed and prepared the APOE-ε3 donor DNA for gene editing; M.E.B. helped with genetic screening; G.L. helped with miPSC studies; Z.A.M., B.L.M. and M.J.M. provided the APOE-null human skin biopsy; and C.W. and Y.H. wrote the manuscript.

Competing interests

Y.H. is a cofounder and scientific advisory board member of E-Scape Bio, Inc.

Additional information

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Generating and characterizing isogenic ApoE3/3 hiPSC lines by zinc-finger nuclease (ZFN)-mediated gene editing. To generate isogenic ApoE3/3 hiPSC lines, we dissociated the parental ApoE4/4 hiPSCs with Accutase (Millipore) and placed nuclease (ZFN)-mediated gene editing.

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MGE-cell-derived GABAergic neurons, were fixed in 4% paraformaldehyde and stained with primary antibodies to the following proteins: nestin (MAB5326, EMD Millipore), PAX6 (Developmental Studies Hybridoma Bank), FOXG1 (sc-48788, Santa Cruz Biotechnology), MAP2 (MAB3418, AB5622, EMD Millipore), TUJ1 (MAB1637, EMD Millipore; MRB-435P, Covance), APOE (178479, Calbiochem), PHF1 (gift from Peter Davies), AT8 (MN1020, Thermo Fisher Scientific), AT180 (MN1040, Thermo Fisher Scientific), TAUS (778001, EMD Millipore), total Tau (T6402, Sigma), NNX2.1 (sc-13040, Santa Cruz Biotechnology), GABA (A2052, Sigma) and cleaved caspase-3 (D3E9, Cell Signaling Technology). The secondary antibodies were IgG conjugated to Alexa Fluor 488 or Alexa Fluor 594 (against rabbit, mouse or goat IgG (catalog no. A-21206, A-21207, A-21202, A-21203, A-11058, A-11055, from Life Technologies, a Thermo Fisher Scientific Brand)). Nuclei were stained with DAPI. Images were taken with a Leica epifluorescence microscope, a Keyence BZ-9000E fluorescence microscope or a Leica confocal imaging system.

Western blot analysis of hiPSC-derived neurons. hiPSC-derived neurons, including MGE-cell-derived GABAergic neurons, in culture were washed with PBS and collected in the presence of a high-detergent buffer consisting of 50 mM Tris, 150 mM sodium chloride, 2% Nonidet P-40, 1% sodium deoxycholate and 4% sodium dodecyl sulfate, and supplemented with complete protease inhibitor cocktail (Roche), phosphatase inhibitor cocktail 1 (P89850, Sigma) and phosphatase inhibitor cocktail 2 (P5726, Sigma). The total protein in cell lysates was quantified with the BCA protein assay kit (cat. no. 23227, Pierce). In some experiments, the medium was also collected for analysis of APOE and soluble APP levels by western blot. The samples were separated by SDS-PAGE on 4–20% Bis-Tris polyacrylamide gels (Life Technologies) or Criterion XT 4–12% Bis-Tris gels (Bio-Rad) and transferred to nitrocellulose membranes (Bio-Rad). The membranes were then blocked in Odyssey Blocking Buffer (PBS) (LI-COR) and probed with primary antibodies to the following proteins: APOE (178479, Calbiochem, 1:4,000), actin (A5060, Sigma, 1:2,000), TUJ1 (MAB1637, EMD Millipore; MRB-435P, Covance, 1:1,000,000), GAP (20334, Dako, 1:3,000), PHF1 (gift from Peter Davies, 1:2,000), AT8 (MN1020, Thermo Fisher Scientific, 1:2,000), AT180 (MN1040, Thermo Fisher Scientific, 1:2,000), AT270 (MN1050, Thermo Fisher Scientific, 1:2,000), TAUS (778001, EMD Millipore, 1:5,000), soluble APP-ß (SIG-39138, Covance, 1:1,000), 22C11 (MAB3484, EMD Millipore, 1:3,000), GAD65 and GAD67 (AB1511, EMD Millipore, 1:1,000) and GAD67 (MAB5406, EMD Millipore, 1:1,000). The secondary antibodies were IgG labeled with IRDye 800 or IRDye 680 (LI-COR), including donkey anti-rabbit 680 (cat. no. 926-68023, 1:10,000); donkey anti-mouse 680 (cat. no. 926-68072, 1:10,000); donkey anti-goat 680 (cat. no. 926-68024, 1:10,000); donkey anti-mouse IgG 800 (cat. no. 926-32212, 1:10,000); donkey anti-goat 800 (cat. no. 926-32214, 1:10,000) and donkey anti-rabbit 800 (cat. no. 926-32213, 1:10,000). The blotted membranes were scanned with an Odyssey CLX Imaging System (LI-COR). Signals were analyzed with Image Studio Lite 4.0 (LI-COR). Unprocessed original scans of blots are shown in Supplementary Fig. 15.

Measurement of Aβ in the medium from hiPSC- and miPSC-derived neuron cultures. Medium from neuronal cultures was harvested and stored at −80°C. Cell lysates were collected for normalization with cellular protein amounts and analysis of p-tau levels by western blot analysis. Human and mouse Aβ peptides were measured with MSD Human V-PLEX Aβ Peptide Panel 1 (6E10) Kit (K15200E, Meso Scale Discovery) and Thermo Fisher Mouse Aβ Peptide ELISA Kits (KMB3481 and KMB3441, Thermo Fisher Scientific), respectively, according to the manufacturer’s instructions. For human Aβ measurement, the plates were read with a Sector Imager 2400, and the data were acquired and analyzed with Discovery Workbench software. For mouse Aβ measurement, the plates were read with a FlexStation-III, and the data were analyzed with Prism-6 software. For experiments with secretase inhibitors, neuronal cultures were treated with a γ-secretase inhibitor (compound E, Cspd-E; at a final concentration of 200 nM), a β-secretase inhibitor (OM992, final concentration of 750 nM), or DMSO (vehicle) as described19. All inhibitors were from EMD Millipore.

Astrocyte differentiation of hiPSCs and treatment of neurons from APOE-deficient hiPSCs with astrocyte-conditioned medium. hiPSCs were differentiated into astrocyte progenitors and astrocytes as previously reported with some modifications25. The astrocyte progenitors were generated using the procedure as described for neuronal differentiation of hiPSCs. Instead, these progenitor spheres were kept in suspension for further expansion in the presence of 10 ng/ml bFGF (PeproTech) and 10 ng/ml EGF (PeproTech), and they were passaged by disaggregation into small clusters with a Pasteur pipette for ~90 d. For astroglial differentiation, progenitor spheres were dissociated with Accutase to single cells and seeded onto plates coated with PLL and laminin in neural differentiation medium without bFGF and EGF. The ACM was collected from the astrocyte culture, and the APOE levels in the medium were measured with the Human APOE (AD2) ELISA Kit (cat. no. EHAPOE, Thermo Scientific). The amount of ACM for treatment of the neurons derived from APOE-deficient hiPSCs was adjusted to the same APOE concentration (0.35 nM or 1.47 nM), based on APOE levels in the ACM collected from APOE3/3 or APOE4/4 astrocyte culture. After ACM treatment for 1 week, the medium and cell lysates of neurons derived from APOE-deficient hiPSCs were collected and further analyzed.

Treatment of hiPSC-derived neurons with a small-molecule structure corrector. Neuronal cultures derived from hiPSC lines were treated with a small-molecule structure corrector, PH002 (final concentration, 100 nM; prepared in DMSO) as described27,28. After 7 d of treatment, culture medium was collected for measurement of Aβ levels and cells were homogenized for western blot analyses. For the dose-effect experiments, different doses of PH002 (i.e., 0 nM, 10 nM, 30 nM and 100 nM) were used to treat the cultured neurons for 1 week.

Treatment of hiPSC-derived neurons with recombinant human APOE. Neuronal cultures derived from APOE−/− hiPSCs were treated with purified recombinant human APOE3 or APOE4 (final concentration, 220 nM or 7.5 μg/ml). After 2 d of treatment with recombinant human APOE, culture medium was collected for the measurement of Aβ and Aβ42, and cells were homogenized for protein normalization and western blot analysis.

Statistical analyses. Power analysis of a pilot cohort (n = 3) showed that a sample size of three would be sufficient to show genotype effects; all groups had n ≥ 3. Values are expressed as mean ± s.e.m. All n numbers represent the numbers of biological replicates or fields used for image analysis. The distribution of data was assessed with the Shapiro–Wilk normality test; most of the data were normally distributed. The variances between groups were similar as shown by Bartlett’s test. Statistical significance was calculated with GraphPad Prism (GraphPad Software). Differences between groups were determined with the unpaired two-sided t-test. For multiple comparisons, ANOVA with Tukey’s post-test was used. P < 0.05 was considered to be significant. Researchers were not blinded to the genotypes while performing the experiments.

Compliance with relevant ethical regulations and animal use guidelines. All experimental and animal protocols and procedures were in compliance with the university and institutional ethical regulations and animal use guidelines.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All of the data generated or analyzed during this study are included in this published article (and its Supplementary Information file) and are available at the corresponding author’s lab.

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Experimental design

1. Sample size
   Describe how sample size was determined.
   - Power analysis of a pilot cohort (n = 3) showed that a sample size of three would be sufficient to show genotype effects; all groups have n ≥ 3.

2. Data exclusions
   Describe any data exclusions.
   - No data were excluded.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   - All attempts of replication were successful.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   - Samples were randomly allocated to groups.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - Investigators were not blinded to apoE genotypes during data collection and/or analysis. Eight hiPSC lines and four miPSC lines with different apoE genotypes were used in this study. Multiple iPSC lines were used in each experiment for differentiating into different types of neurons, and multiple investigators were doing similar experiments. Blinding could easily bring in confusions/mistakes across experiments and among investigators.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   | n/a | Confirmed |
   |-----|-----------|
   | ☐   | ☒         |
   | ☐   | ☐         |
   | ☐   | ☐         |
   | ☐   | ☐         |
   | ☐   | ☐         |
   | ☐   | ☐         |
   | ☐   | ☐         |

   - The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   - A statement indicating how many times each experiment was replicated
   - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   - A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   - The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
   - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   - Clearly defined error bars

See the web collection on [statistics for biologists](#) for further resources and guidance.
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Prism 7, BD FACS Diva, and FlowJo 10.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials used are readily available from the authors.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Primary antibodies used in this study are: apoE (178479, Calbiochem), actin (A5060, Sigma), Tuj1 (MAB1637, EMD Millipore; MRB-435P, Covance), GFAP (Z0334, Dako), PHF1 (gift from Peter Davies), AT8 (MN1020, Thermo Fisher Scientific), AT180 (MN1040, Thermo Fisher Scientific), AT270 (MN1050, Thermo Fisher Scientific), Tau-5 (S77801, EMD Millipore), soluble APP-ß (SIG-39138, Covance), 22C11 (MAB348, EMD Millipore), GAD65/67 (AB1511, EMD Millipore), GAD67 (MAB5406, EMD Millipore), Nanog (ab21624, Abcam), human nuclei (MAB1281, EMD Millipore), Sox2 (sc-17320, Santa Cruz Biotechnology), Oct-3/4(sc-5279, Santa Cruz Biotechnology), SSEA4 (ab16287, Abcam), TRA-1-81 (MAB4381, EMD Millipore), TRA-1-60 (MAB4360, EMD Millipore), nestin (MAB5326, EMD Millipore), Pax6 (Developmental Studies Hybridoma Bank), FoxG1 (sc-48788, Santa Cruz Biotechnology), MAP2 (MAB3418, AB5622, EMD Millipore), Tuj1 (MAB1637, EMD Millipore; MRB-435P, Covance), NNX2.1 (sc-13040, Santa Cruz Biotechnology), GABA (A2052, Sigma), and cleaved Caspase-3 (D3E9, Cell Signaling Technology). The secondary antibodies were IgG conjugated with Alexa Fluor 488 or Alexa Fluor 594 (Life Technologies) as well as IgG labeled with IRDye 800 or IRDye 680 (LI-COR).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Eight human iPSC lines were generated and used in this study, including ApoE3/3-A, ApoE3/3-B, ApoE3/3-C, ApoE4/4-A, ApoE4/4-B, Isogenic ApoE3/3, and ApoE-/- . See Supplementary Table 1 for details.

b. Describe the method of cell line authentication used.

All human iPSC lines were characterized for normal pluripotency gene expression, apoE genotypes, karyotypes, and capability of differentiating into neural stem cells, different types of neurons, and astrocytes in culture.

c. Report whether the cell lines were tested for mycoplasma contamination.

All hiPSC lines were tested negative for mycoplasma.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cells lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Two mouse iPSC lines (apoE3/3-miPSC and apoE4/4-miPSC) were generated from MEF cells of apoE3-KI and apoE4-KI mice on a C57/BL6 genetic background. The apoE3/3-miPSC and apoE4/4-miPSC were characterized for normal pluripotency gene expression, apoE genotypes, karyotypes, and capability of differentiating into neural stem cells, different types of neurons, and astrocytes in culture. Immunodeficient mice were used for teratoma formation test of hiPSC lines.
12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Human skin fibroblasts were used to generated iPSC lines with different apoE genotypes. The general information of subjects from whom skin fibroblasts were collected are presented in Supplementary Table 1.
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

▶ Data presentation

For all flow cytometry data, confirm that:

☐ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

▶ Methodological details

5. Describe the sample preparation. hiPSC-derived MGE progenitor cells were stained with anti-NKX2.1 (1:250) and anti-GABA (1:1000).

6. Identify the instrument used for data collection. BD LSRII Flow Cytometer.

7. Describe the software used to collect and analyze the flow cytometry data. BD FACS Diva was used for data collection, and FlowJo Version 10 was used for data analysis.

8. Describe the abundance of the relevant cell populations within post-sort fractions. Over 95% NKX2.1+ cell population and over 90% GABA+ cell population.

9. Describe the gating strategy used. FSC/SSC gates were applied to specify NKX2.1+ and GABA+ cell populations. Cells stained with the secondary antibody alone were used as a negative control to set the positive/negative gates.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☐