APP-Induced Patterned Neurodegeneration Is Exacerbated by APOE4 in Caenorhabditis elegans

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ABSTRACT Genetic and epidemiological studies have found that variations in the amyloid precursor protein (APP) and the apolipoprotein E (APOE) genes represent major modifiers of the progressive neurodegeneration in Alzheimer’s disease (AD). An extra copy of or gain-of-function mutations in APP correlate with early onset AD. Compared to the other variants (APOE2 and APOE3), the ε4 allele of APOE (APOE4) hastens and exacerbates early and late onset forms of AD. Convenient in vivo models to study how APP and APOE4 interact at the cellular and molecular level to influence neurodegeneration are lacking. Here, we show that the nematode C. elegans can model important aspects of AD including age-related, patterned neurodegeneration that is exacerbated by APOE4. Specifically, we found that APOE4, but not APOE3, acts with APP to hasten and expand the pattern of cholinergic neurodegeneration caused by APP. Molecular mechanisms underlying how APP and APOE4 synergize to kill some neurons while leaving others unaffected may be uncovered using this convenient worm model of neurodegeneration.

Alzheimer’s disease (AD) is a progressive neurodegenerative disease which cannot be prevented, cured, or decelerated. About 10–20% of people older than 45 in the US are at risk of developing AD in their life (Chêne et al. 2015). Genetic and epidemiological studies have associated variation in several genes strongly to AD. Gain-of-function mutations in amyloid precursor protein (APP) or in presenilin genes related with processing APP are associated to early-onset AD (Goate et al. 1991). Possession of extra wild-type copies of APP, such as found in Down Syndrome (DS) or in rare individuals without DS, is also associated to early-onset AD (Prasher et al. 1998; Cabrejo et al. 2006). Additionally, a distinct variant of APP appears protective against AD (Jonsson et al. 2012). The strongest genetic risk factor for the more common late-onset AD is the ε4 allele of apolipoprotein E (APOE4) (Corder et al. 1993; Liu et al. 2013). Over 65–80% of AD patients carry an APOE4 allele (Saunders et al. 1993; Farrer et al. 1997). The lifetime risk for AD in people homozygous for APOE4 is extremely high (91%) relative to those with the more common APOE3 allele (20%) or the less common APOE2 allele (2.8–4.5%) (Corder et al. 1993; Corder et al. 1994). APOE4 is associated with more pronounced neurodegeneration (Holtzman et al. 2012); consequently, each copy of APOE4 predicts shorter lifespan relative to the more common APOE3 variant (Liu et al. 2013). Even for early-onset AD, the APOE4 variant is associated with earlier onset and harsher severity, including elevating levels of the amyloid-β1-40 peptide and faster spread of neurodegeneration in the case of Down syndrome (Patel et al. 2011; Head et al. 2011). This suggests that APP and APOE4 may contribute an additive or synergistic risk for AD onset and progression.

Despite the substantial influence of APOE4 on the progression of AD, how APOE4 modulates the molecular mechanisms underlying neurodegeneration remains elusive. Study of genetically modified mice has contributed to our understanding of the susceptibility to AD and other neurodegenerative diseases conferred by mutations or variants in APP, APOE, and related genes (Di Battista et al. 2016). However, progress toward understanding the mechanistic underpinnings of some AD-related pathologies, particularly neurodegeneration, has been slower (LaFerla and Green 2012). Many mouse models
of AD do not show neurodegeneration that is central to the human condition (Jankowsky and Zheng 2017). Moreover, it is more expensive and time-consuming to study age-related diseases in mice.

To surmount some of these limitations, we explored whether APOE could be studied in the context of APP-related neurodegeneration with the genetically tractable model nematode, Caenorhabditis elegans (Yi et al. 2017). This minimal in vivo animal model offers several advantages for basic and applied research for AD. First, worms mature rapidly to adulthood, reaching “middle-age” of adulthood in only 5 days as reproduction declines. Despite this compressed lifespan, C. elegans shares many of the genetic, cellular, and molecular processes of aging with humans and mice (Arey and Murphy 2017). Thus, age-dependent processes can be studied within the span of one week with C. elegans. Second, forward and reverse genetics as well as transgenesis studies are extremely rapid with C. elegans. Third, every cell in the tiny (1 mm) worm is identified, including its 302 neurons, which can be examined individually using fluorescent reporters in the living, transparent worm. The function and health of even single identified neurons can be further probed by quantifying simple behaviors such as egg laying and locomotion. Fourth, several models of neurodegeneration related to AD have been generated using C. elegans (Griffin et al. 2017). We recently described a transgenic worm strain that expresses a single copy of human APP and displays degeneration of a subset of cholinergic neurons in middle-age adulthood (Yi et al. 2017; Mondal et al. 2018). Using this APP-expressing strain, we discovered small molecules that both prevent degeneration of neurons in worm via a conserved signaling pathway and boost cognition in a mouse model of AD (Yi et al. 2017). Thus, although the simple worm cannot be used to study the important decline in cognition and memory as in mouse models, it can be used to directly study degeneration of identified neurons and to indirectly predict effective pharmacological approaches in rodent models of AD.

In this study, we tested how expressing variants of human APOE altered health and function of neurons in our worm model of APP-related neurodegeneration. We observed several key characteristics associated with AD. First, APOE4 caused a higher level of neurodegeneration than APOE3 and, APOE4 but not APOE3 acted in concert with APP to further increase neurodegeneration. This retains the variant-specific effect of APOE that is well-documented in humans (Corder et al. 1993). Second, APOE4 accelerated neurodegeneration in C. elegans, lowering the age of onset from late to early adulthood. This mirrors the expedited onset of degeneration in humans who carry APOE4 (Holtzman et al. 2012). Lastly, despite deliberate expression of APP and APOE4 throughout the nervous system, the pattern of neurodegeneration in the worm model was restricted. This result is similar to the initially restricted degeneration of specific brain regions, i.e., the hippocampus and the entorhinal cortex, despite the near-ubiquitous expression of APP and APOE in the brain seen in AD patients (Holtzman et al. 2012). Because APOE variant expression influences C. elegans neurodegeneration in a manner similar to several key aspects found in human AD, our worm model may facilitate both the discovery of molecular pathways involved in the development of AD as well as novel drugs for the treatment of AD.

MATERIALS AND METHODS

Plasmid and transgenic animals

The plasmid constructs for human APP or APOE transgenes under a pan-neuronal promoter (prab-3::APP::mCherry::UNC-54 UTR; prab-3::APOE4::UNC54 UTR; prab-3::APOE3::UNC54 UTR) were generated using Multi-site Gateway technology (Invitrogen, Carlsbad, CA). Middle entry clones were made using the MultiSite Gateway Three-Fragment Vector Construction Kit (Invitrogen). cDNA for human APP695 or APOE variants (APOE3 or APOE4) was amplified using the High Fidelity Phusion Polymerase (NEB) and recombined into pDONR221 using the BP clonase (Invitrogen). To generate the expression clones, the middle entry clones were previously fused into pCFJ130 along with the 5′-rab-3-pan-neuronal promoter and 3′-unc-54 UTR entry clones (Yi et al. 2017) using the LR clonase II (Invitrogen). Resulting plasmids were verified by sequencing. The construct for the GFP reporter for HSN neurons was made by a PCR fusion of a ~2 kb region upstream of tph-1 amplified from genomic DNA and GFP::unc-54 UTR amplified from pPD95.75. The construct for the GFP reporter for VA and VB neurons was made by a PCR fusion of a ~1.8 kb region upstream of del-1 amplified from genomic DNA and GFP::unc-54 UTR amplified from pPD95.75. The constructs to express APOE4 in the intestine and coelomocytes were made via NEBuilder HiFi DNA Assembly. The promoter of fat-7 (~1.2 kb upstream of the gene) was amplified from genomic DNA while the promoter of unc-122 (~300 bp) was amplified from pCFJ68.

Single copy transgenic strains for human APP and C. elegans apl-1 were generated via MosSCI methods as described by Frokjaer-Jensen et al. (2008). A single copy of human APP or apl-1 under a rab-3 promoter was inserted into the chromosome II. The EG4322 strain was selected for the insertion of the construct at ttt5605 II. Insertion of APP or apl-1 was confirmed by PCR and sequencing of the modified region on the chromosome. Extrachromosomal array strains in this study were made by injecting expression plasmids and co-injection markers (1.2 ng/μl for pCF190, pmyo-2:mCherry, and 30 ng/μl for pCFJ68, punc-122::GFP, ptph-1::GFP) described by (Mello et al. 1991). The extrachromosomal array for ptph-1::GFP was subsequently integrated using standard UV-integration techniques and outcrossed 6 times; this JPS617 strain served as the wild-type (WT) background for all other strains.

We made three attempts to integrate the extrachromosomal array expressing APOE4 using traditional UV-integration techniques. However, the attempts were unsuccessful, at least in part due to a low survival rate after UV exposure. Instead, the array [prab-3::APOE4::UNC54 UTR; pmyo-2::mCherry] was integrated using CRISPR-cas9-based methods modified from Yoshina et al. (2015). Briefly, two plasmids containing the cas9 gene (pDD162) and a guide RNA targeting either the genomic region of integration (LG X:22.84, a location previously used for MosSCI insertions (Frokjaer-Jensen et al. 2008)) or a region on the extrachromosomal array (β-lactamase gene), and a co-injection marker (pCF104, pmyo-2::mCherry) were injected into worms carrying the APOE4 array. The sequences for guide RNAs used in the integration are as follow, 5′-TTAATAGCTGGATGAGG3′ (β-lactamase gene), 5′-ATGTTGATAAGTCAACAAGAC3′ and 5′-TTATGTAGTCTTTCTCAGTG3′ (X:22.84). The guide RNAs were made according to Ward (2015). After integration of the linearized array, we chose a strain with a similar intensity of red pharynx as that seen prior to integration. The same method was employed by SunyBiotech (Fuzhou City, China) to generate an integrated APOE3 strain PHX2443 [prab-3::APOE3::unc-54 UTR, prab-3::mCherry::unc-54 UTR].

The integrated APOE4 and APOE3 lines were crossed to JPS617 to make the APOE4 (JPS844) and APOE3 (JPS1312) strains with GFP-labeled VC and HSN neurons. JPS844 was then crossed to JPS809 to make APOE4+APP (JPS845) strains with GFP-labeled VC and HSN neurons. Subsequently, JPS845 was crossed with MTS802 (ced-3(n1286) IV) to obtain an APOE4+APP strain with a ced-3 null
Bag of worms assay
Worms were maintained at 20° as previously described by Brenner (1974). A total of 50 worms were age-synchronized by picking L4-stage larvae onto NGM agar plates seeded with OP50 bacteria. Adult worms were carefully examined every day for up to four days (Day 1-4 of adulthood) for the bag-of-worms (BW) phenotype indicated by the presence of hatched larvae within their body. Groups of healthy worms with no hatched larvae were transferred to a freshly seeded plate each day. The reported percentage BW represents the number of BW observed over the period of observation (2-4 days) relative to the total number of assayed worms. The assay was repeated 3-4 times for each genotype at different times to control for conditions that may vary by date. BW phenotype was quantified as a percentage ± 95% confidence interval. Group comparisons were made with planned χ²-tests.

Scoring of neurodegeneration
Worms were maintained at 20° and synchronized by picking L4-stage larvae onto NGM plates seeded with OP50 bacteria and containing 0.35 mM 5-fluoro-2-deoxyuridine-5'-phosphate (FUDR) to sterilize adults and prevent non-specific consequences of larvae hatching inside adults. Prior to scoring neuron health, worms were stained by transferring them to an unseeded plate until they left no residual tracks of bacteria, a process that took ~10 min. Worms were mounted on 2% agarose pads, immobilized with 30-mM sodium azide and imaged on an Olympus IX51 inverted microscope equipped with an X-Cite FIRE LED Illuminator (Excitella Technologies Corp.) and an Olympus UPlanFL N 40X/0.75 NA objective. Epifluorescence images were taken with a Retiga 2000R CCD camera (QImaging) and QCapture Pro 6.0 software. For Figures 2C and 3C worms were identically maintained, but immobilized mechanically without azide on a microfluidic vivoChip (Newormics, Austin, TX) in filtered M9 buffer and imaged at 40X. For strains with no or extrachromosomal expression of APOE (Figure 1), HSN neurons were considered healthy when the scored HSN cell body was clearly present and the processes were intact. HSNs were considered degenerated when the scored neuron was absent and/or displayed significant morphological abnormalities (e.g., blebbing, beading and absence of processes). Examples of degenerated HSN neurons following these criteria can be found in Fig. S1. We observed dimmer GFP expression in the HSNs of integrated APOE4 strains (Figure 3) making it more difficult to visualize the HSN processes. Thus, HSN health in these strains (JPS844 and JPS845) was based exclusively on the presence or absence of the HSN cell-bodies. That is, HSNs were considered to be degenerated when the scored HSN neurons were absent. Neuron health was scored with the observer blind to genotype. An average of 50 worms were scored per strain for each trial. Scoring was repeated 3-4 times for each genotype and expressed as percent HSN degeneration ± 95% confidence interval. Group comparisons were made with planned χ²-tests.

Locomotion assay
Worms were cleaned of bacteria as described above. Approximately 15 worms were moved into a 5/8-inch-diameter copper ring sealed on a standard unseeded NGM agar plate. Movement was recorded for 2 min at 2 frames/sec with a FLEA digital camera (Point Gray, Richmond, BC, Canada). The distance that the worms crawled during 1 min was measured using a semi-automated procedure in ImagePro Plus (Media Cybernetics, Rockville, MD) to objectively calculate overall speed of individual worms. Speed for each group was quantified as group mean ± SEM. Group comparisons were made with planned Student’s t-tests.

Semi-quantitative PCR
Five worms of each genotype (WT, integrated APOE3, integrated APOE4, extrachromosomal APOE3, extrachromosomal APOE4) were lysed and digested in buffer + proteinase K for one hour. PCR was performed using 2 μl of template for each reaction with 2x DreamTaq Green Master Mix (FisherSci) and primers: forward 5'-CGGACATG-GAGGACGTG-3', reverse 5'-AGCCGTTACGGAGCTTG-3'. Samples were run on a 1.5% agarose gel at 150V for 40min and imaged on a BioRad UV Imager. Images were exported into Image Lab (BioRad), where lanes and bands were manually selected, and band intensity was measured. Adjusted band intensities were exported into Prism 8 (GraphPad), and data are presented as mean ± SEM. Three independent biological replicates were measured for each genomic preparation.

RT-qPCR
Worms were washed from near-starved plates (3 plates for WT and integrated, 9 plates for extrachromosomal) and pelleted. Each pellet was flash frozen in liquid nitrogen, thawed on ice, and vortexed 6 times. The pellet was further disrupted by needle and syringe in RNA extraction buffer. RNA was isolated (Zymo Quick-RNA Mini-prep; QIAGEN RNasy Mini Kit), and 1ug was reverse transcribed into cDNA with the Protoscript II cDNA synthesis kit (NEB). qPCR was performed using SYBR Green (FisherSci) on a Roche 480 Lightcycler (Roche) using primers: APOE forward 5'-CCTGACGAGGT-GAAGAGCA-3', reverse 5'-CTCGAACGCCTCTGAGGC-3' and tba-1 forward 5'-ATCTCTGTGCAAGGCTTCATC-3', reverse 5'-GTCAACAGGGCAAAGGCG-3'. Ct values were exported and analyzed using the ΔCt method. No values were detected for no template and no RT controls. Data represents the mean of two biological replicates with three technical replicates each. The housekeeping gene tba-1 was used as a control, and relative expression was normalized to this reference gene.

Data availability
Strains and plasmids are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. Table S1 contains genotypes for each strain used in this study. Supplemental material available at figshare: https://doi.org/10.25387/g3.12493769.

RESULTS
We previously developed a C. elegans model of neurodegeneration that expressed a single-copy of human APP and showed age-related degeneration of a subset of cholinergic neurons (Yi et al. 2017; Mondal et al. 2018). Specifically, six VC-class neurons located in the middle of the ventral nerve cord begin to die as the worm advances past Day 3 of adulthood. To investigate whether variants in APP modify neurodegeneration phenotypes in our APP worm model, we generated strains that expressed human APP43 or APOE3, with or without human APP. Both APP and APOE transgenes were expressed throughout the nervous system using the conventional pan-neuronal rab-3 promoter to mimic widespread brain expression.
in mammals (Stefanakis et al. 2015). APP was tagged with mCherry at the C-terminus and was found to be expressed throughout the nervous system of the worm (Fig. S2). APOE expression was verified via RT-qPCR (Fig. S3). APP was integrated while initially APOE transgenes were expressed with extrachromosomal arrays. Comparisons were made between these strains and a wild-type background (WT). While studies have shown a neuroprotective effect of APOE2 in humans and other models (Conejero-Goldberg et al. 2014; Wu and Zhao 2016; Griffin et al. 2019; Reiman et al. 2020), our study focused only on the effects of human APOE3 or APOE4 expression.

Despite the degeneration of VC neurons in our APP-expressing strain, the expression of human APP alone did not confer any obvious behavioral defects (Mondal et al. 2018). To test if APOE variants caused gross phenotypic differences that may be indicative of neuronal degeneration or dysfunction beyond that observed in our strain only expressing APP, we evaluated the behavior of APOE3- or APOE4-expressing strains. We observed an increase in a behavioral phenotype called ‘bag-of-worms’ (BW) in strains expressing APOE4 but not APOE3 (Figure 1A). C. elegans normally lays eggs that hatch outside the parent. However, eggs are retained inside the parent under stressful conditions of starvation, or when the neurons that mediate egg-laying become dysfunctional or die (Schafer 2005). If eggs are retained too long in the worm, they hatch and fill the parent with writhing larvae (Angelo and Van Gilst 2009). This BW phenotype can be unambiguously detected at low power via a stereomicroscope and has proven convenient to study many biological processes (e.g., Trent et al. 1983; Conradt and Horvitz 1998).

When we quantified the amount of BW by observing worms for 3 days throughout the C. elegans reproductive period, we found that WT worms displayed a low level of BW as expected. The incidence of BW was not raised in strains expressing APP or APOE3. However, APOE4-expressing strains had a significantly higher percentage of BW than WT, APP-, and APOE3-expressing strains (Figure 1A). Worms expressing both APOE4 and APP (APOE4+APP) displayed an even higher incidence of BW than worms expressing either APOE4 or APP alone (Figure 1A). In contrast, the percentage BW in strains expressing APOE3+APP remained low and was not significantly higher than that found in strains expressing either gene individually (Figure 1A). The lack of effect of APOE3 could not be easily explained by a lower number of APOE3 transgene copies on the extrachromosomal vs. APOE4 copies because an independent strain made with a higher (2x) transformative dose (exAPOE3h) showed a low level of BW indistinguishable from the APP strain (Figure 1A).

Because the APOE4-expressing strains displayed the BW phenotype even when well-fed, this suggested that one or more of the egg-laying neurons were dysfunctional or dying. Egg-laying is controlled primarily by the left-right pair of HSN neurons and to a much lesser extent by VC4 and VC5 neurons (Schafer 2005). The HSN and VC4 and VC5 cholinergic neurons connect reciprocally, as well as to egg-laying muscles by synapses (Altun and Hall 2018). We checked the health of these neurons by directly visualizing them with a fluorescent reporter through the worm’s transparent body (see Methods). We considered HSN neurons “degenerated” when the neuron was absent and/or displayed significant morphological abnormalities (e.g., blebbing, beading and absence of processes) (see Figures 1B and S1). Additionally, we saw clear degeneration of HSN neurons in APOE4-expressing strains with or without co-expression of APP. Consistent with our observed BW behavior, the incidence of HSN neuron degeneration was significantly higher in APOE4+APP worms than those expressing either APP or APOE4 alone (Figure 1C).
Intriguingly, although expression of APOE4 increased degeneration of HSN neurons, the expression of APOE3 with or without APP co-expression did not increase HSN degeneration relative to WT (Figure 1C). Even when the APOE3 transgene was transformed at double the concentration of the APOE4 transgene and combined with APP expression, the resulting strains exhibited a percentage BW and HSN neurodegeneration that was not significantly higher than WT (Figure 1A,C).

Reminiscent to our previous findings with APP alone, APOE4-induced neurodegeneration increased in an age-dependent manner, from few at Day 1 of adulthood to many by Day 3 of adulthood (Yi et al. 2017; Mondal et al. 2018). As BW behavior results in death of the adult, our sample size was too diminished to study older (> day 4) adults.

Others have found that APOE4 may worsen outcomes in AD patients and models via several mechanisms, including some that depend on APP. For instance, APOE4 exacerbates Aβ deposition in transgenic models, though a direct interaction between APOE4 and APP has not been shown (Ye et al. 2005; Bales et al. 2009; Bien-Ly et al. 2011; Kim et al. 2011; Liu et al. 2017). Although C. elegans does not have a clear ortholog of APP, it has a related gene, apl-1, with 71% sequence similarity to the intracellular domain of APP (Figure 2A; Daigle and Li 1993). C. elegans lacks a clear β-secretase ortholog, suggesting an Aβ-like fragment would not be processed from either human APP or APL-1. Importantly, APL-1 has no Aβ domain. Nevertheless, we decided to test whether APOE4-induced degeneration of HSN neurons depended on apl-1. However, we could not easily test whether APOE4-induced neurodegeneration required apl-1 by knocking it out, because apl-1 is essential for development (Hornsten et al. 2007). Instead, we tested whether knocking in an extra copy of apl-1 mimicked the synergistic effects of knocking in APP. Specifically, we integrated a single additional copy of apl-1 expressed pan-neuronally and tagged with mCherry (SC_APL-1). The SC_APL-1 strain also served as a control for knocking a single mCherry-tagged transgene into a specific locus on the second chromosome in our APP model.

We generated strains expressing both SC_apl-1 and APOE4 and tested for BW and HSN neurodegeneration. Overexpression of apl-1 alone did not result in an incidence of BW above WT levels. Interestingly, expression of SC_apl-1 with APOE4 showed an unexpected, significant reduction in BW compared to APOE4 alone (Figure 2B) suggesting that HSN function was retained. Consistent with these behavioral results, we found that HSN neurodegeneration was reduced in APOE4+SC_APL-1 strains compared to APOE4+APP strains (Figure 2C). We conclude that the BW and neurodegeneration phenotypes seen in our APOE4-expressing strain is not exacerbated by over-expression of apl-1. This suggests that APOE4 likely does not interact with APL-1 in the same way it does with APP to give rise to these phenotypes.

Taken together, these results demonstrate that the pan-neuronal expression of APOE4 causes degeneration of the HSN neurons which happen to synapse with VC neurons. Further, co-expression...
of APOE4 with APP extends the level of HSN neurodegeneration beyond that observed when either gene is expressed singly.

**Neuronal APOE4 causes selective neurodegeneration**

Thus far, we used extrachromosomal arrays to express the APOE transgenes. Although extrachromosomal arrays represent a convenient approach to express transgenes, these arrays are not perfectly carried through cell division which gives rise to mosaic individuals (Leung-Hagestein et al. 1992). Moreover, the expression of extrachromosomal transgenes is sometimes suppressed (Hsieh and Fire 2000). To control for these potential caveats, we sought to integrate the APOE3 and APOE4 transgene arrays into the worm’s X chromosome and re-evaluate BW phenotype and HSN degeneration. In this way, the worm would be expected to faithfully express the APOE3 and APOE4 transgenes throughout the nervous system.

We again assayed BW and HSN neurodegeneration, breaking down the incidence as a function of age. The integrated APOE4 and APOE4+APP strains retained a comparable percentage of BW as observed in the extrachromosomal array strains by Day 3 of adulthood, while the integrated APOE3 strain did not exhibit significant differences (Figure 1A vs. Figure 3A). Interestingly, not only was the total incidence of BW higher in the APOE4+APP strain by Day 3 of adulthood, but the total incidence of BW was already substantially raised by Day 2 of adulthood relative to that seen in the APOE4 strain (Figure 3A). Though both of the APOE4 and APOE4+APP strains showed age-dependent degeneration of HSN neurons, the degree of degeneration was highest in the APOE4+APP strain (Figure 3C). These results are consistent with our findings for the extrachromosomal strains. Differences in how APOE4 but not APOE3 strains displayed degeneration could not be simply attributed to higher levels of expression of the APOE4 transgene compared to APOE3. Both semi-quantitative genomic PCR and RT-qPCR revealed subtle but no significant differences in expression of both integrated and extrachromosomal transgenes (Fig. S3).

When we expressed APOE4 and APP throughout the nervous system, we originally expected to observe gross defects across multiple behaviors. However, aside from the BW phenotype, no gross movement defects were apparent in the APOE4+APP strain. Moreover, APOE4+APP worms exhibited no difference in crawling speed compared to WT worms (Figure 4A). The crawling speed was indistinguishable on both Day 1 and Day 3 of adulthood between WT and APOE4+APP worms (Figure 4A). Consistent with this behavioral observation, when we looked at GFP-labeled VA and VB cholinergic motor neurons, which contribute to locomotion, we saw no qualitatively discernable differences in morphology and number of VA and VB neurons between WT and APOE4+APP worms (Figure 4B).

Next, we asked whether APOE4 induces neurodegeneration when expressed outside the nervous system in a pan-neuronal APP background. Using extrachromosomal arrays, we expressed APOE4 in organs involved in metabolic and excretory functions, the intestine and coelomocytes, using the fat-7 and unc-122 promoters, respectively. Unlike with pan-neuronally expressed APOE4, the incidence of BW was as low for strains expressing APOE4 in either coelomocytes or intestine as in the background APP strains (Figure 5A). This low percentage of BW for strains with APOE4 expressed in coelomocytes or intestine corresponded with low levels of HSN degeneration in these strains (Figure 5B,C).

To better understand the mechanism of degeneration, we investigated whether HSN neurons degenerate in a manner dependent on apoptosis. We crossed the ced-3(n1286) null mutation into the integrated APOE4+APP strain. CED-3 is an executioner caspase that is part of the core apoptotic machinery in worm (Yuan et al. 1993). We found that the incidence of both BW and...
HSN neurons do not appear to degenerate when degeneration of the HSN neurons in C. elegans engineered on an amyloid-mouse models. In APOE-target replacement (TR) mouse models alone. Similar APOE variant-specific modulation of degeneration, age-dependent degeneration, and cell-specific patterned degeneration. In humans, the allele ε4 of APOE (APOE4) increases the risk as well as the mean age of clinical onset of AD in a copy-dependent manner (Corder et al. 1993). The more common APOE variant in humans, APOE3, does not confer this relative risk. Our results show that APOE4, but not APOE3, causes degeneration in a subset of neurons in C. elegans. APP co-expression enhances this APOE4-related degeneration of the HSN neurons in C. elegans. By contrast, the HSN neurons do not appear to degenerate when APP is expressed alone. Similar APOE variant-specific effects have been observed in mouse models. In APOE-target replacement (TR) mouse models engineered on an amyloid-β transgenic background, pathological phenotypes, including amyloid-β deposition, are observed at a higher level in APOE4-TR mice than APOE3-TR mice (Bales et al. 2009; Fryer et al. 2005). Further, compared to APOE2 and APOE3, APOE4 does not ameliorate neurodegeneration caused by amyloid-β deposition in the glutamatergic tail neurons of another C. elegans AD model (Griffin et al. 2019). However, studies that show a direct mechanistic link between APP and APOE4 for neurodegeneration are still lacking.

In our APOE4-expressing strains, GFP-labeled HSN neurons display neurodegeneration by becoming dim, shrinking, forming blebs, and disappearing in many animals. We also found that the long axonal processes of HSN neurons, which extend anteriorly to synapse onto neurons in the nerve ring, undergo beading. This is reminiscent of how neuronal processes degenerate after laser ablation (Yanik et al. 2006). Indeed, a recently published C. elegans AD model describes similar neurodegeneration patterns in the five glutamatergic tail neurons in the presence of Aβ (Griffin et al. 2019). In mice, targeted replacement of ApoE with human APOE causes dendrites of neurons in the amygdala to shrink compared to the dendrites of APOE3-TR mice (Klein et al. 2010). These shared morphological changes in the processes of dying neurons suggest that there might be conserved pathways for degeneration.

Notably, we found that expression of APOE4 on its own was sufficient to induce significant HSN neurodegeneration and its associated bag-of-worms behavioral phenotype. In contrast, Griffin et al. (2019) found that APOE4 expression did not appear to cause neurodegeneration in C. elegans. These different results may be explained by our pan-neuronal vs. their restricted glutamatergic expression of APOE4. Prevailing hypotheses suggest the interaction between APOE4 and the Aβ fragment drives AD progression. However, a number of studies in mice have shown that APOE4 alone can influence AD progression by affecting numerous, Aβ-independent processes including impaired learning behaviors, reduced synaptic plasticity and neurogenesis, increased neuroinflammation, neurotoxicity of fragmented APOE4, impaired mitochondrial function, and increased tauopathy (Yadong Huang 2011; Yamazaki et al. 2016). Many of these processes may be tested in worm. In fact, C. elegans contains an ortholog to human tau, PTL-1, that shows 50% sequence conservation in the microtubule-binding repeats (Goedert et al. 1996). Future studies may test how ptt-1 influences APOE4-induced neurodegeneration in our model. One of the prominent characteristics of AD is age-dependent neurodegeneration. Co-expression of APP with APOE4 throughout the nervous system in C. elegans enhances the incidence of APOE4-related neurodegeneration. Interestingly, the cumulative effect of APP and APOE4 expression manifests more strongly in Day 3 of adulthood, as reproduction declines. This suggests that the additive insult may arise from an age-related process. In patients with Down syndrome who carry an extra copy of APP, APOE4 appears to hasten the onset of AD and accelerate the progression of neurodegeneration (Patel et al. 2011; Head et al. 2011).

The synergy we observed for enhanced neurodegeneration by combining APOE4 with APP did not extend to an APP-related gene in C. elegans called apl-1. In fact, we found a modest protective effect of overexpressing an extra copy of apl-1. APL-1 is highly homologous to the intracellular portion of APP (Figure 2A), but not the extracellular or Aβ portions of APP (Daigle and Li 1993). Because several clinical trials targeting Aβ have failed to ameliorate cognitive decline, further studies may shift to the role of the APP intracellular domain (AICD). The AICD has been implicated in enhancing AD through its roles in transcriptional...
activation, pro-apoptotic functions, cytoskeletal modifications, calcium homeostasis modulation, and tau phosphorylation (Müller et al. 2008; Chang and Suh 2010; Ghosal et al. 2016). In our model, a single extra copy of APL-1 appears mildly protective, but multicopy overexpression of apl-1 has shown defects in movement, brood size, development, chemotaxis, and learning paradigms (Hornsten et al. 2007; Ewald et al. 2012). This suggests expression level may also play a role in disease progression. Future studies will need to determine whether neuroprotection against APOE4 stems from the homologous intracellular side and/or other pathways while paying careful attention to expression levels.

In humans, neurodegeneration occurs more extensively in certain regions of the brain, such as the hippocampus and entorhinal cortex (Saxena and Caroni 2011). This aspect is one of the more curious observations in AD patients because APP and APOE4 are both expressed throughout the brain (Huang and Mucke 2012). Our results show that numerous VA and VB cholinergic neurons did not degenerate despite the pan-neuronal expression of APP and APOE4 transgenes and their proximity to the vulnerable VC and HSN neurons. The restricted pattern of APOE4-dependent degeneration in worm suggests that there may be cellular and/or molecular characteristics that confer resistance (e.g., VA and VB) or vulnerability (HSNs) to APOE4.

Although APOE4 is expressed in both the liver and the nervous system in human (Holtzman et al. 2012; Liu et al. 2013; Mahley et al. 2006), it is unclear whether hepatic, neuronal, or both types of APOE4 contribute to neurodegeneration. We were interested in exploring whether APOE4-induced degeneration is tissue-specific in worm. Recent studies have demonstrated clear examples of cell non-autonomous signaling between neuronal and non-neuronal tissue in C. elegans (van Oosten-Hawle et al. 2013; Taylor and Dillin 2013; Melentijevic et al. 2017). For instance, unfolded protein responses, which may underlie neurodegeneration, are interdependently co-activated between the nervous systems and the intestine (van Oosten-Hawle et al. 2013; Taylor and Dillin 2013). Additionally, when under stress worm neurons jettison particles extracellularly to become absorbed by coelomocytes (Melentijevic et al. 2017). Taken together, these studies suggest that APOE4 could very well influence neurodegeneration when expressed outside of the neurons. However, after probing expression in both intestine and coelomocytes, our results indicate that APOE4 mainly induces degeneration when expressed in the nervous system of C. elegans. These results are consistent with other work showing the sufficiency of neuronal APOE4 expression in causing exacerbated cellular or behavioral deficits. For example, in mice neuronal APP can cause degeneration and memory deficits when co-expressed with APOE4 (Bien-Ly et al. 2011; Harris et al. 2003).

To begin to understand the basis for neurodegeneration in our model, we asked whether degeneration of HSN neurons required apoptosis. CED-3 is the primary executioner caspase that initiates the cascade of apoptosis (Yuan et al. 1993). When a ced-3 null mutation was crossed into the APOE4+APP strain, we saw no reduction of the bag-of-worms phenotype or HSN neurodegeneration. This indicates that apoptosis is likely not involved in APOE4-dependent degeneration in C. elegans. Other C. elegans neurodegenerative models similarly show that ced-3 and other broad apoptotic mechanisms do not play a role in models of degeneration (e.g., Liachko et al. 2010). Necrosis has been previously observed in models of neurodegeneration caused by a number of insults including hyperactive ion channels, increased intracellular Ca\(^{2+}\), and protein aggregate-induced stress (Nikoletopolou and Tavernarakis 2014). Genetic and pharmacological manipulation of necrosis pathways have been previously described in worm, and the drug thapsigargin, a modulator of cytosolic Ca\(^{2+}\), has been shown to moderately

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**Figure 5** Pan-neuronal, but not non-neuronal, expression of APOE4 promotes HSN neurodegeneration in a pan-neuronal APP background. A, Histogram shows the cumulative percent bag-of-worms (BW) phenotype by Day 3 of adulthood. The frequency of the BW increased in worms with pan-neuronal APP when APOE4 was expressed in neurons, but not in coelomocytes or intestine. Two strains of each genotype were made (signified by groups of paired solid and open bars) with independent extrachromosomal arrays containing a pmyo-2::mCherry and APOE4 transgene. The APP transgene was integrated. For statistical comparisons, shaded bars and open bars were each treated as a set. Within a set, each exAPOE4 strain was compared with the background APP strain using \(\chi^2\) tests. Alpha was set at 0.008 to correct for multiple comparisons (\(P < 0.008\)). B, Fluorescent images of the neurons, HSNL and HSNR, in Day 3 adults. Many worms expressing pan-neuronal APOE4 on the APP background show morphological abnormalities or a total loss of one or both HSN neurons. Label indicates the location of APOE4 expression. Arrowheads indicate healthy HSN neurons. Dotted circles indicate degenerated neurons. C, Histogram showing the percent HSN neurodegeneration on Day 3 of adulthood for a set of strains assayed in A. Each exAPOE4 strain was compared with the background APP strain using \(\chi^2\) tests. Alpha was set at 0.015 to correct for multiple comparisons (\(P < 0.015\)).
repress neurodegeneration in worms expressing APOE4 and Aβ (Caraveo et al. 2014; Griffin et al. 2019). We plan to use these and other methods to elucidate the mechanism of neurodegeneration in our APOE4 model in C. elegans. Our model is well-positioned to be used to explore how patterned neurodegeneration may arise in AD and other neurodegenerative diseases.

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LITERATURE CITED

Altun, Z. F., and D. H. Hall, 2018 Reproductive system. In WormAtlas.
doi:10.3908/wormatlas.1.17

Angelo, G., and M. R. Van Gilst, 2009 Starvation protects germline stem cells and extends reproductive longevity in C. elegans. Science 326: 954–958. https://doi.org/10.1126/science.1178343

Arey, R.N., and C.T. Murphy, 2017 Conserved regulators of cognitive aging: From worms to humans. Behav Brain Res 322: 299–310. https://doi.org/10.1016/j.bbr.2016.06.035

Bales, K. R., F. Liu, S. Wu, S. Lin, D. Koger et al., 2009 Human APOE isoform-dependent effects on brain beta-amyloid levels in PDAPP transgenic mice. J. Neurosci. 29: 6771–6779. https://doi.org/10.1523/JNEUROSCI.0887-09.2009

Bien-Ly, N., Y. Andrews-Zwilling, Q. Xu, A. Bernardo, C. Wang et al., 2011 C-terminal-truncated apolipoprotein (apo) E4 inefficiently clears amyloid-beta (Abeta) and acts in concert with Abeta to elicit neuronal and behavioral deficits in mice. Proc. Natl. Acad. Sci. USA 108: 4236–4241. https://doi.org/10.1073/pnas.1018381108

Brenner, S. 1974 The genetics of Caenorhabditis elegans. Genetics 77: 71–94.

Cabrejo, L., L. Guyant-Maréchal, A. Laquerrière, M. Vercelletto, F. de la Fournière et al., 2006 Phenotype associated with APP duplication in five families. Brain 129: 2966–2976. https://doi.org/10.1093/brain/awd237

Caraveo, G., P. K. Auluck, L. Whitesell, C. Y. Chung, V. Baru et al., 2014 Calcineurin determines toxic versus beneficial responses to α-synuclein. Proc. Natl. Acad. Sci. USA 111: E3544–E3552. https://doi.org/10.1073/pnas.1413201111

Chang, K. A., and Y. H. Suh, 2010 Possible roles of amyloid intracellular domain of amyloid precursor protein. BMB Rep. 43: 656–663. https://doi.org/10.5483/BMBRep.2010.43.10.656

Chêne, G., A. Beiser, R. Au, S. R. Preis, P. A. Wolf et al., 2015 Gender and incidence of dementia in the Framingham Heart Study from mid-adult life. Alzheimers Dement. 11: 310–320. https://doi.org/10.1016/j.jad.2013.10.005

Conejero-Goldberg, C., I. J. Gomar, T. Bobes-Bascaran, T. M. Hyde, J. E. Kleinman et al., 2014 APOE enhances neuroprotection against Alzheimer’s disease through multiple molecular mechanisms. Mol. Psychiatry 19: 1243–1250. https://doi.org/10.1038/mp.2013.194

Conradt, B., and H. R. Horvitz, 1998 The C. elegans protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. Cell 93: 519–529. https://doi.org/10.1016/S0092-8674(00)81182-4

Corder, E. H., A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell et al., 1993 Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. Science 261: 921–923. https://doi.org/10.1126/science.8346443

Corder, E. H., A. M. Saunders, N. J. Risch, W. J. Strittmatter, D. E. Schmechel et al., 1994 Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat. Genet. 7: 180–184. https://doi.org/10.1038/ng0694-180

Daigle, I., and C. Li, 1993 apl-1, a Caenorhabditis elegans gene encoding a protein related to the human β-amyloid protein precursor. Proc. Natl. Acad. Sci. USA 90: 12045–12049. https://doi.org/10.1073/pnas.90.24.12045

Di Battista, A. M., N. M. Heinsinger, and G. W. Rebeck, 2016 Alzheimer’s Disease Genetic Risk Factor APOE-ε4 Also Affects Normal Brain Function. Curr. Alzheimer Res. 13: 1200–1207. https://doi.org/10.2174/1567205013661604011115127

Ewald, C. Y., R. Cheng, L. Tolcn, V. Shah, A. Gillani et al., 2012 Pan-neuronal expression of APL-1, an APP-related protein, disrupts olfactory, gustatory, and touch plasticity in Caenorhabditis elegans. J. Neurosci. 32: 10156–10169. https://doi.org/10.1523/JNEUROSCI.0495-12.2012

Figure 6 Neurodegeneration induced by pan-neuronal co-expression of APOE4 and APP is not mediated by CED-3. A, Histogram shows the cumulative percent bag-of-worms (BW) phenotype by day 3 of adulthood. The frequency of BW in the APOE4+APP strain is not altered in a ced-3 null background. Both APP and APOE4 transgenes were integrated. B, Likewise, ced-3 expression does not alter neurodegeneration in the APOE4+APP strain. Fluorescent images of HSNL and HSNR. Arrowheads indicate healthy HSN neurons. Dotted circles indicate degenerated neurons. C, Histogram showing the percent HSN neurodegeneration for Day 3 adults. Comparisons were made with χ² tests.
Farrer, L. A., L. A. Cupples, J. L. Haines, B. Hyman, W. A. Kukull et al., 1997 Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278: 1349–1356. https://doi.org/10.1001/jama.1997.03550160060401

Frøkjaer-Jensen, C., M. W. Davis, C. E. Hopkins, B. J. Newman, J. M. Thonnell et al., 2008 Single-copy insertion of transgenes in Caenorhabditis elegans. Nat. Genet. 40: 1375–1383. https://doi.org/10.1038/ng.248

Fryer, J. D., K. Simmons, M. Parsadanian, K. R. Bales, S. M. Paul et al., 2005 Human apolipoprotein E4 alters the amyloid-beta 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model. J. Neurosci. 25: 2803–2810. https://doi.org/10.1523/JNEUROSCI.0531-04.2005

Ghosal, K., Q. Fan, H. N. Dawson, and S. W. Pimplikar, 2016 Tau protein mediates APP Intracellular Domain (AICD)-induced Alzheimer’s-like pathological features in mice. PLoS One 11: e0159435. https://doi.org/10.1371/journal.pone.0159435

Goate, A., M. C. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford et al., 1991 Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer’s disease. Nature 349: 704–706. https://doi.org/10.1038/349704a0

Goedert, M., C. P. Baur, J. Ahirnger, R. Lakes, M. Hasegawa et al., 1996 PTL-1, a microtubule-associated protein with tau-like repeats from the nematode Caenorhabditis elegans. J. Cell Sci. 109: 2661–2672.

Griffin, E. F., K. A. Caldwell, and G. A. Caldwell, 2017 Genetic and Pharmacological Discovery for Alzheimer’s Disease Using Caenorhabditis elegans. ACS Chem. Neurosci. 8: 2596–2606. https://doi.org/10.1021/acschemneuro.7b00361

Griffin, E. F., S. E. Scopel, C. A. Stephen, A. C. Holzhauser, M. A. Vaji et al., 2019 ApoE-associated modulation of neuroprotection from β-amyloid-mediated neurodegeneration in transgenic Caenorhabditis elegans. Dis. Model. Mech. 12: dmm037218. https://doi.org/10.1242/dmm.037218

Harris, F. M., W. J. Brecht, Q. Xu, I. Tesseur, L. Kekonius et al., 2013 UNC-5, a transmembrane protein with immunoglobulin and thrombospondin type 1 domains, guides cell and pioneer axon migrations in C. elegans. Cell 71: 289–299. https://doi.org/10.1016/0092-8674(92)90357-1

Liáčko, N. F., C. R. Guthrie, and B. C. Kraemer, 2010 Phosphorylation promotes neurotoxicity in a Caenorhabditis elegans model of TDP-43 proteinopathy. J. Neurosci. 30: 16208–16219. https://doi.org/10.1523/JNEUROSCI.2911-10.2010

Liu, C. C., C. C. Liu, T. Kanekiyo, H. Xu, and B. Bu, 2013 ApoE4 in Alzheimer and Alzheimer disease: risk, mechanisms and therapy. Nat. Rev. Neurol. 9: 106–118. https://doi.org/10.1038/nrneurol.2012.263

Liu, C. C., N. Zhao, Y. Fu, N. Wang, C. Linares et al., 2017 ApoE4 accelerates early seeding of amyloid pathology. Neuron 96: 1024–1032.e3. https://doi.org/10.1016/j.neuron.2017.11.013

Mahley, R. W., K. H. Weisgraber, and Y. Huang, 2006 Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer’s disease. Proc. Natl. Acad. Sci. USA 103: 5644–5651. https://doi.org/10.1073/pnas.0600549103

Menteljovic, I., M. L. Toth, M. L. Arnold, R. J. Guasp, G. Harinath et al., 2017 C. elegans neurons jettison protein aggregates and mitochondria under neurotoxic stress. Nature 542: 367–371. https://doi.org/10.1038/nature21362

Mello, C. C., J. M. Kramer, D. Stinchcomb, and V. Ambros, 1991 Efficient gene transfer in C. elegans: extrachromosomal maintenance and integration of transforming sequences. EMBO J. 10: 3959–3970. https://doi.org/10.1002/j.1460-2075.1991.tb0496x.x

Mondal, S., E. Hegarty, J. J. Sahih, L. L. Scott, S. K. Göcke et al., 2018 High-Content Microfluidic Screening Platform Used To Identify or2R/Tmem97 Binding Ligands That Reduce Age-Dependent Neurodegeneration in C. elegans SC_APP Model. ACS Chem. Neurosci. 9: 1014–1026. https://doi.org/10.1021/acschemneuro.7b00428

Müller, T., H. E. Meyer, R. Egenesperger, and K. Marcus, 2008 The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics – relevance for Alzheimer’s disease. Prog. Neurobiol. 85: 393–406. https://doi.org/10.1016/j.pneurobio.2008.05.002

Nikoletopoulou, V., and N. Tavernarakis, 2014 Necrotic cell death in Caenorhabditis elegans. Methods Enzymol. 545: 125–155. https://doi.org/10.1016/B978-0-12-801430-1.00006-8

Pericak-Vance, M. A., and S. D. Rees, M. A. Kelly, S. C. Bain, A. H. Barnett et al., 2014 Necrotic cell death in Caenorhabditis elegans SC_APP Model. ACS Chem. Neurosci. 9: 1014–1026. https://doi.org/10.1021/acschemneuro.7b00428

Patel, A., S. D. Rees, M. A. Kelly, S. C. Bain, A. H. Barnett et al., 2011 Association of variants within APOE, SORL1, RUNX1, BACE1 and ALDH1A1 with dementia in Alzheimer’s disease in subjects with Down syndrome. Neurosci. Lett. 487: 144–148. https://doi.org/10.1016/j.neulet.2010.01.010

Prasher V. P., M. J. Farrer, A. M. Kessling, E. M. Fisher, R. J. West, et al., 1993 Molecular mapping of Alzheimer-type dementia in Down’s syndrome. Ann Neurol. 1994 43: 380–383. https://doi.org/10.1002/ana.41043016

Saunders, A. M., W. J. Strittmatter, D. Schmechel, P. H. George-Hyslop, M. A. Pericak-Vance et al., 1993 Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer’s disease. Neurology 43: 1467–1472. https://doi.org/10.1212/WNL.43.8.1467

Saxena, S., and P. Caroni, 2011 Selective neuronal vulnerability in neurodegenerative diseases: from stressor thresholds to degeneration. Neuron 71: 35–48. https://doi.org/10.1016/j.neuron.2011.06.031
Schafer, W. R., 2005 Egg-laying. WormBook, edited by The C. elegans Research Community, WormBook, Available at doi/10.1895/wormbook.1.38.1, http://www.wormbook.org. https://doi.org/10.1895/wormbook.1.38.1

Stefanakis, N., I. Carrera, and O. Hobert, 2015 Regulatory Logic of Pan-Neuronal Gene Expression in C. elegans. Neuron 87: 733–750. https://doi.org/10.1016/j.neuron.2015.07.031

Taylor, R. C., and A. Dillin, 2013 XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. Cell 153: 1435–1447. https://doi.org/10.1016/j.cell.2013.05.042

Trent, C., N. Tsuing, and H. R. Horvitz, 1983 Egg-laying defective mutants of the nematode Caenorhabditis elegans. Genetics 104: 619–647.

van Oosten-Hawle, P., R. S. Porter, and R. I. Morimoto, 2013 Regulation of organismal proteostasis by transcellular chaperone signaling. Cell 153: 1366–1378. https://doi.org/10.1016/j.cell.2013.05.015

Ward, J. D., 2015 Rapid and precise engineering of the Caenorhabditis elegans genome with lethal mutation co-conversion and inactivation of NHEJ repair. Genetics 199: 363–377. https://doi.org/10.1534 Genetics.114.172361

Wu, L., and L. Zhao, 2016 ApoE2 and Alzheimer’s disease: time to take a closer look. Neural Regen. Res. 11: 412–413. https://doi.org/10.4103/1673-5374.179044

Yamazaki, Y., M. M. Painter, G. Bu, and T. Kanekiyo, 2016 Apolipoprotein E as a therapeutic target in Alzheimer’s disease: a review of basic research and clinical evidence. CNS Drugs 30: 773–789. https://doi.org/10.1007/s40263-016-0361-4

Yanik, M. F., H. Cinar, H. N. Cinar, A. Gibby, A. D. Chisholm et al., 2006 Nerve regeneration in Caenorhabditis elegans after femtosecond laser axotomy. IEEE J. Sel. Top. Quantum Electron. 12: 1283–1291. https://doi.org/10.1109/JSTQE.2006.879579

Ye, S., Y. Huang, K. Müllendorff, L. Dong, G. Giedt et al., 2005 Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target. Proc. Natl. Acad. Sci. USA 102: 18700–18705. https://doi.org/10.1073/pnas.0508693102

Yi, B. J. J., J. J. Sahn, P. M. Ardestani, A. K. Evans, L. L. Scott et al., 2017 Small molecule modulator of sigma 2 receptor is neuroprotective and reduces cognitive deficits and neuroinflammation in experimental models of Alzheimer’s disease. J. Neurochem. 140: 561–575. https://doi.org/10.1111/jnc.13917

Yoshina, S., Y. Suchiro, E. Kage-Nakada, and S. Mitani, 2015 Locus-specific integration of extrachromosomal transgenes in C. elegans with the CRISPR/Cas9 system. Biochem. Biophys. Rep. 5: 70–76. https://doi.org/10.1016/j. bbrep.2015.11.017

Yuan, J., S. Shaham, S. Ledoux, H. M. Ellis, and H. R. Horvitz, 1993 The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 75: 641–652. https://doi.org/10.1016/0092-8674(93)90485-9

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