**Perspective**

**APOE4 expression is associated with impaired autophagy and mitophagy in astrocytes**

Schmukler Eran, Pinkas-Kramarski Ronit

Among the risk factors for late onset sporadic Alzheimer’s disease (AD) is the expression of ε4 allele of apolipoprotein E (APOE4) gene (Mahley et al., 2006). Elevated amyloid processing and reduced degradation of Aβ, which lead to Aβ plaque deposition, are evident in APOE4-positive AD patients and mice (Mahley et al., 2006). These features correlate with neuronal cell loss. Impaired mitochondrial activity and increased oxidative stress have long been recognized as additional hallmarks of AD pathology (Mahley et al., 2006). The effect of APOE4 expression on autophagy and mitochondrial dynamics and activity in astrocytes is discussed in this perspective and is summarized in Figure 1.

Impaired autophagy and APOE4 are both strongly associated with AD, and the possibility that the pathological effects of APOE4 in AD, especially in the context of Aβ clearance, are related to autophagy was recently investigated (Simonovitch et al., 2016). Astrocytes have major protective roles under neuro-pathological conditions, such as neurotoxicity and neurodegeneration. Autophagy also acts as a protective mechanism, keeping cell homeostasis and promote survival under stress conditions (Klionsky and Emr, 2000). Therefore, the effects of APOE3 and APOE4 expression on autophagy in astrocytes under resting and stimulating conditions were determined, as well as the effect of APOE4 on beta-amyloid plaques clearance and digestion (Simonovitch et al., 2016). In these astrocytes, autophagy was found to be defective at the initiation step (autophagosome formation) and at the cargo degradation step. The APOE4-expressing astrocytes were also impaired in the uptake of soluble Aβ and in the ability to clear insoluble Aβ plaques from brain sections of 5xFAD mice, compared to astrocytes expressing APOE3 (Simonovitch et al., 2016). Chloroquine, an inhibitor of autophagosome-lysosome fusion, abolished the clearance of Aβ plaques by the APOE4 astrocytes, while rapamycin, an autophagy inducer, enhanced plaques removal by APOE4 astrocytes. These results pointed a possible link between autophagy and Aβ clearance. Importantly, additional studies demonstrated that APOE4 expression may be associated with impaired autophagy, especially in astrocytes (Parcon et al., 2017). Parcon et al. (2017) have shown that APOE4 AD patients exhibit lower brain mRNA levels of the autophagic proteins LC3 and p62, as well as of the lysosomal glycoprotein LAMP2. Consistently, mRNA levels of LC3, p62 and LAMP2 were upregulated following starvation-induced autophagy in APOE4-expressing but not in APOE4-expressing human glioblastoma cells and this upregulation was accompanied by clearance of protein aggregates (Parcon et al., 2017). Also, the autophagy inducer rapamycin was found to reduce learning impairment in APOE4 mice (Lin et al., 2017). The downregulation of autophagy by APOE4 may involve several mechanisms. Parcon et al. (2017) have suggested that apoE4 downregulates autophagy by acting as a transcriptional repressor of autophagic/lysosomal genes, which are normally upregulated by the transcription factor TFEB. Alternatively, the deleterious effect of apoE4 on autophagy may be due to its interaction and disruption of lysosome/autophagosome membranes disruption.

Since autophagy plays a key role in the clearance of redundant and damaged organelles including mitochondria (a process known as mitophagy), the effect of APOE4 expression on mitochondrial dynamics was subsequently examined (Simonovitch et al., 2019; Schmukler et al., 2020). Using APOE3 and APOE4 targeted replacement mice, the mitochondrial dynamics and mitophagy related proteins expression was determined in brain sections and homogenates (Simonovitch et al., 2019). The levels of the mitochondrial fusion protein Mfn1 were higher, whereas the mitochondrial fission protein Drp1 were lower in the APOE4 brains indicating increased mitochondrial fusion and decreased fission. APOE4-dependent decrease in Drp1 was also observed in brain homogenates of AD patients (Simonovitch et al., 2019). In agreement with these results, altered mitochondrial dynamics in APOE4-expressing astrocytes, including changes in the synthesis, mitochondrial recruitment, ubiquitination and degradation of proteins involved in mitochondrial fission, fusion and mitophagy was recently demonstrated (Schmukler et al., 2020). Reduced mitochondrial fission was evident by lower levels of Drp1 protein and mRNA levels as well as its interaction with the mitochondria in APOE4 astrocytes. Additionally, it was demonstrated that APOE4-expressing astrocytes exhibit higher levels of Mfn1, which are driven by reduced proteasomal degradation, and may interfere with normal mitophagy (Schmukler et al., 2020). Taken together, these findings suggest that APOE4 is associated with decreased mitochondrial

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**Figure 1** | Schematic presentation of the effect of APOE4 on various aspects of autophagy, mitophagy and mitochondrial dynamics.

Aβ: Amyloid beta; apoE4: apolipoprotein E; Drp1: Dynamin-1-like protein 1; LC3: microtubule-associated proteins light chain 3; Mfn1: mitofusin-1; p62: p62/SQSTM1.
fission and elevated mitochondrial fusion in astrocytes and possibly in other CNS cells. Parkin labels mitochondria for mitophagy by ubiquitination of specific mitochondrial proteins. APOE4 mice brains exhibited higher levels of parkin compared with APOE3 mice (Simonovitch et al., 2019), and, consistently, APOE4 astrocytes displayed increased levels of total and mitochondrial parkin (Schmukler et al., 2020). These finding suggest that parkin recruitment to mitochondria is intact in APOE4 astrocytes and might indicate impaired degradation of mitochondrial parkin, leading to its accumulation. Indeed, in these astrocytes, reduced proteasomal and lysosomal turnover of parkin underlie increased parkin levels (Schmukler et al., 2020), which was shown to impede its activity and result in mitophagy impairment (Durcan et al., 2014). In addition, the interaction of the autophagic proteins LC3-II and p62 with the mitochondria, and the lysosomal degradation of mitochondrial LC3-II and p62 were reduced in APOE4 astrocytes. Mitophagy induction following mitochondrial damage (CCCP treatment) was also reduced in APOE4, further indicating altered mitochondrial dynamics and mitophagy deficiency. Indeed, the mitochondrial network of APOE4 astrocytes was shown to be hyperfused and mitochondria from APOE4 mice brain sections were more elongated compared with APOE3 (Simonovitch et al., 2019; Schmukler et al., 2020). In agreement, fibroblast from AD patients also exhibit elongated mitochondria, highly connected network and decreased Drp1 levels (Zhu et al., 2013). The mechanism by which APOE4 affects mitochondrial dynamics is yet unclear and may involve a detrimental interaction of apoE4 itself with the mitochondria, as was shown in both astrocytes and neurons (Schmukler et al., 2020). Alternatively, apoE4 may affect mitochondrial dynamics indirectly, by interfering with the basic autophagy/lysosomal machineries as discussed above.

Balanced mitochondrial dynamics and mitochondrial degradation through mitochondria, which presumably underlies APOE4 astrocytes mitochondrial dysfunction, could be partially corrected by mitophagy induction using rapamycin treatment (Schmukler et al., 2020). Therefore, rapamycin-induced mitophagy in the APOE4 astrocytes may mediate the removal of dysfunctional mitochondria.

In this context of APOE4, autophagy and mitochondrial dynamics/function, therapeutic approaches could be employed. For example, it will be intriguing to examine the effect of other autophagy inducers on APOE4-related pathology in vitro and in vivo. Similarly, the naturally occurring compounds, utorilin A and actininon, which were shown to improve mitochondrial activity and to induce mitophagy (Ryu et al., 2016; Fang et al., 2019) might prove to be beneficial in APOE4 models of AD. In fact, both utorilin A and actininon were shown to have a therapeutic potential in other models of AD. Finally, the effect of apoE4-targeted agents, such as CS-6253 and baxetorone ( Boehm-Cagan et al., 2016), on autophagy/mitophagy and mitochondrial dynamics/function could also be examined to further substantiate the relationship between apoE4 and these cellular features.

Collectively, the studies reviewed here indicate that the pathological effects of APOE4 include autophagy/mitophagy impairments and could pave the way for identification of novel APOE4-related therapeutic targets.

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