

REVIEW ARTICLE

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# A key role for MAM in mediating mitochondrial dysfunction in Alzheimer disease

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## Abstract

In the last few years, increased emphasis has been devoted to understanding the contribution of mitochondria-associated endoplasmic reticulum (ER) membranes (MAM) to human pathology in general, and neurodegenerative diseases in particular. A major reason for this is the central role that this subdomain of the ER plays in metabolic regulation and in mitochondrial biology. As such, aberrant MAM function may help explain the seemingly unrelated metabolic abnormalities often seen in neurodegeneration. In the specific case of Alzheimer disease (AD), besides perturbations in calcium and lipid homeostasis, there are numerous documented alterations in mitochondrial behavior and function, including reduced respiratory chain activity and oxidative phosphorylation, increased free radical production, and altered organellar morphology, dynamics, and positioning (especially perinuclear mitochondria). However, whether these alterations are primary events causative of the disease, or are secondary downstream events that are the result of some other, more fundamental problem, is still unclear. In support of the former possibility, we recently reported that C99, the C-terminal processing product of the amyloid precursor protein (APP) derived from its cleavage by  $\beta$ -secretase, is present in MAM, that its level is increased in AD, and that this increase reduces mitochondrial respiration, likely via a C99-induced alteration in cellular sphingolipid homeostasis. Thus, the metabolic disturbances seen in AD likely arise from increased ER-mitochondrial communication that is driven by an increase in the levels of C99 at the MAM.

## Facts

- Mitochondrial bioenergetic function is decreased in AD, but the reason for this decline is unknown.
- A “mitochondrial cascade hypothesis” has been put forward to explain AD pathogenesis.
- ER-mitochondrial communication and MAM function are increased significantly in AD.
- C99 is present in MAM, and accumulates above

normal levels in AD cells and animal models.

- Increased C99-mediated MAM activity induces bioenergetic dysfunction in AD cells.

## Open questions

- How does C99 modulate MAM function in general and bioenergetic output in particular?
- What is the mechanism of mitochondrial dysfunction due to alterations in MAM behavior?
- How do these alterations occur in sporadic AD, in which APP processing is presumably normal?

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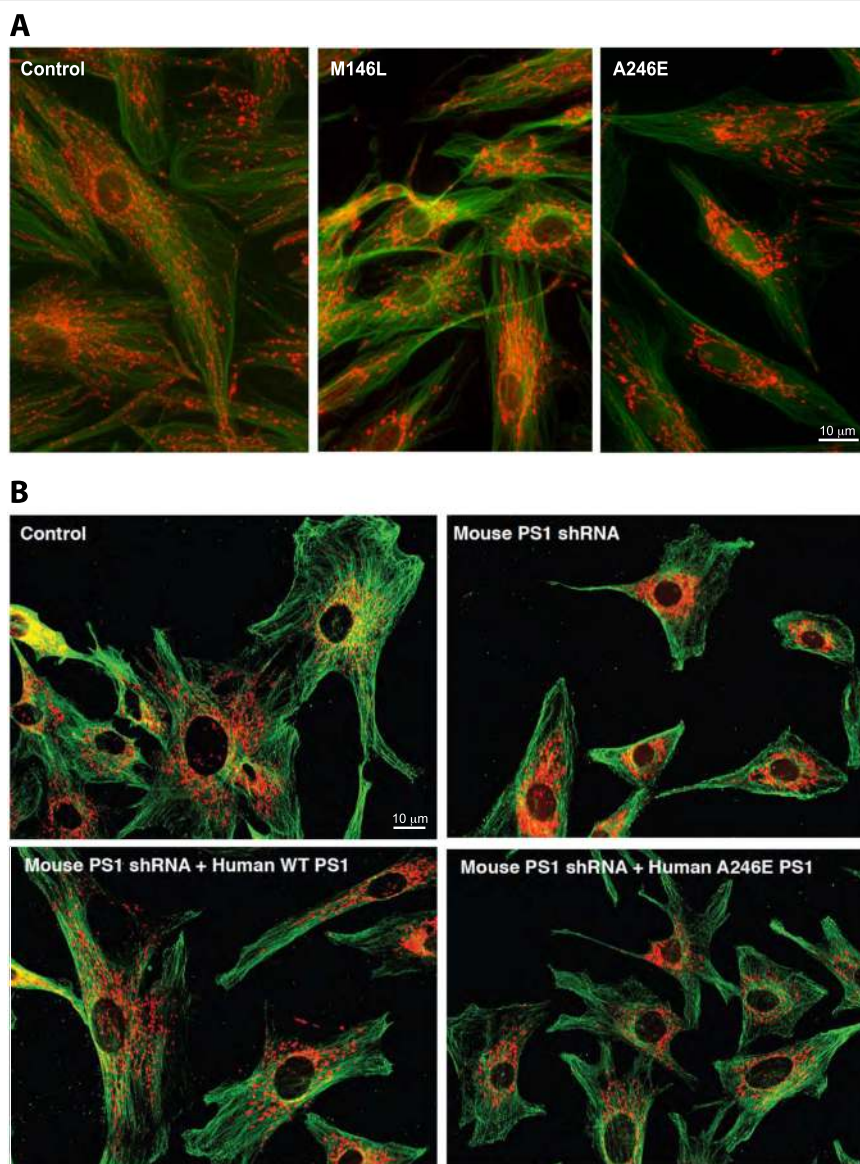


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## Introduction

Alzheimer disease (AD) is the most common adult neurodegenerative disorder<sup>1</sup>. Pathologically, it is characterized by progressive neuronal loss in the hippocampus and cortex, with the accumulation in the brain of extracellular neuritic plaques and intracellular neurofibrillary tangles. Prominent among the proteins deposited in the plaques is  $\beta$ -amyloid ( $A\beta$ ), which is produced by cleavage of the amyloid precursor protein (APP) by presenilin-1 (PS1) and/or presenilin-2 (PS2), both of which are active

components of the  $\gamma$ -secretase complex<sup>2</sup>. Notably, dominantly inherited mutations both in the presenilins and in APP are currently the only known causes of the familial form of AD (FAD), which has led to the most widely accepted hypothesis to explain the pathogenesis of AD, namely, the “amyloid cascade,” which proposes that deposition of  $A\beta$  in the brain is the precipitating pathological event in AD<sup>3</sup>. However, while the amyloid cascade hypothesis helps explain the development of the plaques and perhaps also the tangles, it sheds little light on the



**Fig. 1** **a** Representative mitochondrial morphology in AD-mutant cells. Human fibroblasts were stained with MitoTracker (red) and anti-tubulin (green). Note the relatively dispersed distribution of the mitochondria in the control, whereas they are more perinuclear in the FAD-PS1<sup>M146L</sup> and FAD-PS1<sup>A246E</sup> cells. **b** MEFs in which PS1 was knocked down (by small hairpin RNA). Cells were stained as in **a**. Note relatively dispersed distribution of the mitochondria in the control, whereas they are more perinuclear in the PS1-knockdown cells. This phenotype could be rescued by overexpression of WT human PS1, but not by expression of a human pathogenic PS1 mutation (A246E)

impact of other aspects of the disease, some of which occur years before the appearance of those plaques and tangles<sup>4–6</sup>. Those other aspects include altered metabolism of phospholipids and fatty acids<sup>7,8</sup>, increased levels of circulating cholesterol<sup>9</sup>, the deposition of lipid droplets within cells<sup>10–12</sup>, alterations in glucose levels<sup>13</sup>, aberrant calcium homeostasis<sup>14</sup>, increased ER stress<sup>15</sup>, and mitochondrial dysfunction<sup>16,17</sup>, the focus of our discussion here.

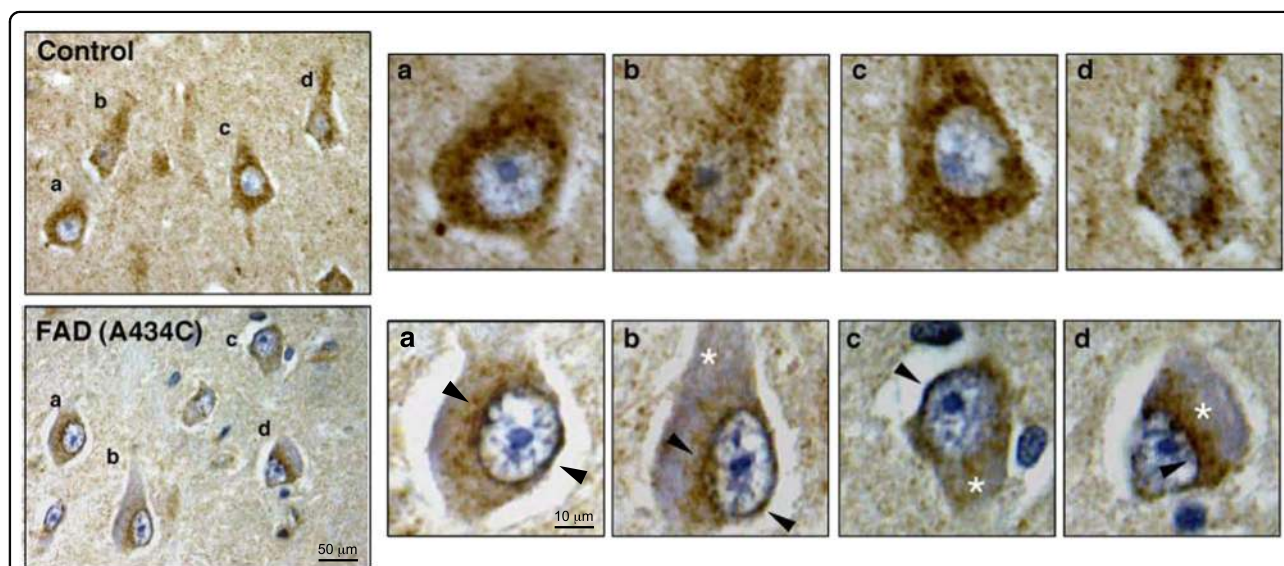
### Mitochondrial alterations in AD

In the last few decades, many reports have demonstrated the impairment of mitochondrial function in AD. Moreover, a number of lines of biochemical and cell biological evidence have been marshaled in support of a “mitochondrial cascade hypothesis” for the pathogenesis of AD, which proposes that mitochondrial alterations initiate the cascade of pathologies characteristic of the disease<sup>18–25</sup>. However, while this possibility is intriguing, it is currently unclear whether the impairment of mitochondrial function in AD<sup>26–33</sup> is the cause, the consequence, or merely a “bystander effect” of the biochemical and morphological changes seen in AD<sup>34,35</sup>. While mitochondria are clearly altered in AD, we believe that the mitochondrial cascade hypothesis has a number of flaws, discussed in greater detail below, that have led us to the conclusion that mitochondrial dysfunction is an early disturbance in the pathogenesis of AD but is not the driver of the pathogenesis.

### Mitochondrial biochemical and dynamic alterations

As alluded to above, mitochondrial bioenergetic function is reduced in AD. Specifically, AD patients and animal models of AD exhibit reduced respiratory chain activity and lower ATP production<sup>26,28,31,36–39</sup>. Additionally, many reports have described a significant decrease in enzymes of the mitochondrial tricarboxylic acid cycle in AD patients<sup>40–42</sup>. Moreover, the levels of free radicals and reactive oxygen species, which are produced mainly by mitochondria, are elevated in AD cells<sup>38,43–46</sup>.

Besides the effects on bioenergetics, there are also significant changes in mitochondrial dynamics and localization in AD cells, namely, dysfunctional mitochondrial axonal transport<sup>19,47–49</sup>, deregulated organellar dynamics (e.g., mitochondrial fission and fusion)<sup>17,50–53</sup>, and a more perinuclear distribution of the organelles<sup>17,50,54,55</sup>. On this latter point, we too have observed perinuclear mitochondria in AD patient cells (Fig. 1a) and were also able to reproduce the perinuclear phenotype in mouse embryonic fibroblasts (MEFs) in which endogenous PS1 mRNA was knocked down (Fig. 1b). Equally important, we observed a reversal of the perinuclear phenotype upon overexpression of the wild-type allele of human PS1, but not a pathogenic AD-mutant allele (A246E) (Fig. 1b). Consistent with this, we obtained a similar result in the most clinically relevant tissue, namely human brain. Specifically, we used immunohistochemistry to detect mitochondria in the hippocampal CA1 region of an autaptic brain from an AD patient with a pathogenic PS1 mutation



**Fig. 2 Immunohistochemistry to detect mitochondria (FeS subunit of complex III of the mitochondrial respiratory chain) in the hippocampus (CA1 region) from a FAD patient with a PS1 mutation (A434C).** (Upper panels) Control subject (left), with four indicated neurons (a–d) magnified at right. Note relatively uniform stain (brown), indicating a homogeneous distribution of mitochondria in the cell body. (Lower panels) FAD-PS1<sup>A434C</sup> patient. Notation as in upper panel. Note the perinuclear distribution of immunostain (brown rings; arrowheads), with a relative paucity of immunostain in the distal regions of the cell body (asterisks)



(A434C)<sup>56</sup>. Mitochondria were uniformly distributed in the cytosol of control hippocampus, as expected. In contrast, we detected a “ring” of mitochondria around the nucleus of the patient neurons, and depletion of the organelles in the distal region of hippocampal cell bodies (Fig. 2). Thus, we believe that the observation of perinuclear mitochondria in patient cells in vitro likely reflects what is occurring clinically in vivo. Moreover, it appears that  $\gamma$ -secretase activity and/or APP processing play a role in the altered distribution of mitochondria in at least some forms of AD<sup>17,50</sup>, but the relationship, if any, of mitochondrial maldistribution to altered APP processing has been obscure; this issue is addressed below.

Taken together, it is clear that there are numerous functional alterations in mitochondrial behavior in both familial and sporadic AD. However, even though many of these mitochondrial phenotypes are evident before the appearance of plaques<sup>57–59</sup>, we believe that mitochondrial dysfunction likely will not be found to be the underlying cause of the disease, for a number of reasons.

First and foremost, patients with authentic mitochondrial diseases (by which we mean diseases where bioenergetic deficits are the initiating cause of the pathology), whether due to mutations in the mitochondrial or nuclear genomes, do not evince the symptomatology of AD, even in those patients who live into their fourth and fifth decades. Generally speaking, mitochondrial diseases are characterized by numerous defects (e.g., encephalopathy, myopathy, endocrinopathy, retinopathy, and gastrointestinal and kidney disorders) that are simply not seen with any frequency in AD.

Second, mitochondrial deficiency is a common consequence of other insults, such as tissue injury. In fact, the altered mitochondrial function and dynamics seen in AD are also seen in a number of other neurodegenerative disorders that are distinct from AD. For example, perinuclear mitochondria are seen in Huntington disease<sup>60,61</sup> and in amyotrophic lateral sclerosis<sup>62–65</sup>, and was seen in a patient with a mutation in the mitochondrial fission protein DRP1<sup>66</sup>.

Finally, perinuclear mitochondria can be induced by overexpression of the mitochondrial fission protein FIS1<sup>67</sup> and also, notably, by tau<sup>68</sup>. Thus, these findings imply minimally that mitochondrial dysfunction and altered mitodynamics, while occurring early, are probably downstream consequences of other specific primary events in AD progression, and are not a fundamental cause of pathogenesis.

### Mitochondria and genetics

Genetically, alterations in mitochondrial DNA (mtDNA) have been found in AD cells and tissues<sup>69</sup>.

These include both qualitative changes, such as the association of specific mtDNA haplogroups with the risk of developing AD<sup>70–73</sup> and the presence of mtDNA deletions<sup>74,75</sup> and point mutations<sup>76–78</sup> in patient cells and tissues, and quantitative changes, such as reduced mtDNA levels in AD cerebrospinal fluid<sup>79</sup>. At the gene expression level, one study showed that the transcription of a number of nuclear-encoded oxidative phosphorylation (OxPhos) subunits was reduced in AD blood, whereas transcription of most mtDNA-encoded subunits was elevated in AD blood<sup>80</sup>.

Nevertheless, from the genetic point of view, in spite of the implication that mtDNA mutations, and especially point mutations, should result in maternally inherited AD, only a tiny number of mtDNA variants have been ascribed specifically to the development of the disease<sup>76</sup>, and even this is controversial<sup>81–83</sup>; in fact, there is little evidence for maternal inheritance in AD at all<sup>84,85</sup>.

Finally, genetic association studies have identified numerous nuclear loci associated with increased risk for developing AD<sup>86</sup>. Along with the three FAD-linked genes (*APP*, *PSEN1*, and *PSEN2*), the most commonly accepted sporadic AD (SAD) risk loci (i.e. linkage to mutations either near or within known genes) are *ABCA7*, *APOE*, *BIN1*, *CASS4*, *CD2AP*, *CD33*, *CELF1*, *CLU*, *CRI*, *EPHA1*, *FERMT2*, *INPP5D*, *MEF2C*, *MS4A*, *NME8*, *PICALM*, *PLD3*, *PTK2B*, *SLC24A4*, *SORL1*, *TREM2*, and *ZCWPW1*. Notably, none of the proteins encoded by these genes are targeted to mitochondria. Of course, this lack of correlation does not prove that mitochondria are not involved in AD pathogenesis, but by the same token it provides little genetic support to the mitochondrial cascade hypothesis.

### Mitochondria and $\gamma$ -secretase

In support of the mitochondrial cascade hypothesis, there is an intriguing connection between mitochondria and the  $\gamma$ -secretase complex. First, APP and/or A $\beta$ <sup>44,46,87–94</sup>, as well as components of the  $\gamma$ -secretase complex<sup>95–98</sup>, have been reported to be at or in mitochondria, presumably implicating the organelle directly in AD. Second, there are mitochondria-mediated alterations in APP processing in AD cells and tissues<sup>39</sup>. Third, PS1 enhances the expression of PGC-1 $\alpha$ , the master regulator of mitochondrial biogenesis, and this effect is reduced in PS1-mutated cells<sup>99</sup>. Finally, incubation of cultured cells and/or isolated mitochondria with A $\beta$  has been shown to have deleterious effects on mitochondrial functions, including effects on respiration<sup>100–104</sup>, protein import<sup>105</sup>, organellar transport<sup>19,47–49</sup>, organellar localization<sup>106</sup>, and organellar dynamics (e.g., mitochondrial fission and fusion)<sup>17,50–53</sup>. These and other reported data reveal an intriguing association between mitochondrial regulation and  $\gamma$ -secretase activity, but whether this link is direct or is mediated by some other, more indirect, mechanism, is not clear.

Regarding the localization studies, it is true that components of the  $\gamma$ -secretase complex can be found on mitochondria, but their relative concentration is very low compared to that in other membranes, such as ER. Moreover, data from our laboratory<sup>107</sup> and others<sup>108</sup> showed that presenilins are not imported into mitochondria, implying that while presenilins and  $\gamma$ -secretase exert direct effects on mitochondria, they do not behave as canonical mitochondrial proteins. Another argument in opposition to APP or  $\gamma$ -secretase activity being localized to mitochondria is that this protein complex is only activated in lipid raft domains<sup>109–112</sup>, which do not exist on mitochondrial membranes<sup>113</sup>.

Regarding the toxic effects of A $\beta$  on mitochondria, previous studies showing inhibitory effects of A $\beta$  treatment on mitochondrial function could have been due to the use of unphysiological concentrations of A $\beta$ <sup>101</sup>. Indeed, inhibitory effects on mitochondria were observed with A $\beta$  concentrations that were 10–100 times higher than those found in the entorhinal cortex or cerebrospinal fluid from AD patients<sup>114</sup>.

We therefore think that the pathogenesis of AD cannot be explained as resulting from those alterations in mitochondrial function that are similar to those seen in authentic mitochondrial disorders. Nevertheless, mitochondrial dysfunction is an undeniable early symptom of the disease that needs to be investigated and understood if we are to understand better the course of AD pathogenesis. How might this conundrum be reconciled?

### Mitochondria-associated ER membranes, APP processing, and bioenergetics in AD

Some years ago we hypothesized that the phenotypes seen in AD, including the mitochondrial disturbances, were the downstream consequences of some primary insult in AD arising prior to plaque and tangle formation, and triggered by mutations in PS1, PS2, and APP in the case of FAD, and by unknown causes in the case of SAD<sup>115,116</sup>.

With that in mind, we first tried to clarify the subcellular localization of presenilins and  $\gamma$ -secretase activity and its spatial relationship to mitochondria. Intriguingly, our group found<sup>107</sup>, and others confirmed<sup>117–119</sup>, that presenilins and  $\gamma$ -secretase activity, while localized at the ER, as described previously<sup>120</sup>, are enriched in mitochondria-associated ER membranes or MAM. MAM is a specialized subdomain of the ER that, as opposed to the rest of the ER, has the features of a lipid raft and is rich in cholesterol and sphingomyelin<sup>121,122</sup>. MAM is critical for processes that occur at the interface between mitochondria and ER, including phospholipid biosynthesis, cholesterol esterification, calcium transport, and communication between the two organelles<sup>123</sup>.

After this initial finding, our group (and subsequently others<sup>124–126</sup>) became interested in the potential role that MAM might play in the pathogenesis of AD<sup>115,116,127,128</sup>. We therefore measured MAM activity and ER-mitochondrial connectivity in AD cell models and in cells from AD patients, and found both to be increased significantly compared to controls<sup>122</sup>.

This connection between MAM and AD, at least on theoretical grounds, is appealing, because besides its close apposition to mitochondria, many of MAM's known functions are among the functions that are perturbed in AD beyond the accumulation of amyloid plaques and tangles<sup>129,130</sup>. These include the regulation of phospholipid, cholesterol, and calcium homeostasis<sup>123</sup>, increased ER stress<sup>131–133</sup>, and perturbed calcium homeostasis, driven, in part, by interactions between p53 and the sarco-ER calcium pump at the MAM<sup>132</sup>. In addition, at least one known MAM-localized enzyme, acyl-CoA:cholesterol acyltransferase (ACAT1; gene *SOAT1*)<sup>134</sup> appears to be required for the production of A $\beta$ <sup>135,136</sup>. Moreover, altered mitochondrial function might somehow be connected to APP processing<sup>137</sup>, at least in the familial form of the disease where APP processing is clearly perturbed<sup>2</sup>. Finally, given the physical proximity of ER to mitochondria<sup>123,134,138</sup>, it is possible that the mitochondrial disturbances that are found in AD might also be due to perturbed MAM morphology and behavior<sup>133,139–141</sup>. For example, given the fact that the subcellular localization of the majority of ER is perinuclear, the increased apposition between ER and mitochondria in AD<sup>122</sup> could help explain why mitochondria accumulate in the perinuclear region in the disease. In order to address whether perturbations in MAM are responsible for mitochondrial dysfunction in AD, we focused on the relationships among APP processing, MAM behavior, and mitochondrial regulation.

In the non-amyloidogenic pathway, full-length APP (~700 amino acids (aa) in length) is first cleaved by  $\alpha$ -secretase at the plasma membrane to produce a long soluble N-terminal fragment (sAPP $\alpha$ ) and a short membrane-bound 83-aa C-terminal fragment, called C83; C83 is cleaved by the  $\gamma$ -secretase complex to produce two peptides, P3 and the APP intracellular domain (AICD)<sup>142</sup>. In the alternative amyloidogenic pathway, full-length APP is first cleaved by  $\beta$ -secretase (BACE1) within endosomes to produce a slightly shorter soluble N-terminal fragment (sAPP $\beta$ ) and a slightly longer 99-aa membrane-bound C-terminal fragment, called C99. C99 is then delivered to the ER, via a currently unknown mechanism, to be cleaved by the  $\gamma$ -secretase complex, producing two peptides, A $\beta$  and AICD. In unaffected individuals, C99 is cleaved rapidly to A $\beta$ <sub>40</sub>, which is ~40 aa in length. In AD, C99 is cleaved to A $\beta$ <sub>42</sub>, which is ~42 aa in length, and there is an increase in the ratio of A $\beta$ <sub>42</sub>:A $\beta$ <sub>40</sub>. Since A $\beta$  has been

found to be produced in MAM<sup>107,118</sup>, it was logical to assume that the substrate for MAM-localized  $\gamma$ -secretase, namely C83 and/or C99, must be present in this compartment. In agreement with this supposition, we found that C99 (but not C83) was present not only in endosomes, as expected, but in MAM as well, both in cells and in tissues<sup>143</sup>, where it undergoes cleavage by  $\gamma$ -secretase to generate  $A\beta$ <sup>107,117,118</sup>.

Our data further showed that in cellular models of AD, in cells and tissues from AD animal models, and in cells from FAD and SAD patients, there were significant increases in C99 in MAMs that correlated with alterations in MAM structure and function<sup>143</sup>. Specifically, we found that the accumulation of C99 at MAM resulted in the upregulation of sphingomyelin hydrolysis by sphingomyelinases (SMases) within this ER subdomain, but the identity of the specific SMases that are upregulated (there are at least five) is currently unknown. We were also able to replicate the increase in SMase activity at MAM domains in SH-SY5Y cells by inhibiting  $\gamma$ -secretase activity (thereby promoting the accumulation of C99). Supporting this result, the inhibition of BACE1 activity (which reduces C99 formation) resulted in an attenuation of SMase activity<sup>143</sup>.

This increase in SMase activity resulted not only in reductions in the content of sphingomyelin but also in a notable elevation of the sphingomyelin hydrolysis product, ceramide<sup>143</sup>. This finding was noteworthy because ceramide is not only a pro-apoptotic molecule<sup>144</sup> but is also an inhibitor of mitochondrial respiration<sup>145–149</sup>. Indeed, we found that in our presenilin-mutant and AD patient cells, ceramide levels were elevated and respiratory chain function was decreased, as was respiratory supercomplex formation and function<sup>143</sup>. Importantly, manipulation of the ceramide pathway (both pharmacologically and genetically) to reduce ceramide levels in these cells reversed the bioenergetic defects<sup>143</sup>. In addition, reduction of C99 levels, either via inhibition of BACE1 activity (again, both pharmacologically and genetically) or via ablation of the *APP* gene, also reversed the bioenergetic deficits, concomitant with a renormalization of the sphingolipid profiles<sup>143</sup>. Importantly, these phenotypes could not be replicated by the addition of physiological concentration ratios of  $A\beta_{42}$ : $A\beta_{40}$ , physiological concentrations of  $A\beta_{42}$  oligomers, or by the overexpression of AICD. We therefore believe that the bioenergetic defects in AD are likely to be the consequence of upregulated sphingolipid turnover and increased ceramide content triggered by the accumulation of C99 at the MAM. This elevation in ceramide levels alters mitochondrial membrane properties, hindering the assembly and activity of respiratory supercomplexes, resulting or exacerbating, at least in part, in bioenergetic deficiencies. Importantly, these findings implicating C99 are consistent with the findings of others, who showed that altered APP

processing, especially via MAM-localized PS2<sup>126,150</sup>, decreased bioenergetics. Interestingly, deletion of a portion of the C99 transmembrane region altered mitochondrial morphology and function in HeLa cells, including decreased ATP levels and decreased membrane potential<sup>151</sup>.

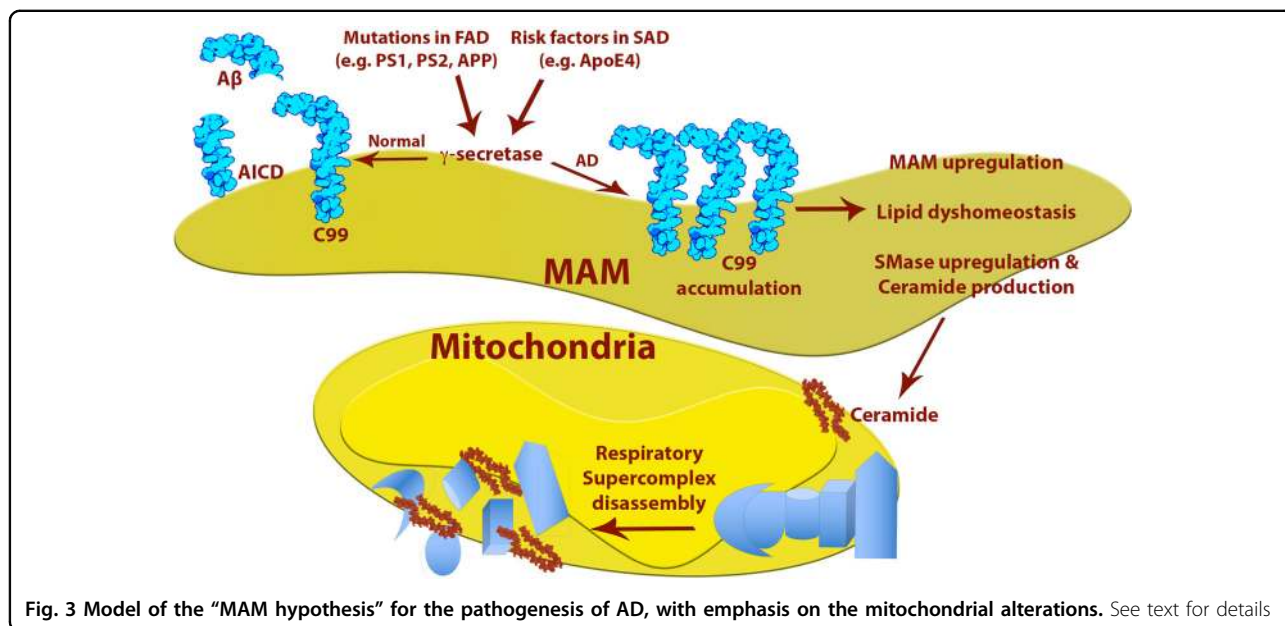
These findings are particularly important because the sphingolipid and mitochondrial phenotypes that we found in PS-mutant cells and in cells from FAD patients were also observed in cells from SAD patients<sup>143</sup>, in which the *PSEN1*, *PSEN2*, and *APP* genes are normal. This latter result implies that from a mitochondrial point of view, both the familial and sporadic forms of the disease have a common pathogenic origin. In this regard, we note that the most important genetic risk factor for developing SAD is a variant of apolipoprotein E (ApoE), a protein required to ferry cholesterol within lipoprotein particles: the  $\epsilon 4$  allele (ApoE4) confers a significantly higher risk of developing AD than does the  $\epsilon 3$  allele (ApoE3)<sup>152</sup>. It is noteworthy, therefore, that ER-mitochondrial communication and MAM function were increased significantly in fibroblasts and neurons treated with ApoE4-containing astrocyte-conditioned media as compared to those treated with ApoE3-containing astrocyte-conditioned media<sup>153</sup>. Moreover, in spite of no obvious qualitative defect in the *APP* or presenilin genes in SAD, C99 is nevertheless elevated in these patients<sup>154–156</sup>.

In summary, previous data and our own results point to a direct connection between APP processing and OxPhos deficiency via C99, both in FAD and SAD. Moreover, these data imply that at least from the mitochondrial point of view, it is C99, and not  $A\beta$  (nor any other APP processing product (e.g., sAPP $\alpha$ , C83, or P3)<sup>137</sup>), that is the key APP-processing intermediate that is required for pathogenicity.

### Concluding remarks—the “MAM hypothesis”

We believe that while mitochondrial dysfunction is an early and significant defect in AD, it is not a primary insult in the pathogenesis of the disease, but rather is a consequence of MAM dysfunction that is driven by an increased presence of C99 at MAM.

These results also imply that from the genetic standpoint, dominant mutations in PS1 likely result in haploinsufficiency<sup>157–159</sup> or behave in a dominant-negative manner<sup>160</sup> rather than resulting in a gain of function<sup>161</sup>, with the reduced PS1 activity as the likely cause of the increased levels of C99. In turn, increased MAM-localized C99 promotes the various features of the disease, including the calcium and lipid dyshomeostasis, the mitochondrial perturbations, and ultimately the plaque and tangle formation. In agreement with this view, the accumulation of C99 in mitochondrial fractions, and mitochondrial respiratory chain deficiency, has been detected in brains from animal models of AD<sup>162</sup>, and was reversed following deletion of *BACE1* (thereby preventing



**Fig. 3** Model of the “MAM hypothesis” for the pathogenesis of AD, with emphasis on the mitochondrial alterations. See text for details

the formation of C99)<sup>162</sup>. Finally, the idea that mitochondrial dysfunction is the result of the accumulation of C99 in the MAM rather than being a consequence of higher levels of A $\beta_{42}$  helps explain previous results showing that mitochondrial alterations occur early in the pathogenesis of the disease<sup>58</sup>, well before any pathophysiological hallmark of AD becomes apparent<sup>57</sup>. Nevertheless, we believe that even though mitochondrial dysfunction is an early event upstream of plaque and tangle formation, we do not consider the organelle to be a reasonable target for therapeutic intervention, as the mitochondrial perturbations observed in AD are themselves consequences of an even earlier precipitating process, namely elevated C99 and altered lipid homeostasis (Fig. 3). Thus, it is possible that increased ER-mitochondrial connectivity and upregulated MAM behavior underlie the metabolic disturbances (and probably the other phenotypes) seen in AD<sup>122,143,163</sup> (the “MAM hypothesis”<sup>115,116,127,128</sup>). We are currently actively engaged in deducing the mechanism(s) underlying these changes.

Finally, alterations in ER-mitochondrial communication and in MAM behavior may not be confined to AD. Other neurodegenerative disorders, such as Parkinson disease and amyotrophic lateral sclerosis, also evince altered mitochondrial function and disturbances in calcium and lipid homeostasis<sup>164</sup>. Notably, alterations in MAM behavior have also been found in both of these disorders, and are especially prominent in the familial form of these diseases, where a connection between the culprit gene and altered MAM behavior can be drawn<sup>165,166</sup>. Thus, altered ER-mitochondrial communication has the potential to play a critical, and hitherto unappreciated, role in the pathogenesis of many of the most common and devastating diseases of advanced age<sup>129</sup>.

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#### Conflict of interest

The authors declare that they have no conflict of interest.

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#### References

1. Alzheimer's Association. Alzheimer's disease facts and figures. *Alzheimers Dement.* **7**, 208–244 (2011).
2. Goedert, M. & Spillantini, M. G. A century of Alzheimer's disease. *Science* **314**, 777–781 (2006).
3. Hardy, J. A. & Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**, 184–185 (1992).
4. Nunomura, A. et al. Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **60**, 759–767 (2001).
5. Manczak, M., Sheiko, T., Craigen, W. J. & Reddy, P. H. Reduced VDAC1 protects against Alzheimer's disease, mitochondria, and synaptic deficiencies. *J. Alzheimers Dis.* **37**, 679–690 (2013).
6. Morris, G. P., Clark, I. A. & Vissel, B. Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. *Acta Neuropathol. Commun.* **2**, 135 (2014).



7. Pettegrew, J. W., Panchalingam, K., Hamilton, R. L. & McClure, R. J. Brain membrane phospholipid alterations in Alzheimer's disease. *Neurochem. Res.* **26**, 771–782 (2001).
8. Fraser, T., Tayler, H. & Love, S. Fatty acid composition of frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease. *Neurochem. Res.* **35**, 503–513 (2010).
9. Stefani, M. & Liguri, G. Cholesterol in Alzheimer's disease: unresolved questions. *Curr. Alzheimer Res.* **6**, 15–29 (2009).
10. Gómez-Ramos, P. & Asunción Morán, M. Ultrastructural localization of intraneuronal A $\beta$ -peptide in Alzheimer disease brains. *J. Alzheimers Dis.* **11**, 53–59 (2007).
11. Pani, A. et al. Altered cholesterol ester cycle in skin fibroblasts from patients with Alzheimer's disease. *J. Alzheimers Dis.* **18**, 829–841 (2009).
12. Pani, A. et al. Accumulation of neutral lipids in peripheral blood mononuclear cells as a distinctive trait of Alzheimer patients and asymptomatic subjects at risk of disease. *BMC Med.* **7**, 66–77 (2009).
13. Mosconi, L. Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. *Eur. J. Nucl. Med. Mol. Imaging* **32**, 486–510 (2005).
14. Bezprozvanny, I. & Mattson, M. P. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci.* **31**, 454–463 (2008).
15. Lindholm, D., Wootz, H. & Korhonen, L. ER stress and neurodegenerative diseases. *Cell Death Differ.* **13**, 385–392 (2006).
16. Gillardon, F. et al. Proteomic and functional alterations in brain mitochondria from Tg2576 mice occur before amyloid plaque deposition. *Proteomics* **7**, 605–816 (2007).
17. Wang, X. et al. The role of abnormal mitochondrial dynamics in the pathogenesis of Alzheimer's disease. *J. Neurochem.* **109**, 153–159 (2009).
18. Chen, X., Stern, D. & Yan, S. D. Mitochondrial dysfunction and Alzheimer's disease. *Curr. Alzheimer Res.* **3**, 515–520 (2006).
19. Reddy, P. H. Amyloid beta, mitochondrial structural and functional dynamics in Alzheimer's disease. *Exp. Neurol.* **218**, 286–292 (2009).
20. Moreira, P. I., Carvalho, C., Zhu, X., Smith, M. A. & Perry, G. Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. *Biochim. Biophys. Acta* **1802**, 2–10 (2010).
21. Swerdlow, R. H. Brain aging, Alzheimer's disease, and mitochondria. *Biochim. Biophys. Acta* **1812**, 1630–1639 (2011).
22. Spuch, C., Ortolano, S. & Navarro, C. New insights in the amyloid-beta interaction with mitochondria. *J. Aging Res.* **2012**, 324968 (2012).
23. Coskun, P. et al. A mitochondrial etiology of Alzheimer and Parkinson disease. *Biochim. Biophys. Acta* **1820**, 553–564 (2012).
24. Swerdlow, R. H., Burns, J. M. & Khan, S. M. The Alzheimer's disease mitochondrial cascade hypothesis: progress and perspectives. *Biochim. Biophys. Acta* **1842**, 1219–1231 (2014).
25. Demetrius, L. A. & Driver, J. A. Preventing Alzheimer's disease by means of natural selection. *J. R. Soc. Interface* **12**, 20140919 (2015).
26. Sims, N. R., Finegan, J. M. & Blass, J. P. Altered metabolic properties of cultured skin fibroblasts in Alzheimer's disease. *Ann. Neurol.* **21**, 451–457 (1987).
27. Stokin, G. B. et al. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science* **307**, 1282–1288 (2005).
28. Atamna, H. & Frey, W. H. 2nd Mechanisms of mitochondrial dysfunction and energy deficiency in Alzheimer's disease. *Mitochondrion* **7**, 297–310 (2007).
29. Santos, R. X. et al. A synergistic dysfunction of mitochondrial fission/fusion dynamics and mitophagy in Alzheimer's disease. *J. Alzheimers Dis.* **20** (Suppl. 2), S401–S412 (2010).
30. Su, B. et al. Abnormal mitochondrial dynamics—a novel therapeutic target for Alzheimer's disease? *Mol. Neurobiol.* **41**, 87–96 (2010).
31. Young-Collier, K. J., McArdle, M. & Bennett, J. P. The dying of the light: mitochondrial failure in Alzheimer's disease. *J. Alzheimers Dis.* **28**, 771–781 (2012).
32. DuBoff, B., Feany, M. & Gotz, J. Why size matters—balancing mitochondrial dynamics in Alzheimer's disease. *Trends Neurosci.* **36**, 325–335 (2013).
33. Cai, Q. & Tammineni, P. Alterations in mitochondrial quality control in Alzheimer's disease. *Front. Cell. Neurosci.* **10**, 24 (2016).
34. Mancuso, M., Siciliano, G., Filosto, M. & Murri, L. Mitochondrial dysfunction and Alzheimer's disease: new developments. *J. Alzheimers Dis.* **9**, 111–117 (2006).
35. Cabezas-Opazo, F. A. et al. Mitochondrial dysfunction contributes to the pathogenesis of Alzheimer's disease. *Oxid. Med. Cell. Longev.* **2015**, 509654 (2015).
36. Redjems-Bennani, N. et al. Abnormal substrate levels that depend upon mitochondrial function in cerebrospinal fluid from Alzheimer patients. *Gerontology* **44**, 300–304 (1998).
37. Bosetti, F. et al. Cytochrome c oxidase and mitochondrial F<sub>1</sub>F<sub>0</sub>-ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease. *Neurobiol. Aging* **23**, 371–376 (2002).
38. Ohta, S. & Ohsawa, I. Dysfunction of mitochondria and oxidative stress in the pathogenesis of Alzheimer's disease: on defects in the cytochrome c oxidase complex and aldehyde detoxification. *J. Alzheimers Dis.* **9**, 155–166 (2006).
39. Wilkins, H. M. & Swerdlow, R. H. Amyloid precursor protein processing and bioenergetics. *Brain Res. Bull.* **133**, 71–79 (2017).
40. Gibson, G. E. & Huang, H. M. Mitochondrial enzymes and endoplasmic reticulum calcium stores as targets of oxidative stress in neurodegenerative diseases. *J. Bioenerg. Biomembr.* **36**, 335–340 (2004).
41. Bubber, P., Haroutunian, V., Fisch, G., Blass, J. P. & Gibson, G. E. Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. *Ann. Neurol.* **57**, 695–703 (2005).
42. Gibson, G. E. et al. Deficits in the mitochondrial enzyme  $\alpha$ -ketoglutarate dehydrogenase lead to Alzheimer's disease-like calcium dysregulation. *Neurobiol. Aging* **33**, 1121.e1113–1124 (2012).
43. Hirai, K. et al. Mitochondrial abnormalities in Alzheimer's disease. *J. Neurosci.* **21**, 3017–3023 (2001).
44. Manczak, M. et al. Mitochondria are a direct site of A $\beta$  accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum. Mol. Genet.* **15**, 1437–1449 (2006).
45. Nunomura, A. et al. Involvement of oxidative stress in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **65**, 631–641 (2006).
46. Du, H. et al. Early deficits in synaptic mitochondria in an Alzheimer's disease mouse model. *Proc. Natl Acad. Sci. USA* **107**, 18670–18675 (2010).
47. Wang, X., Perry, G., Smith, M. A. & Zhu, X. Amyloid- $\beta$ -derived diffusible ligands cause impaired axonal transport of mitochondria in neurons. *Neurodegener. Dis.* **7**, 56–59 (2010).
48. Calkins, M. J. & Reddy, P. H. Amyloid beta impairs mitochondrial anterograde transport and degenerates synapses in Alzheimer's disease neurons. *Biochim. Biophys. Acta* **1812**, 507–513 (2011).
49. Riemer, J. & Kins, S. Axonal transport and mitochondrial dysfunction in Alzheimer's disease. *Neurodegener. Dis.* **12**, 111–124 (2013).
50. Wang, X. et al. Amyloid- $\beta$  overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins. *Proc. Natl Acad. Sci. USA* **105**, 19318–19323 (2008).
51. Nakamura, T., Cieplak, P., Cho, D. H., Godzik, A. & Lipton, S. A. S-nitrosylation of Drp1 links excessive mitochondrial fission to neuronal injury in neurodegeneration. *Mitochondrion* **10**, 573–578 (2010).
52. Manczak, M., Calkins, M. J. & Reddy, P. H. Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. *Hum. Mol. Genet.* **20**, 2495–2509 (2011).
53. Han, X. J. et al. Amyloid  $\beta$ -42 induces neuronal apoptosis by targeting mitochondria. *Mol. Med. Rep.* **16**, 4521–4528 (2017).
54. Wang, X., Su, B., Fujioka, H. & Zhu, X. Dynamin-like protein 1 reduction underlies mitochondrial morphology and distribution abnormalities in fibroblasts from sporadic Alzheimer's disease patients. *Am. J. Pathol.* **173**, 470–482 (2008).
55. Bonda, D. J., Wang, X., Perry, G., Smith, M. A. & Zhu, X. Mitochondrial dynamics in Alzheimer's disease: opportunities for future treatment strategies. *Drugs Aging* **27**, 181–192 (2010).
56. Devi, G. et al. Novel presenilin 1 mutations associated with early onset of dementia in a family with both early-onset and late-onset Alzheimer disease. *Arch. Neurol.* **57**, 1454–1457 (2000).
57. Yao, J. et al. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **106**, 14670–14675 (2009).
58. Ballelli, M. et al. Early selective vulnerability of synapses and synaptic mitochondria in the hippocampal CA1 region of the Tg2576 mouse model of Alzheimer's disease. *J. Alzheimers Dis.* **34**, 887–896 (2013).
59. Wang, X. et al. Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochim. Biophys. Acta* **1842**, 1240–1247 (2014).
60. Khalil, B. et al. PINK1-induced mitophagy promotes neuroprotection in Huntington's disease. *Cell Death Dis.* **6**, e1617 (2015).



61. Guo, X. et al. VCP recruitment to mitochondria causes mitophagy impairment and neurodegeneration in models of Huntington's disease. *Nat. Commun.* **7**, 12646 (2016).
62. Shan, X., Chiang, P. M., Price, D. L. & Wong, P. C. Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. *Proc. Natl Acad. Sci. USA* **107**, 16325–16330 (2010).
63. Xu, Y. F. et al. Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. *J. Neurosci.* **30**, 10851–10859 (2010).
64. Wang, W. et al. The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons. *Hum. Mol. Genet.* **22**, 4706–4719 (2013).
65. Magrane, J., Cortez, C., Gan, W. B. & Manfredi, G. Abnormal mitochondrial transport and morphology are common pathological denominators in SOD1 and TDP43 ALS mouse models. *Hum. Mol. Genet.* **23**, 1413–1424 (2014).
66. Waterham, H. R. et al. A lethal defect of mitochondrial and peroxisomal fission. *N. Engl. J. Med.* **356**, 1736–1741 (2007).
67. Frieden, M. et al. Ca<sup>2+</sup> homeostasis during mitochondrial fragmentation and perinuclear clustering induced by hFis1. *J. Biol. Chem.* **279**, 22704–22714 (2004).
68. Li, X. C. et al. Human wild-type full-length tau accumulation disrupts mitochondrial dynamics and the functions via increasing mitofusins. *Sci. Rep.* **6**, 24756 (2016).
69. Mancuso, M., Calsolaro, V., Orsucci, D., Siciliano, G. & Murri, L. Is there a primary role of the mitochondrial genome in Alzheimer's disease? *J. Bioenerg. Biomembr.* **41**, 411–416 (2009).
70. Lakatos, A. et al. Association between mitochondrial DNA variations and Alzheimer's disease in the ADNI cohort. *Neurobiol. Aging* **31**, 1355–1363 (2010).
71. Santoro, A. et al. Evidence for sub-haplogroup H5 of mitochondrial DNA as a risk factor for late onset Alzheimer's disease. *PLoS ONE* **5**, e12037 (2010).
72. Maruszak, A. et al. The impact of mitochondrial and nuclear DNA variants on late-onset Alzheimer's disease risk. *J. Alzheimers Dis.* **27**, 197–210 (2011).
73. Ridge, P. G. et al. Mitochondrial haplotypes associated with biomarkers for Alzheimer's disease. *PLoS ONE* **8**, e74158 (2013).
74. Corral-Debrinski, M. et al. Marked changes in mitochondrial DNA deletion levels in Alzheimer brains. *Genomics* **23**, 471–476 (1994).
75. Krishnan, K. J., Ratnaik, T. E., De Gruyter, H. L., Jaros, E. & Turnbull, D. M. Mitochondrial DNA deletions cause the biochemical defect observed in Alzheimer's disease. *Neurobiol. Aging* **33**, 2210–2214 (2012).
76. Brown, M. D. et al. Mitochondrial DNA sequence analysis of four Alzheimer's and Parkinson's disease patients. *Am. J. Med. Genet.* **61**, 283–289 (1996).
77. Hutchin, T. & Cortopassi, G. A mitochondrial DNA clone is associated with increased risk for Alzheimer disease. *Proc. Natl Acad. Sci. USA* **92**, 6892–6895 (1995).
78. Coskun, P. E., Beal, M. F. & Wallace, D. C. Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc. Natl Acad. Sci. USA* **101**, 10726–10731 (2004).
79. Podlesniy, P. et al. Low cerebrospinal fluid concentration of mitochondrial DNA in preclinical Alzheimer disease. *Ann. Neurol.* **74**, 655–668 (2013).
80. Lunnon, K. et al. Mitochondrial genes are altered in blood early in Alzheimer's disease. *Neurobiol. Aging* **53**, 36–47 (2017).
81. Zsurka, G. et al. No mitochondrial haplotype was found to increase risk for Alzheimer's disease. *Biol. Psychiatry* **44**, 371–373 (1998).
82. Rodriguez Santiago, B., Casademont, J. & Nunes, V. Is there a relation between Alzheimer's disease and defects of mitochondrial DNA? *Rev. Neurol.* **33**, 301–305 (2001).
83. Hudson, G. et al. No consistent evidence for association between mtDNA variants and Alzheimer disease. *Neurology* **78**, 1038–1042 (2012).
84. Payami, H. & Hoffbuhr, K. Lack of evidence for maternal effect in familial Alzheimer's disease. *Genet. Epidemiol.* **10**, 461–464 (1993).
85. Ehrenkrantz, D. et al. Genetic epidemiological study of maternal and paternal transmission of Alzheimer's disease. *Am. J. Med. Genet.* **88**, 378–382 (1999).
86. Karch, C. M. & Goate, A. M. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biol. Psychiatry* **77**, 43–51 (2015).
87. Ankarcona, M. & Hultenby, K. Presenilin-1 is located in rat mitochondria. *Biochem. Biophys. Res. Commun.* **295**, 766–770 (2002).
88. Anandatheerthavarada, H. K., Biswas, G., Robin, M. A. & Avadhani, N. G. Mitochondrial targeting and a novel transmembrane arrest of Alzheimer's amyloid precursor protein impairs mitochondrial function in neuronal cells. *J. Cell Biol.* **161**, 41–54 (2003).
89. Lustbader, J. W. et al. AβAD directly links Aβ to mitochondrial toxicity in Alzheimer's disease. *Science* **304**, 448–452 (2004).
90. Devi, L., Prabhu, B. M., Galati, D. F., Avadhani, N. G. & Anandatheerthavarada, H. K. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J. Neurosci.* **26**, 9057–9068 (2006).
91. Hansson Petersen, C. A. et al. The amyloid beta-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. *Proc. Natl Acad. Sci. USA* **105**, 13145–13150 (2008).
92. Pagani, L. & Eckert, A. Amyloid-beta interaction with mitochondria. *Int. J. Alzheimers Dis.* **2011**, 925050 (2011).
93. Walls, K. C. et al. Swedish Alzheimer mutation induces mitochondrial dysfunction mediated by HSP60 mislocalization of amyloid precursor protein (APP) and beta-amyloid. *J. Biol. Chem.* **287**, 30317–30327 (2012).
94. Xie, H. et al. Mitochondrial alterations near amyloid plaques in an Alzheimer's disease mouse model. *J. Neurosci.* **33**, 17042–17051 (2013).
95. Hansson, C. A. et al. Nicastrin, presenilin, APH-1, and PEN-2 form active γ-secretase complexes in mitochondria. *J. Biol. Chem.* **279**, 51654–51660 (2004).
96. Behbahani, H. et al. Association of Omi/HtrA2 with γ-secretase in mitochondria. *Neurochem. Int.* **57**, 668–675 (2010).
97. Pavlov, P. F. et al. Mitochondrial γ-secretase participates in the metabolism of mitochondria-associated amyloid precursor protein. *FASEB J.* **25**, 78–88 (2011).
98. Hayashi, H. et al. HIG1, a novel regulator of mitochondrial γ-secretase, maintains normal mitochondrial function. *FASEB J.* **26**, 2306–2317 (2012).
99. Robinson, A. et al. Upregulation of PGC-1α expression by Alzheimer's disease-associated pathway: presenilin 1/amyloid precursor protein (APP)/intracellular domain of APP. *Aging Cell* **13**, 263–272 (2014).
100. Peterson, C. & Goldman, J. E. Alterations in calcium content and biochemical processes in cultured skin fibroblasts from aged and Alzheimer donors. *Proc. Natl Acad. Sci. USA* **83**, 2758–2762 (1986).
101. Casley, C. S., Canevari, L., Land, J. M., Clark, J. B. & Sharpe, M. A. β-Amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J. Neurochem.* **80**, 91–100 (2002).
102. Keil, U. et al. Mitochondrial dysfunction induced by disease relevant AβPP and tau protein mutations. *J. Alzheimers Dis.* **9**, 139–146 (2006).
103. Rhein, V. et al. Amyloid-β leads to impaired cellular respiration, energy production and mitochondrial electron chain complex activities in human neuroblastoma cells. *Cell. Mol. Neurobiol.* **29**, 1063–1071 (2009).
104. Schaefer, P. M., von Einem, B., Walther, P., Calzia, E. & von Arnim, C. A. Metabolic characterization of intact cells reveals intracellular amyloid beta but not its precursor protein to reduce mitochondrial respiration. *PLoS ONE* **11**, e0168157 (2016).
105. Mossmann, D. et al. Amyloid-β peptide induces mitochondrial dysfunction by inhibition of preprotein maturation. *Cell Metab.* **20**, 662–669 (2014).
106. Iijima-Ando, K. et al. Mitochondrial mislocalization underlies Aβ42-induced neuronal dysfunction in a *Drosophila* model of Alzheimer's disease. *PLoS ONE* **4**, e8310 (2009).
107. Area-Gomez, E. et al. Presenilins are enriched in endoplasmic reticulum membranes associated with mitochondria. *Am. J. Pathol.* **175**, 1810–1816 (2009).
108. Mamada, N., Tanokashira, D., Ishii, K., Tamaoka, A. & Araki, W. Mitochondria are devoid of amyloid β-protein (Aβ)-producing secretases: evidence for unlikely occurrence within mitochondria of Aβ generation from amyloid precursor protein. *Biochem. Biophys. Res. Commun.* **486**, 321–328 (2017).
109. Wada, S. et al. γ-Secretase activity is present in rafts but is not cholesterol-dependent. *Biochemistry* **42**, 13977–13986 (2003).
110. Vetrivel, K. S. et al. Association of γ-secretase with lipid rafts in post-Golgi and endosome membranes. *J. Biol. Chem.* **279**, 44945–44954 (2004).
111. Urano, Y. et al. Association of active γ-secretase complex with lipid rafts. *J. Lipid Res.* **46**, 904–912 (2005).
112. Cordy, J. M., Hooper, N. M. & Turner, A. J. The involvement of lipid rafts in Alzheimer's disease. *Mol. Membr. Biol.* **23**, 111–122 (2006).
113. Zheng, Y. Z., Berg, K. B. & Foster, L. J. Mitochondria do not contain lipid rafts, and lipid rafts do not contain mitochondrial proteins. *J. Lipid Res.* **50**, 988–998 (2009).
114. Lue, L. F. et al. Soluble amyloid β peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am. J. Pathol.* **155**, 853–862 (1999).

115. Schon, E. A. & Area-Gomez, E. Is Alzheimer's disease a disorder of mitochondria-associated membranes?. *J. Alzheimers Dis.* **20**(Suppl. 2), S281–S292 (2010).
116. Schon, E. A. & Area-Gomez, E. Mitochondria-associated ER membranes in Alzheimer disease. *Mol. Cell. Neurosci.* **55**, 26–36 (2013).
117. Newman, M. et al. Differential, dominant activation and inhibition of Notch signalling and APP cleavage by truncations of PSEN1 in human disease. *Hum. Mol. Genet.* **23**, 602–617 (2014).
118. Schreiner, B., Hedskog, L., Wiehager, B. & Ankarcrona, M. Amyloid- $\beta$  peptides are generated in mitochondria-associated endoplasmic reticulum membranes. *J. Alzheimers Dis.* **43**, 369–374 (2015).
119. Del Prete, D. et al. Localization and processing of the amyloid- $\beta$  protein precursor in mitochondria-associated membranes. *J. Alzheimers Dis.* **55**, 1549–1570 (2017).
120. Annaert, W. G. et al. Presenilin 1 controls  $\gamma$ -secretase processing of amyloid precursor protein in pre-Golgi compartments of hippocampal neurons. *J. Cell Biol.* **147**, 277–294 (1999).
121. Hayashi, T. & Su, T. P. Cholesterol at the endoplasmic reticulum: roles of the sigma-1 receptor chaperone and implications thereof in human diseases. *Subcell. Biochem.* **51**, 381–398 (2010).
122. Area-Gomez, E. et al. Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. *EMBO J.* **31**, 4106–4123 (2012).
123. Hayashi, T., Rizzuto, R., Hajnoczky, G. & Su, T. P. MAM: more than just a housekeeper. *Trends Cell Biol.* **19**, 81–88 (2009).
124. Hedskog, L. et al. Modulation of the endoplasmic reticulum-mitochondria interface in Alzheimer's disease and related models. *Proc. Natl Acad. Sci. USA* **110**, 7916–7921 (2013).
125. Pinho, C. M., Teixeira, P. F. & Glaser, E. Mitochondrial import and degradation of amyloid- $\beta$  peptide. *Biochim. Biophys. Acta* **1837**, 1069–1074 (2014).
126. Contino, S. et al. Presenilin 2-dependent maintenance of mitochondrial oxidative capacity and morphology. *Front. Physiol.* **8**, 796 (2017).
127. Area-Gomez, E. & Schon, E. A. Mitochondria-associated ER membranes and Alzheimer disease. *Curr. Opin. Genet. Dev.* **38**, 90–96 (2016).
128. Area-Gomez, E. & Schon, E. A. On the pathogenesis of Alzheimer's disease: the MAM Hypothesis. *FASEB J.* **31**, 864–867 (2017).
129. Krols, M. et al. Mitochondria-associated membranes as hubs for neurodegeneration. *Acta Neuropathol.* **131**, 505–523 (2016).
130. Paillusson, S. et al. There's something wrong with my MAM; the ER-mitochondria axis and neurodegenerative diseases. *Trends Neurosci.* **39**, 146–157 (2016).
131. Simmen, T., Lynes, E. M., Gesson, K. & Thomas, G. Oxidative protein folding in the endoplasmic reticulum: tight links to the mitochondria-associated membrane (MAM). *Biochim. Biophys. Acta* **1798**, 1465–1473 (2010).
132. Giorgi, C. et al. Mitochondria-associated membranes: composition, molecular mechanisms, and physiopathological implications. *Antioxid. Redox Signal.* **22**, 995–1019 (2015).
133. Giacomello, M. & Pellegrini, L. The coming of age of the mitochondria-ER contact: a matter of thickness. *Cell Death Differ.* **23**, 1417–1427 (2016).
134. Rusinol, A. E., Cui, Z., Chen, M. H. & Vance, J. E. A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins. *J. Biol. Chem.* **269**, 27494–27502 (1994).
135. Puglielli, L. et al. Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid  $\beta$ -peptide. *Nat. Cell Biol.* **3**, 905–912 (2001).
136. Puglielli, L., Ellis, B. C., Ingano, L. A. & Kovacs, D. M. Role of acyl-coenzyme A: cholesterol acyltransferase activity in the processing of the amyloid precursor protein. *J. Mol. Neurosci.* **24**, 93–96 (2004).
137. Lopez Sanchez, M. I. G. et al. Amyloid precursor protein drives down-regulation of mitochondrial oxidative phosphorylation independent of amyloid beta. *Sci. Rep.* **7**, 9835 (2017).
138. Csordas, G. et al. Structural and functional features and significance of the physical linkage between ER and mitochondria. *J. Cell Biol.* **174**, 915–921 (2006).
139. Patergnani, S. et al. Calcium signaling around mitochondria associated membranes (MAMs). *Cell. Commun. Signal.* **9**, 19 (2011).
140. Tasseva, G. et al. Phosphatidylethanolamine deficiency in mammalian mitochondria impairs oxidative phosphorylation and alters mitochondrial morphology. *J. Biol. Chem.* **288**, 4158–4173 (2013).
141. Theurey, P. et al. Mitochondria-associated endoplasmic reticulum membranes allow adaptation of mitochondrial metabolism to glucose availability in the liver. *J. Mol. Cell Biol.* **8**, 129–143 (2016).
142. Passer, B. et al. Generation of an apoptotic intracellular peptide by  $\gamma$ -secretase cleavage of Alzheimer's amyloid  $\beta$  protein precursor. *J. Alzheimers Dis.* **2**, 289–301 (2000).
143. Pera, M. et al. Increased localization of APP-C99 in mitochondria-associated ER membranes causes mitochondrial dysfunction in Alzheimer disease. *EMBO J.* **36**, 3356–3371 (2017).
144. Haimovitz-Friedman, A., Kolesnick, R. N. & Fuks, Z. Ceramide signaling in apoptosis. *Br. Med. Bull.* **53**, 539–553 (1997).
145. Yu, J. et al. JNK3 signaling pathway activates ceramide synthase leading to mitochondrial dysfunction. *J. Biol. Chem.* **282**, 25940–25949 (2007).
146. Monette, J. S. et al. R)- $\alpha$ -Lipoic acid treatment restores ceramide balance in aging rat cardiac mitochondria. *Pharmacol. Res.* **63**, 23–29 (2011).
147. Trounce, I. A., Crouch, P. J., Carey, K. T. & McKenzie, M. Modulation of ceramide-induced cell death and superoxide production by mitochondrial DNA-encoded respiratory chain defects in *Rattus* xenocybrid mouse cells. *Biochim. Biophys. Acta* **1827**, 817–825 (2013).
148. Zigdon, H. et al. Ablation of ceramide synthase 2 causes chronic oxidative stress due to disruption of the mitochondrial respiratory chain. *J. Biol. Chem.* **288**, 4947–4956 (2013).
149. Kogot-Levin, A. & Saada, A. Ceramide and the mitochondrial respiratory chain. *Biochimie* **100**, 88–94 (2014).
150. Behbahani, H. et al. Differential role of presenilin-1 and -2 on mitochondrial membrane potential and oxygen consumption in mouse embryonic fibroblasts. *J. Neurosci. Res.* **84**, 891–902 (2006).
151. Wang, Y. et al. Lost region in amyloid precursor protein (APP) through TALEN-mediated genome editing alters mitochondrial morphology. *Sci. Rep.* **6**, 22244 (2016).
152. Holtzman, D. M., Herz, J. & Bu, G. Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* **2**, a006312 (2012).
153. Tambini, M. D. et al. ApoE4 upregulates the activity of mitochondria-associated ER membranes. *EMBO Rep.* **17**, 27–36 (2016).
154. Fukumoto, H., Cheung, B. S., Hyman, B. T. & Irlizarry, M. C.  $\beta$ -secretase protein and activity are increased in the neocortex in Alzheimer disease. *Arch. Neurol.* **59**, 1381–1389 (2002).
155. Li, R. et al. Amyloid  $\beta$  peptide load is correlated with increased  $\beta$ -secretase activity in sporadic Alzheimer's disease patients. *Proc. Natl Acad. Sci. USA* **101**, 3632–3637 (2004).
156. Pera, M. et al. Distinct patterns of APP processing in the CNS in autosomal-dominant and sporadic Alzheimer disease. *Acta Neuropathol.* **125**, 201–213 (2013).
157. Heilig, E. A., Gutti, U., Tai, T., Shen, J. & Kelleher, R. J. 3rd Trans-dominant negative effects of pathogenic PSEN1 mutations on  $\gamma$ -secretase activity and A $\beta$  production. *J. Neurosci.* **33**, 11606–11617 (2013).
158. Kepp, K. P. Alzheimer's disease due to loss of function: a new synthesis of the available data. *Prog. Neurobiol.* **143**, 36–60 (2016).
159. Sun, L., Zhou, R., Yang, G. & Shi, Y. Analysis of 138 pathogenic mutations in presenilin-1 on the in vitro production of A $\beta$ 42 and A $\beta$ 40 peptides by  $\gamma$ -secretase. *Proc. Natl Acad. Sci. USA* **114**, E476–E485 (2017).
160. Zhou, R., Yang, G. & Shi, Y. Dominant negative effect of the loss-of-function  $\gamma$ -secretase mutants on the wild-type enzyme through heterooligomerization. *Proc. Natl Acad. Sci. USA* **114**, 12731–12736 (2017).
161. Kretner, B. et al. Generation and deposition of A $\beta$ 43 by the virtually inactive presenilin-1 L435F mutant contradicts the presenilin loss-of-function hypothesis of Alzheimer's disease. *EMBO Mol. Med.* **8**, 458–465 (2016).
162. Devi, L. & Ohno, M. Mitochondrial dysfunction and accumulation of the  $\beta$ -secretase-cleaved C-terminal fragment of APP in Alzheimer's disease transgenic mice. *Neurobiol. Dis.* **45**, 417–424 (2012).
163. Leal, N. S. et al. Mitofusin-2 knockdown increases ER-mitochondria contact and decreases amyloid  $\beta$ -peptide production. *J. Cell. Mol. Med.* **20**, 1686–1695 (2016).
164. Schon, E. A. & Przedborski, S. Mitochondria: the next (neuro)generation. *Neuron* **70**, 1033–1053 (2011).
165. Watanabe, S. et al. Mitochondria-associated membrane collapse is a common pathomechanism in SIGMAR1- and SOD1-linked ALS. *EMBO Mol. Med.* **8**, 1421–1437 (2016).
166. Hattori, N., Arano, T., Hatano, T., Mori, A. & Imai, Y. Mitochondrial-associated membranes in Parkinson's disease. *Adv. Exp. Med. Biol.* **997**, 157–169 (2017).