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Mitochondria-associated ER membranes and Alzheimer Disease

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Abstract

The series of events underlying the pathogenesis of Alzheimer disease (AD) in unknown. The most widely-accepted hypothesis is called the amyloid cascade, based on the observation that the brains of AD patients contain high levels of extracellular plaques, composed mainly of β-amyloid (Aβ), and intracellular tangles, composed of hyperphosphorylated forms of the microtubule-associated protein tau. However, AD is also characterized by other features, including aberrant cholesterol, phospholipid, and calcium metabolism, and mitochondrial dysfunction, all ostensibly unrelated to plaque and tangle formation. Notably, these "other" aspects of AD pathology are functions related to mitochondria-associated ER membranes (MAM), a subdomain of the endoplasmic reticulum (ER) that is apposed to, and communicates with, mitochondria. Given the potential relationship between MAM and AD, we explored the possibility that perturbed MAM function might play a role in AD pathogenesis. We found that γ-secretase activity, which processes the amyloid precursor protein to generate Aβ, is located predominantly in the MAM, and that ER-mitochondrial apposition and MAM function are increased significantly in cells from AD patients. These observations may help explain not only the aberrant Aβ production, but also many of the "other" biochemical and morphological features of the disease. Based on these, and other, data we propose that AD is fundamentally a disorder of ER-mitochondrial hyperconnectivity.

Alzheimer disease

Alzheimer disease (AD) is the most common neurodegenerative dementia of aging [1]. It is defined operationally as a disorder in which there is an accumulation of extracellular neuritic plaques and intracellular tangles in the brain [1]. The plaques are composed of numerous proteins [2,3], but foremost among them is β-amyloid (Aβ). The tangles are more homogeneous and consist mainly of aggregates of hyperphosphorylated forms of the microtubule-associated protein tau [1,4].

The familial form of AD (FAD), which affects ~1% of all patients, is inherited as an autosomal-dominant trait, and is caused by mutations in one of three genes: presenilin-1 (PS1), presenilin-2 (PS2), and the amyloid precursor protein (APP). PS1 and PS2 are aspartyl proteases that are the enzymatically active components of the γ-secretase complex

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that, together with β-secretase (BACE1), processes APP to produce Aβ, thereby likely explaining the deposition of amyloid in these patients (Fig. 1). In sporadic AD (SAD), which comprises the vast majority of cases, the mechanistic connection to amyloid deposition is far less clear. However, individuals harboring the ε4 allele of apolipoprotein E (hereinafter ApoE4), which is a component of lipoproteins that are involved in intercellular cholesterol trafficking, are at significantly higher risk for developing AD than those harboring the more common ε3 allele [5,6]; the reason for this elevated risk is unknown.

The amyloid cascade hypothesis

The pathogenetic mechanism that causes AD is unknown, but the discovery that mutations in presenilins (which cleave APP) and in APP itself (which is a substrate of the presenilin-containing γ-secretase complex) in FAD patients gave rise to the most dominant, and commonly-accepted, hypothesis to explain pathogenicity in both FAD and SAD, namely the "amyloid cascade" [7]. The hypothesis proposes that the disease arises when APP is cleaved by BACE1 to produce a 99-aa C-terminal fragment (C99), which is then cleaved by γ-secretase to produce a ~50-aa APP intracellular domain (AICD) and a range of Aβ fragments that average ~40-aa in length in normal individuals but ~42-aa in AD (Fig. 1), with a concomitant increase in the ratio of $A\beta_{42}$:$A\beta_{40}$. As opposed to $A\beta_{40}$, $A\beta_{42}$ is fibrillogenic and accumulates in the plaques. This amyloid is toxic to cells, and the resulting stress promotes tau hyperphosphorylation, leading to the tangles, with both the extraneuritic plaques and the intraneuronal tangles conspiring to cause the disease by promoting cell death [4,7] (Fig. 2).

The amyloid cascade hypothesis is compelling, not only because it unites findings from many different approaches to the disease, but also because it explains why mutations in both the γ-secretase enzyme (i.e. PS1, PS2) and its substrate (i.e. APP) cause FAD. However, besides the obvious problem of the lack of mutations in these three proteins in SAD, a nagging concern has been that the amyloid cascade does not address a number of issues that are central to both understanding and explaining AD pathogenesis. In particular, AD is associated with other features that have received less attention in the field, because there is no unifying conceptual framework within the amyloid cascade that would explain their occurrence [8,9]. These include altered cholesterol [10], fatty acid [11], glucose [12,13], and phospholipid [14] metabolism, aberrant calcium homeostasis [15], and mitochondrial dysfunction [16] (Fig. 3).

Mitochondria-associated ER membranes

As a group focused predominantly on human mitochondrial biology, function, and disease [17], we noticed that there was a common theme that had the potential to unite these disparate features of AD under a single rubric, namely mitochondria-associated ER membranes (MAM). MAM is a subdomain of the endoplasmic reticulum (ER) that communicates with mitochondria, both physically and biochemically [18–21]. Among the proteins enriched in or localized to the MAM are those involved in lipid metabolism (e.g. phosphatidylserine synthase [22,23]), in cholesterol metabolism (e.g. acyl-CoA:cholesterol acyltransferase 1 [ACAT1] [21] and steroidogenic acute regulatory protein [24]), in calcium

homeostasis (e.g. IP3 receptors [25,26]), in lipid transfer between the ER and mitochondria (e.g. fatty acid transfer protein 4 [27]), and in the maintenance of mitochondrial function (e.g. voltage-dependent anion channel 2 [24]) and morphology (e.g dynamin-related protein 1 [28]); notably, these MAM-related functions are the very ones that are perturbed in AD. In addition, other proteins stabilize and regulate the interaction of ER and mitochondria, including mitofusin 2 [29] and phosphofurin acidic cluster sorting protein 2 [30], but the exact "tethering" mechanism is unknown.

Based on the supposition that MAM might play a role in AD pathogenesis, we and others found that PS1 and PS2 [31,32], and γ-secretase activity itself [31,33], are localized predominantly at the MAM. This localization could account for the reported localizations of Aβ [34] and PS1 [35] to mitochondria. In addition, the finding that MAM is an intracellular lipid raft [36,37] supports the view that the lipid rafts in which PS1 and γ-secretase activity are known to reside [38] are located not only at the plasma membrane [31,39] but also intracellularly, at the MAM [36,37].

MAM function is perturbed in AD

Given the enrichment of the presenilins and of γ-secretase activity in the MAM, we explored the possibility that MAM function might be altered in AD, minimally in presenilin-mutant cells and in cells from FAD patients harboring mutations in presenilin (FAD^{PS}). Some AD-relevant aspects of MAM behavior, such as calcium metabolism and mitochondrial function, have been studied in great detail by others, albeit not in the context of MAM. For example, there is a rather large literature showing that calcium trafficking, which is fundamentally a MAM-mediated process [19,40,41], is altered in AD patients [42–44], in patient fibroblasts [45–47], and in PS1-mutant mice [48]. Similarly, mitochondrial function, including bioenergetics and mitochondrial dynamics, have also been shown to be altered significantly in AD [46,49–55]. Thus, the changes found in calcium and mitochondrial function in AD are almost certainly due to perturbed ER-mitochondrial communication at the MAM. We therefore decided to study other aspects of MAM function that are relevant to AD but that have received less attention, focusing on phospholipid [14] and cholesterol [10] homeostasis in mouse embryonic fibroblasts (MEFs) lacking presenilins, in PS1-knockdown MEFs, and in fibroblasts from FAD^{PS} patients.

In broad view, phospholipid synthesis takes place in two compartments: the cytoplasm (via the "Kennedy" pathway) and the MAM [56]. In the MAM, phosphatidylserine (PtdSer) translocates from the ER to mitochondria, where it is converted to phosphatidylethanolamine (PtdEtn); PtdEtn then travels back to the ER to be converted into phosphatidylcholine [57]. We found that PtdSer and PtdEtn synthesis and transport were increased significantly in PS-mutant cells and FAD^{PS} fibroblasts [36], indicating upregulated ER-mitochondrial cross-talk in those cells. These results are consistent with, and may help explain, the altered phospholipid profiles seen in FAD patients [14].

In addition to phospholipid synthesis and transport, we measured another MAM-localized activity [21,36], namely, the intracellular conversion by ACAT1 (gene *SOAT1*) of free cholesterol to cholesteryl esters (CE) that are then stored in cytoplasmic lipid droplets [58].

We found significantly more CE in the PS-mutant and FAD^{PS} fibroblasts than in the corresponding control cells [36]. Notably, the increase in CE in these cells was parallelled by a concomitant increase in cytoplasmic neutral lipid droplets (containing CE) [36], indicating that these cells were trying to maintain cholesterol homeostasis [58] by reducing excess free cholesterol that would otherwise be toxic to the cells [59]. We note that patients with AD have elevated circulating cholesterol [10] and increased lipid droplet formation [60,61], and that ACAT1 activity appears to be required for the production of Aβ [58,62], but the reasons for these observations are currently unknown.

Remarkably, these findings on altered MAM-mediated phospholipid and cholesterol homeostasis and on increased ER-mitochondrial apposition were not limited to presenilin-mutant cells. We obtained essentially identical results in fibroblasts from FAD patients with mutations in APP, and even more notably, in fibroblasts from patients with SAD [36]. The fact that lipid dyshomeostasis and altered MAM morphology could be observed in AD cells in which the presenilin and/or APP genes are normal implies that upregulated ER-mitochondrial communication and dysregulated MAM function are likely to be a general property of cells from essentially all AD patients, and that altered MAM behavior is a pathogenetic event upstream of plaque and tangle formation.

The idea that perturbed MAM behavior is a precipitating event in AD was reinforced by recent work on the differential effects of ApoE4 vs ApoE3 on MAM function [63]. Specifically, human fibroblasts and neuronal-like SH-SY5Y cells treated with astrocyte-conditioned media (ACM) from knock-in mice expressing human ApoE4 upregulated MAM function to a significantly greater extent than did ACM from ApoE3 knock-in mice [63]. Moreover, the effects of ApoE4 were apparently due to its role as a component of lipoproteins, not as the free, unlipidated protein [63], implying that its effects on MAM were related to its function in lipoprotein-mediated cholesterol trafficking and metabolism. We note that ApoE4 is recycled less efficiently than is ApoE3 [64,65], thereby causing cholesterol to accumulate in endosomes and lysosomes, which can then traffic to the ER [66]. Thus, the increase in CE and lipid droplets seen in ApoE4-ACM-treated cells may well have reflected a concomitant increase in cholesterol metabolism at the MAM. Thus, ApoE4 in SAD cells appears to mimic the effects of upregulated cholesterol metabolism in FAD cells, and may help explain, in part, the contribution of ApoE4 as a risk factor in the disease. This supposition is also consistent with the identification of allelic variants in other cholesterol metabolism-related genes as risk factors in AD [67], including *ABCA7* [68], which promotes the cellular efflux of both phospholipids and cholesterol [69].

The MAM hypothesis

Based on the findings summarized above, we propose that the pathogenesis of AD is mediated by increased ER-mitochondrial communication, which in turn alters the function of proteins that reside at the interface of these two organelles, both in degree and in kind (Fig. 4). The increase in ER-mitochondrial apposition would be consistent with, and perhaps explain, the increased calcium trafficking between the two organelles [15,42–44], the aberrant phospholipid profiles [14], the perturbed cholesterol homeostasis [10], the changes in mitochondrial function, morphology, and distribution [16,46,49–55], and the increased

$A\beta_{42}$:$A\beta_{40}$ ratio seen in AD (Fig. 4). On this last point, we believe that the altered ER membrane topology at the MAM in AD [70] could explain the shift in the location of the γ-secretase cleavage site on APP-C99 away from $A\beta_{40}$ and towards $A\beta_{42}$ [9].

Taken together, our findings support the view that the *functional* cause of AD is increased ER-mitochondrial communication and upregulated MAM function. However, the *biochemical* cause of ER-mitochondrial hyperconnectivity, and its relationship to aberrant APP processing, are currently unclear. Work to elucidate these upstream causes of perturbed MAM structure and function is currently under way.

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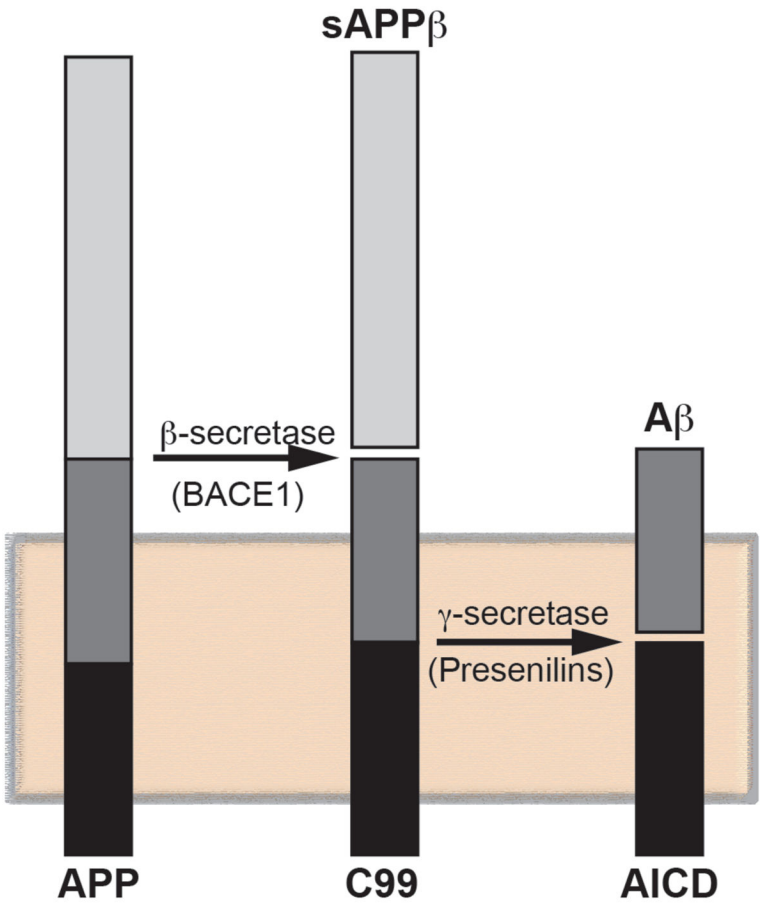

Figure 1. Amyloidogenic processing of APP

Consecutive cleavages of APP, a membrane-tethered protein (shaded box), by BACE1 (to produce C99 and soluble APPβ) and γ-secretase (containing PS1 or PS2) generate Aβ and the APP intracellular domain (AICD).

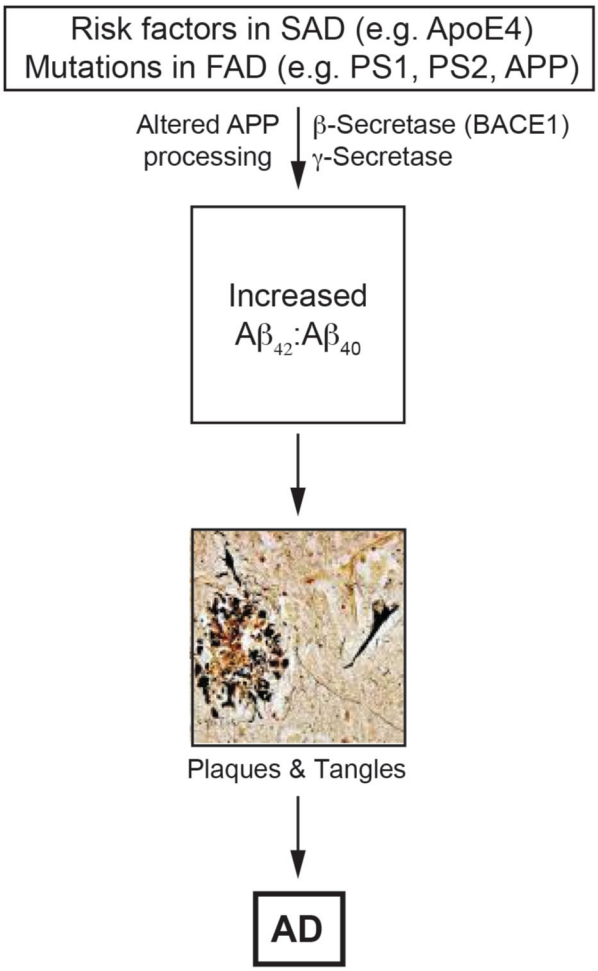

Figure 2. The amyloid cascade hypothesis

Aberrant APP processing gives rise to plaques and then tangles (reproduced from [71], with permission). See text for details.

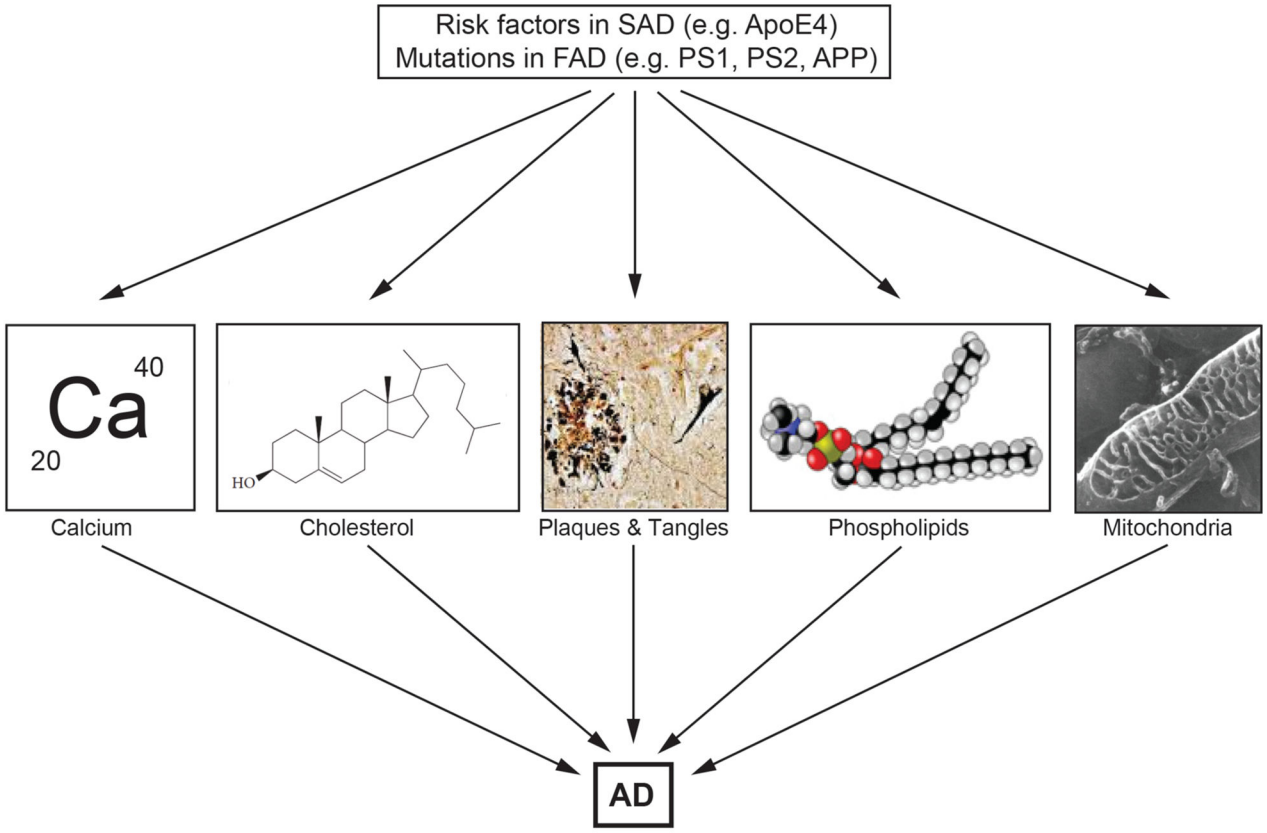

Figure 3. Other features of Alzheimer disease

Besides plaques and tangles [71], AD is also characterized by perturbations in calcium homeostasis, cholesterol metabolism, phospholipid metabolism, and dynamics and function of mitochondria [72].

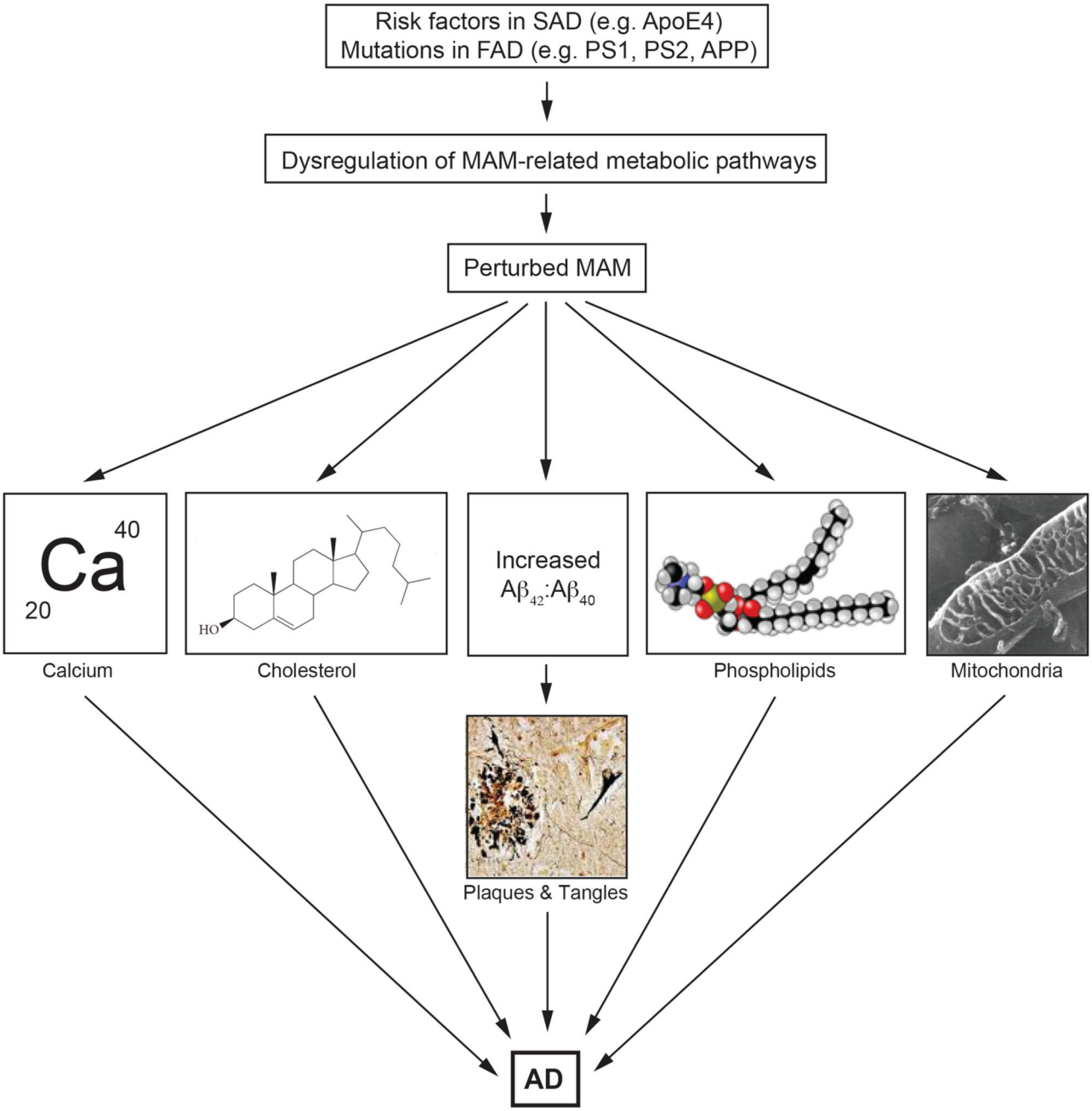

Figure 4. The MAM hypothesis

The hypothesis proposes that the functional cause of AD is upregulated ER-mitochondria communication at the MAM. This results in alterations in the indicated functions, as well as an increase in the $A\beta_{42}$:$A\beta_{40}$ ratio; the plaques and tangles arise as a downstream result of that perturbation. The increased ER-mitochondrial connectivity is the result of a derangement in specific biochemical pathways brought on by mutations in PS's and APP (in the case of FAD) or by other causes (in the case of SAD).