Journal of Alzheimer's Disease 20 (2010) S265–S279
DOI 10.3233/JAD-2010-100339
IOS Press

Review

The Alzheimer's Disease Mitochondrial Cascade Hypothesis

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Accepted 5 April 2010

Abstract. We first proposed the mitochondrial cascade hypothesis of sporadic Alzheimer's disease (AD) in 2004. Our core assumptions were a person's genes determine baseline mitochondrial function and durability, this durability determines how mitochondria change with advancing age, and critical changes in mitochondrial function initiate other pathologies characteristic of AD. Since then several lines of investigation report data consistent with or supportive of our hypothesis. In particular, AD endophenotype studies suggest a strong maternal genetic contribution, and links between mitochondrial function, tau phosphorylation, and amyloid-β (Aβ) amyloidosis are increasingly recognized. As predicted, AD therapies designed to reduce Aβ thus far have had at best very limited clinical benefits; our hypothesis identifies alternative therapeutic targets. While placing mitochondria at the apex of an AD cascade certainly remains controversial, it is increasingly accepted by the AD research community that mitochondria play an important role in the late-onset forms of the disease. Even if the mitochondrial cascade hypothesis proves incorrect, considering its assumptions could potentially advance our understanding of sporadic, late-onset AD.

Keywords: Alzheimer's disease, amyloid-β, cytochrome oxidase, endophenotype, mitochondria, mitochondrial DNA

EXISTING ALZHEIMER'S DISEASE HYPOTHESES

For almost twenty years the amyloid cascade hypothesis has dominated Alzheimer's disease (AD) thinking, modeling, and therapeutic development efforts [1]. Briefly, it proposes sufficient accumulation of an amyloid-β protein precursor (AβPP) derivative, amyloid-β (Aβ), induces the salient biochemical, histologic, and clinical changes AD patients manifest [2]. It has evolved over time; the most important conceptual refinements relate to what particular form of Aβ is felt to occupy the disease pyramid's apex. Currently, oligomers consisting of the 42 amino acid Aβ derivative (Aβ_{42}) are believed to be critical [3].

The amyloid cascade hypothesis was formulated after an AβPP mutation was reported in a family with autosomal dominant amyloid angiopathy, dementia, and AD-typical histology [4]. Two other genes, presenilin (PS) 1 and 2, also contain mutations in autosomal dominant AD and the presenilin proteins are reportedly part of the γ-secretase complex that processes AβPP [5–8]. In autosomal dominant AD, mutations in each of these proteins are believed to shift AβPP processing towards Aβ_{42} [9].

This hypothesis has been extrapolated to sporadic AD. Sporadic AD patients do not have AβPP or PS mutations, so the cause of excessive Aβ_{42} production in sporadic AD is unknown [10]. It is presumed, though, that AD patients have gene variations that subtly steer AβPP processing towards Aβ_{42}.

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ISSN 1387-2877/10/$27.50

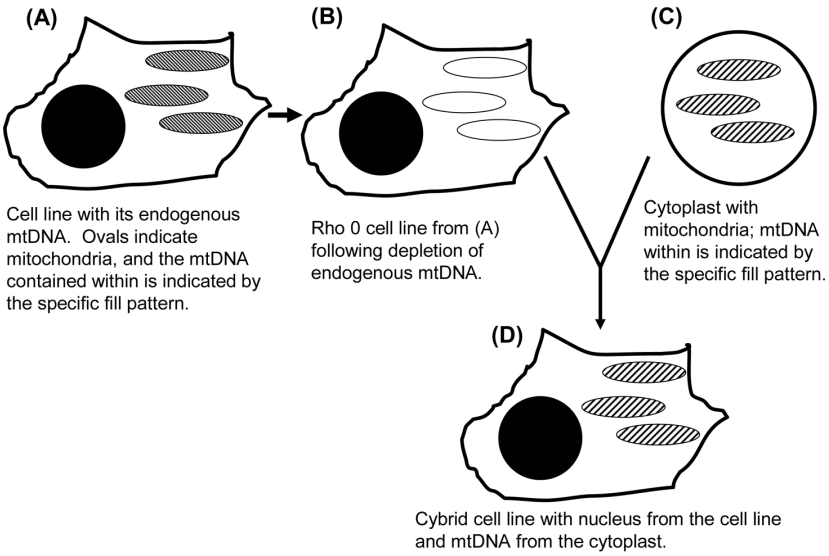

Fig. 1. The cybrid technique. A cultured cell line (A) is depleted of endogenous mtDNA to create $\rho 0$ cells (B). Cytoplasts containing mitochondria (C) but not nuclei are mixed with $\rho 0$ cells in the temporary presence of a detergent that disrupts cell membranes. When cell membranes reform, mixing $\rho 0$ cell and cytoplast contents produces cells with a $\rho 0$ cell line nucleus, cytoplast mitochondria, and cytoplast mtDNA (D).

Other AD hypotheses exist but none have enjoyed the influence of the amyloid cascade hypothesis. Tau, prion, and environmental causes have all been proposed to initiate AD and mediate progression [11–13]. Some believe oxidative stress, either by itself or as part of a "two hit process", causes AD [14]. In 2004 we proposed the mitochondrial cascade hypothesis, which attempts to explain how and why sporadic AD develops [15,16].

OVERVIEW OF THE MITOCHONDRIAL CASCADE HYPOTHESIS

Perceived need for the hypothesis

We first proposed the mitochondrial cascade hypothesis in 2004 [15]. It arose through efforts to understand AD phenomena that, at least as far as we were concerned, were not readily accounted for by the amyloid cascade hypothesis. We wanted to understand the cause or causes of Aβ amyloidosis in sporadic AD patients. We sought a clearer explanation for the continuous correlation between advancing age and AD risk. Also, much of our own research at that time leveraged the fact that AD patients have specific systemic biochemical features. To date, the amyloid cascade hypothesis has done little to address the fact that on a biochemical level, AD is not a brain-limited condition.

AD mitochondrial perturbations and cybrid modeling of AD mitochondrial function

Our mitochondrial cascade hypothesis was heavily influenced by our own work on the causes and consequences of AD-related mitochondrial dysfunction. It is important to emphasize we were by no means the first to describe mitochondrial perturbations in AD patients. For decades it was known that mitochondrial morphology is altered in AD subject brains. Deficiencies of two Krebs cycle enzymes, pyruvate dehydrogenase complex and ketoglutarate dehydrogenase complex, were reported in the 1980s [17–19]. Reduced AD subject cytochrome oxidase activity was first reported in 1990 [20]. The AD cytochrome oxidase defect was first demonstrated in AD subject platelet mitochondria, and later shown to occur in the AD brain [21,22].

During the 1990s we used a cytoplasmic hybrid (cybrid) approach [23] to address whether mitochondrial DNA (mtDNA) could at least partly account for the systemic AD cytochrome oxidase defect (Fig. 1). Cytochrome oxidase, or complex IV of the electron transport chain (ETC), is a 13 subunit multimeric protein localized to the mitochondria inner membrane. Ten subunits are encoded on nuclear genes and imported into the mitochondrial inner membrane. Three subunits are encoded on mtDNA, and these are the most catalytically important subunits. We isolated platelets from AD and age-matched control subjects and fused those platelets with both human neuroblastoma SH-SY5Y cells and

human NT2 teratocarcinoma cells depleted of their endogenous mtDNA [24,25]. The mtDNA-depleted cells, also called $\rho 0$ cells, lack functional ETCs and are not respiration-competent because they cannot produce mtDNA-encoded ETC subunits. After accomplishing platelet mitochondrial transfer to the $\rho 0$ cells, the resulting cybrid cell lines became respiration-competent, but differences between cybrid cell lines that incorporated AD subject mitochondria and cybrid cell lines that incorporated control subject mitochondria were evident. In the AD cybrid cell lines, the cytochrome oxidase activity V_{max} activity was lower, free radical production and oxidative stress markers were elevated, calcium homeostasis was altered, stress signaling pathways were activated, the mitochondrial membrane potential was reduced, apoptosis pathways were activated, and $A\beta_{42}$ production was increased [26].

Based on these data we concluded that at least in a cybrid model system, AD subject mitochondria promote several abnormalities observed in AD subject brains. Our findings further suggested mtDNA at least partly accounts for the AD-associated cytochrome oxidase activity reduction [24]. This is because cybrid cells repeatedly divide in culture flasks, so any non-perpetuating material transferred from platelets degrades and dilutes over time. The biochemical endpoints listed above were present after months in culture and mtDNA is the only recognized perpetuating entity we transferred. Also, in AD subjects, plasma $A\beta$ levels are lower than they are in control subjects [27]. This argues transfer of peripheral $A\beta$ was not responsible for reduced AD cybrid cell line cytochrome oxidase activities.

Clinical contributions to the hypothesis

A phenomenon of age-related cognitive change has long been recognized [28]. For decades it was felt that in the absence of dementia, age-related cognitive change does not predict future AD. In the 1990s this perspective began to change. Non-demented individuals with clinically detectable cognitive domain weaknesses are now routinely characterized as having a mild cognitive impairment (MCI) syndrome. Those given an MCI diagnosis have a substantial chance of receiving a future AD diagnosis. Similarly, cognitive complaints that are unaccompanied by signs (subjective cognitive impairment; SCI) may portend an increased AD risk [29]. The obvious implication is that AD evolves throughout adulthood.

Epidemiologic studies further suggest a majority of persons have the capacity to develop AD should they live long enough. More than half of centenarians meet dementia syndrome criteria, and most have enough senile plaque and neurofibrillary tangle pathology to qualify for an AD diagnosis [28]. Sporadic AD incidence increases with advancing age and prevalence appears to rise at least through the ninth decade and probably beyond [30–32]. To us this suggests common factors drive both aging and AD. The amyloid cascade hypothesis would have to explain this relationship from the perspective that amyloidosis drives aging. Our assumption is that the reverse relationship, that aging drives amyloidosis, is more likely.

AD is currently defined as all plaque-and-tangle dementias [33]. This broad definition is based more on tactics than science. It is presumptuous to assume AD is a homogeneous condition. The simple presence of plaques and tangles does not prove dementing Down's syndrome patients, young adults with autosomal dominant family histories, and elderly individuals have a common etiology.

Many non-demented elderly individuals also have plaques and tangles [28,34]. Some propose such individuals have very early AD in which detectable dementia has not yet manifested [35,36]. Even if this is correct, though, correlation does not establish causality. After all, all persons with myocardial infarctions have elevated serum troponin, but troponin elevation does not cause myocardial infarction.

Core assumptions of the hypothesis

The mitochondrial cascade hypothesis (Fig. 2) proposes a person's genes determine their baseline mitochondrial function and mitochondrial durability [15, 16]. Nuclear and mtDNA genes probably contribute to this. Both parents influence one's lifetime AD risk, but because mtDNA is maternally inherited, mothers have a greater impact than fathers.

It is generally accepted that mitochondrial function declines with age, and data suggest this drives a variety of age-associated physiologic changes [37,38]. It is likely cell physiology initially compensates for and adapts to this change, but eventually a point is reached at which adequate compensation is no longer possible. The mitochondrial cascade hypothesis proposes a person's genetically determined mitochondrial starting line, in conjunction with their genetically and environmentally determined rate of mitochondrial decline, determines the age at which clinical disease ensues.

We propose that some histology changes typical of AD begin to manifest during the compensation phase

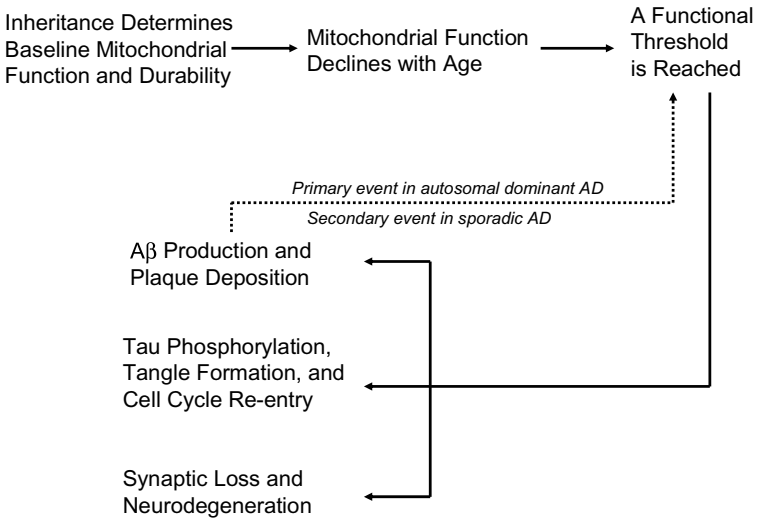

Fig. 2. The mitochondrial cascade hypothesis. In our hypothesis, which applies to sporadic AD, mitochondria are at the apex of the cascade. In autosomal dominant cases we also predict mitochondria may mediate the effects of presenile brain amyloidosis. Used with permission from reference [16].

and arise as a consequence of the compensation response. Other histology changes likely arise after compensation becomes inadequate and are a consequence of failed compensation. Phenomena that occur either during the mitochondrial compensation or failure phases include Aβ production, tau phosphorylation, synaptic loss, cell cycle re-entry, and neurodegeneration.

DATA RELEVANT TO THE MITOCHONDRIAL CASCADE HYPOTHESIS

Genes determine baseline mitochondrial function and durability

Late onset AD typically demonstrates neither clear-cut Mendelian nor maternal inheritance [28]. It is generally considered a sporadic disorder, but this does not mean genes and inheritance are etiologically irrelevant. Whether an individual's parents develop AD does influence the chance of developing AD. For this reason, sporadic AD has occasionally been referred to as a pseudo-sporadic disease. The discussion below refers to late-onset sporadic or pseudo-sporadic AD, not to autosomal dominant AD.

In 1990, Parker and colleagues proposed mtDNA inheritance could influence if not cause late onset AD [20]. The authors postulated mtDNA heteroplasmy and mitotic segregation would allow late-onset AD to present as a sporadic rather than a maternally inherited disorder. This was not entirely consistent with available epidemiologic data indicating both parents contribute to their children's lifetime AD risk [39], but by the mid 1990s several studies reported among late onset AD subjects who report an AD-affected or demented parent, the affected parent is more often a mother [40, 41]. This phenomenon persists after correcting for increased female longevity and higher rates of AD among women [40]. For late-onset AD, epidemiologic data suggest AD mothers contribute more to AD risk than AD fathers.

Several factors complicate the evaluation of intergenerational relationships in late-onset AD. There is some degree of diagnostic uncertainty in persons included in these studies. This uncertainty increases for the parental generation, which cannot be examined and who may have been diagnosed by criteria different to those now used. It is possible some parents diagnosed with AD may not have had AD. It seems more likely, though, that AD would have been under-appreciated in the parental generation. Prior to the 1970s, senile dementia was not typically considered to represent AD, and whether or not someone develops AD highly depends on how long they live. Persons at high AD risk that die at relatively young ages are not classified as having AD, while persons with very low AD risk that live to advanced ages may acquire an AD diagnosis.

To minimize these limitations, some investigators now study AD endophenotypes in persons with an AD parent. An endophenotype is a disease feature or biomarker that can be ascertained in unaffected or undiagnosed persons. For a disease being studied, en-

dophenotype carriers may have an increased risk of developing the disease, may be in the very early stages of developing a disease, or may have an incomplete form of the disease but will never advance to the actual disease. Endophenotypes may be genetically determined. The presence of an endophenotype state in an individual implies that individual inherited at least some degree of genetic risk. Several recent AD endophenotype studies are summarized below.

Positron emission tomography (PET) uses various ligands to assess brain functional or structural parameters. One ligand, a fluoro-labeled glucose analog called fluoro-2-deoxy-D-glucose (FDG), shows differences between AD and control subject brain glucose utilization [42]. In this PET application, FDG is injected intravenously. Some of the injected FDG enters the brain and some of this is phosphorylated by the enzyme hexokinase. Phosphorylated FDG is retained in the brain and un-phosphorylated FDG diffuses out. By determining rates of brain FDG import (K1), brain FDG egress (K2), and FDG phosphorylation (K3), it is possible to calculate a cerebral metabolic rate of glucose (CMRglu) utilization. Mosconi and colleagues used PET to study AD endophenotypes in middle-aged individuals without an AD diagnosis [43]. Subjects were divided into three groups, those with an AD mother (maternal family history, FHm), those with an AD father (paternal family history, FHp), and those in which neither parent developed AD (no family history, FH-). Relative to FH- subjects, FHp subjects showed very limited regional CMRglu declines. Relative to FH- and FHp subjects, FHm subjects showed CMRglu reductions in a number of brain regions including the hippocampus. This study concluded a maternally inherited genetic factor disposes persons to AD-like reductions in CMRglu utilization. A follow-up study performed over a 2 year period also found FHm subjects showed greater longitudinal CMRglu reductions than FH- or FHp subjects [44].

Voxel based morphometry (VBM) is a technique for determining relative regional brain volumes from brain magnetic resonance imaging (MRI) scans of individual subjects. We recently used VBM to analyze MRI scans from non-demented elderly individuals reporting FHm, FHp, and FH- AD family histories [45]. Relative to FH- subjects FHp subjects showed very limited regional volume reductions, while FHm subjects showed extensive volume reductions when compared to either group. Although APOE status can affect regional brain volumes, in this study APOE status was not responsible for VBM reductions unique to the FHm group. From this study we concluded for persons with an AD parent, a maternally inherited genetic factor predisposes to or is responsible for regionally specific brain volume reductions.

Pittsburgh Compound B (PIB) is a PET ligand that binds brain amyloid deposits [46]. Mosconi et al. analyzed PIB PET scans from middle aged individuals with FHm, FHp, and FH- AD family histories [47]. Relative to FH- subjects, FHp subjects showed very limited regional PIB increases, while FHm subjects showed extensive increases when compared to either group. This study concluded for persons with an AD parent, a maternally inherited genetic factor predisposes to or is responsible for brain amyloid deposition.

Debette et al. analyzed cognitive test performance in middle aged subjects from the Framingham Longevity study [48]. This is a well-defined, longitudinal cohort in which various age-relevant parameters are regularly characterized. The investigators grouped cognitive test data in various ways and found among *APOE4* carriers, FHp and FH- test scores were equivalent. On two measures of memory, logical memory and visual reproduction, FHm scores were lower than those from FH- subjects. While the authors were very cautious in their interpretations, this study infers a maternally inherited, AD-associated genetic factor influences mid-life memory performance.

The epidemiologic and endophenotype studies discussed above suggest an individual's risk of developing sporadic or pseudosporadic, late-onset AD is influenced by whether either of their parents developed late-onset AD. The mother's status, though, has a greater impact. For several reasons we previously postulated cytochrome oxidase may contribute to this uneven parental affect [16]. Cytochrome oxidase is unequally inherited; ten cytochrome oxidase subunit genes are bi-parentally inherited on nuclear chromosomes, and three catalytically relevant subunits are strictly maternally inherited via mtDNA. Cytochrome oxidase activity is systemically lower in AD subjects than it is in age-matched controls [22]. While a systemic increase in body Aβ levels could cause this, in AD Aβ levels actually drop in some tissues [27]. Because of this, even though *in vitro* studies have shown Aβ inhibits cytochrome oxidase activity Aβ-induced inhibition is unlikely to be the sole cause. Cybrid studies suggest at least part of the AD cytochrome oxidase defect is a consequence of mtDNA encoding [24,26]. While somatic mtDNA damage within the brain likely contributes to brain mitochondrial dysfunction, cybrid cell lines containing platelet mtDNA from AD subjects ar-

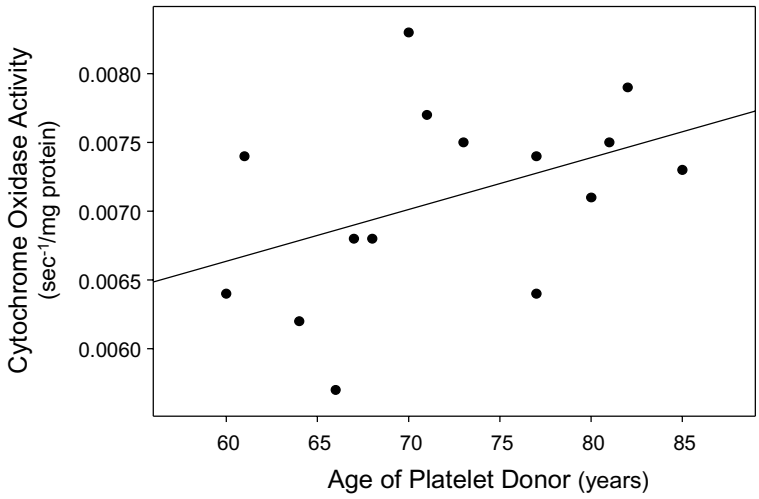

Fig. 3. Relationship between platelet donor age and cytochrome oxidase activity in AD cybrid cell lines. Younger platelet donors likely have a younger age of AD onset than older platelet donors. Cybrid cell lines made using platelets from younger AD subjects tend towards lower cytochrome oxidase V_{max} activities. Used with permission from reference [16].

gues mtDNA inheritance constitutes an AD risk factor. For a series of AD cybrid cell lines we produced on an NT2 nuclear background, cybrid cell lines containing platelet mtDNA from younger donors tended to have a lower cytochrome oxidase activity than cybrid cell lines containing platelet mtDNA from older donors (Fig. 3) [10]. If somatic mtDNA mutation acquisition was driving the AD cybrid cytochrome oxidase activity reduction the opposite relationship would be expected.

To determine the extent of cytochrome oxidase gene variation in the general population, we sequenced 13 cytochrome subunit genes from 50 control subjects [49]. 27 of these 50 subjects had all 13 genes sequenced. When non-synonymous open reading frame (ORF), synonymous ORF, and untranslated region (UTR) polymorphisms were considered, none of these 27 individuals had identical genotypes. Because much nuclear-encoded cytochrome oxidase subunit gene variation was determined by UTR polymorphisms, we studied the impact of a common AGCCCC hexanucleotide deletion found in the COX7A1 5‘UTR region. This deletion forms part of an AGCCCC repeat that constitutes an Sp1 binding site. We used a transcription reporter system to see whether the COX7A1 5‘UTR AGCCCC deletion affected gene expression. When COX7A1 5‘UTR wild type and deletion variants were inserted directly upstream of a green fluorescent protein ORF, the deletion variant produced less fluorescence than the wild type variant. This suggests cytochrome oxidase gene polymorphisms do not need to change amino acids to have functional consequences.

Synonymous polymorphisms were frequently found in the mtDNA COX1, COX2, and COX3 genes. Whether or not synonymous mtDNA polymorphisms are functionally relevant is unknown. Synonymous mtDNA polymorphisms were more frequent in the less conserved COX3 gene than they were in the more conserved COX1 gene, which could indicate even synonymous polymorphisms experience some degree of selection pressure. If so, even synonymous mtDNA cytochrome oxidase polymorphisms may influence cytochrome oxidase function [49].

When just non-synonymous polymorphisms were considered there was still a large degree of inter-individual variation. One common nuclear cytochrome oxidase non-synonymous polymorphism was found, a G to A substitution in the COX4I1 gene that changes an alanine to a threonine. It was seen in 12% of subjects, and functional studies revealed expression of the COX4I1 substitution reduced cytochrome oxidase holoenzyme activity. The majority of the non-synonymous polymorphisms, though, were found in mtDNA COX genes. While no single predominant polymorphism occurred, 20% of individuals had a non-synonymous mtDNA COX gene substitution [49].

To summarize this section, AD endophenotypes may develop long before AD symptoms and signs. If correct, a maternally inherited genetic factor preferentially influences this. Maternal inheritance can occur with X chromosome inheritance, maternal gene imprinting, and mtDNA inheritance. X-chromosome inheritance seems less likely because it preferentially predisposes

males to disease and in AD an increased male incidence is simply not observed. Cybrid studies are consistent with mtDNA inheritance.

Age-related mitochondrial changes are potentially exaggerated in AD

It is generally accepted that mitochondrial function declines with age [37]. Complex I and IV V_{max} activities fall, but the complex II V_{max} activity does not. Complex I and IV have mtDNA encoded subunits but complex II is entirely nuclear-encoded. This has led some investigators to speculate somatic mtDNA mutation contributes to age-related declines in mitochondrial function, and available data support this view [38, 50].

Young control, elderly control, and late-onset AD autopsy brains were evaluated for homoplasmic and heteroplasmic COX2 gene mutations [51]. Low-abundance heteroplasmic mutation frequencies from both elderly control and elderly AD brains exceeded levels in brains from young subjects. The cytochrome oxidase V_{max} activity inversely correlated with the mutation burden; the more mutations there were, the lower the V_{max} was. Mutation frequencies were comparable between AD and the age-matched control subjects.

Frequencies of other somatic mutations may differ between AD and age-matched control brains. Mutation levels of heteroplasmic, presumably somatic mtDNA control region mutations were elevated in AD subject brains [52]. Relative to age-matched control brains, mtDNA deletion levels are elevated in AD subject brains [53,54]. The majority of these deletions involve mtDNA COX genes. Increased mtDNA deletion abundance has also been demonstrated in Parkinson's disease substantia nigra and is believed by some to contribute to that neurodegenerative disease [55,56].

Hirai and colleagues performed a detailed immunochemical and electron microscopy characterization of AD brain tissue [57]. Neuron mitochondrial mass increased during aging, and this increase persisted during AD. Many AD brain mitochondria, though, were found in various states of disrepair and lysosomal degradation. AD neurons overall had fewer normal appearing mitochondria. Mitochondria in AD neurons also contained higher levels of the common 5 kb mtDNA deletion. When degrading mitochondria were considered in the analysis, AD neurons contained more total mtDNA and more COX1 protein than control neurons. The amount of normal mtDNA present within intact mitochondria, though, was diminished. Other investigators have found AD brains contain less PCR-amplifiable mtDNA than control brains, which also suggests levels of retrievable, intact mtDNA are reduced in AD brains [58,59].

Mitochondrial function influences AD histology

Data indicate mitochondria influence tau phosphorylation and AβPP processing. Tau phosphorylation increases in rats and cultured neurons exposed to ETC inhibitors [60–62]. Treating cells with sodium azide, a cytochrome oxidase inhibitor, shifts AβPP processing to an amyloidogenic derivative [63,64]. Cybrid cell lines transplanted with AD subject platelet mitochondria overproduce Aβ_{42} [65].

These findings are not surprising; the effects of oxidative stress on kinase and β-secretase activation are well recognized [66,67]. Mitochondria are the single greatest source of cell free radical production [68]. Azide-mediated cytochrome oxidase inhibition increases free radical production [69], and oxygen radical production is increased in AD cybrid cell lines with reduced cytochrome oxidase V_{max} activities [24, 70].

The mitochondria-Aβ nexus is an area of increasing interest among the AD research community [71–75]. After it was established that cytochrome oxidase V_{max} activities were reduced in AD subjects, several investigators reported Aβ inhibits the ETC in general and cytochrome oxidase specifically [76–78]. While these are important studies, they do not adequately explain lower cytochrome oxidase activity in AD platelets, as Aβ levels are lower in the blood of AD subjects than they are in the blood of control subjects.

Aβ_{42} in particular is regarded to be cell-toxic. This is largely based on numerous *in vitro* studies in which cultured cells were treated with pre-synthesized Aβ_{42} protein. Numerous attempts to explain the cell toxicity of Aβ_{42} have been proposed but there is still no single accepted mechanism and it is unclear whether mechanisms identified in *in vitro* studies apply to human AD. In one study particularly relevant to this discussion, NT2 wild type and NT2 ρ0 cells were exposed to Aβ_{42} protein oligomers [79]. Aβ_{42} reduced wild type but not ρ0 cell viability. This finding suggests that under *in vitro* conditions Aβ_{42} toxicity is mediated through effects on ETC function.

An increasing number of studies report AβPP and Aβ associate with mitochondria [80–87]. These proteins have been found in both the mitochondrial inner membrane and in the mitochondrial matrix. These

studies are not inconsistent with the amyloid cascade hypothesis and potentially reveal how AβPP mutation or Aβ species could at least mediate autosomal dominant AD. Whether or not Aβ species drive or mediate late-onset AD is less clear. This question is unlikely to be settled without a clear explanation of how and why Aβ accumulates in the vast majority (over 99%) of AD cases in which AβPP and PS mutations are not found.

Fukui and collaborators attempted to address this conundrum using genetically altered mice [88]. AD double transgenic mice expressing AβPP and PS1 gene mutations were crossed with mice that develop conditional Cre-loxP mediated knock out of neuron COX10. COX10 does not encode a cytochrome oxidase subunit, but rather a farnesyl transferase that synthesizes a cytochrome oxidase heme component. COX10 knock out was intended to mimic the AD cytochrome oxidase defect, but COX10 knock out induced at least two mitochondrial changes that were opposite to what is observed in AD patient brains. The COX10 knock out mice showed profound reductions in COX1 protein and oxidative stress, whereas AD brains show profound increases in COX1 protein and oxidative stress [57]. The crossed mice had robust reductions in Aβ plaque accumulation and Aβ_{42} protein levels. The authors concluded defects in neuron cytochrome oxidase do not increase oxidative damage and do not promote formation of amyloidgenic AβPP fragments. While true in this experimental model, we hesitate to extrapolate this interpretation to human AD. The nature of the cytochrome oxidase perturbation in COX10 knock out mice does not seem to model the type of cytochrome oxidase perturbation found in AD subjects, and given the known correlation between oxidative stress and β-secretase activity it is not surprising COX10 knock out reduced Aβ levels. Still, the study elegantly demonstrates mitochondrial function influences AβPP processing.

Human studies further establish links between Aβ and neuron metabolism. Unlike the many *in vitro* studies commonly cited to establish Aβ neurotoxicity, in these human studies Aβ does not appear to act as a neurotoxin. On the contrary, neurologic recovery from acute brain injury associates with increased Aβ production [89]. Interstitial fluid Aβ levels also vary with sleep-wake cycles; in mice, wakefulness associates with increased interstitial fluid Aβ, and sleep associates with decreased interstitial fluid Aβ [90].

The debate continues over whether mitochondrial function alters Aβ dynamics or Aβ alters mitochondrial function in late-onset AD. Reduced cytochrome oxidase activity and plaque deposition are evident during the MCI phase of AD [91,92], and even before. PIB PET imaging reveals amyloid accumulates in the middle aged children of asymptomatic at least some AD subjects [47]. Because brain mitochondrial function and Aβ dynamics are potentially entwined, it may be difficult to resolve this question by studying brain. We believe in this case the systemic nature of the AD cytochrome oxidase defect is particularly relevant and informative; the AD cytochrome oxidase defect exists outside the brain in an Aβ-independent fashion. Largely for this reason we suspect mitochondrial function determines amyloidosis in late-onset AD.

RELATIVE STRENGTHS OF THE MITOCHONDRIAL CASCADE HYPOTHESIS

Aging

Both late-onset AD prevalence and incidence rise sharply with advancing age [30–32,93]. In developing our hypothesis, we have therefore considered aging theory and how it may relate to late-onset AD [15,16]. We have intentionally incorporated aging theory into our core principles.

An association between advancing age and declining mitochondrial function has long been noted [37]. Whether declining mitochondrial function is a cause or consequence of aging is an important question. Investigators have tried to address this by using genetically altered mice with defective mtDNA polymerase γ proofreading ability [37,38,50]. These mice accumulate somatic mtDNA mutations at accelerated rates and show accelerated aging. This suggests declining mitochondrial function can drive an aging phenotype.

Genetic association studies report mtDNA polymorphisms and haplogroups influence longevity; mtDNA haplogroups may also influence AD risk [94,95]. One large epidemiology study found that while maternal and paternal longevity both influence an individual's life expectancy, maternal longevity has a greater impact [96, 97]. This pattern is similar to what was observed in the AD endophenotype studies discussed above. Overall, a maternally inherited genetic factor appears to influence both life expectancy and AD risk.

APOE/TOMM40

Although late-onset AD is considered a sporadic or pseudo-sporadic disorder, it is clear that AD risk is genetically influenced. In 1991 an association between

AD and the proximal long arm of chromosome 19 was revealed [98]. Polymorphisms in the *APOE* gene are believed to mediate this association but the physiologic basis of this relationship remains uncertain [99]. Different studies report different potential mechanisms. Apolipoprotein E protein has been shown to influence Aβ trafficking and cell signal transduction, either of which could affect Aβ toxicity [100,101]. Apolipoprotein E or its degradation products also appear to influence mitochondrial function [102].

It now appears polymorphisms in a neighboring gene, *TOMM40*, associate with AD risk [103–105]. This association may arise via effects on *APOE* expression, or may exist independent of *APOE* [106–108]. *TOMM40* encodes a translocase of the outer mitochondrial membrane protein. It would be interesting to know how much late-onset AD risk is specifically determined by *APOE* alleles and how much is specifically determined by *TOMM40* alleles, as well as what degree of association is determined by effects on mitochondrial function.

Systemic manifestations

Weight loss manifests early in AD and during the lead-up to it [109–118]. We extended this finding by showing body composition and cognitive decline rates correlate; MCI subjects with low body mass index (BMI) values progress more rapidly than those with high BMI values [119]. We also found AD subjects have reduced lean (muscle) mass and bone density [120–122]. These changes correlate quantitatively with brain atrophy and cognitive dysfunction.

We determined peak oxygen consumption (VO_2^{peak}) in an AD subject cohort [123]. VO_2^{peak} represents the peak rate of metabolic activity (oxygen utilization) in exercising muscle. Compared to control subjects, AD subjects had reduced VO_2^{peak} values. This correlates with brain atrophy and is not due to differences in cognitive function or baseline physical activity [124,125]. VO_2^{peak} reduction suggests muscle aerobic capacity is fundamentally altered in AD.

Accumulating evidence suggests insulin signaling is altered in AD [126]. AD patients tend towards higher peripheral but lower central insulin levels [127]. We examined relationships between peripheral insulin, cognitive function, and brain volume in non-diabetic AD subjects [128]. As a group, individuals with higher serum insulin levels had less brain atrophy and better cognitive performance. The reasons for this are unclear. Our findings suggest insulin either has a disease modifying effect or else represents a sensitive biomarker of peripheral disease severity. We suspect the latter possibility is more likely.

It is increasingly apparent that AD-affected individuals have altered insulin levels, muscle mass, bone density, and peak oxygen consumption. These metabolism-relevant changes correlate with brain atrophy, correlate with cognitive performance, and are observed during the disease's earliest detectable clinical stages. We predict systemic mitochondrial perturbations are at least partly responsible.

THERAPEUTIC IMPLICATIONS

The mitochondrial cascade hypothesis proposes declining mitochondrial function activates downstream cellular changes observed in late-onset AD. This includes Aβ amyloidosis, tau phosphorylation, oxidative stress, synaptic loss, and neurodegeneration. We believe therapeutic development efforts should target declining mitochondrial function.

Our hypothesis predicts therapies that enhance neuron aerobic metabolism may benefit late-onset AD patients [129,130]. Various approaches may accomplish this; some are technically simple and others far more complex. One simple approach includes the delivery of ketone bodies to the brain. We first proposed the use of betahydroxybutyrate for the treatment of AD in 1989 [131]. Our idea was based on studies of AD rapid autopsy brains. We demonstrated homogenates prepared from these brains consume betahydroxybutyrate. Betahydroxybutyrate feeds acetyl units directly into the Krebs cycle, which in theory could enhance aerobic metabolism by providing ETC reducing equivalents. Almost two decades later, a clinical study reported that increasing serum betahydroxybutyrate levels may benefit cognition in some AD patients [132].

We and others previously proposed using brain mitochondrial biogenesis induction to treat AD [133,134]. Efforts to date have been hindered by an incomplete understanding of the mechanisms that mediate brain mitochondrial biogenesis, as well as a dearth of compounds that induce brain mitochondrial biogenesis at brain-achievable concentrations [133,135]. We have also proposed the enhancement of mitochondrial gene expression to treat AD [136]. To this end we are developing a chimeric recombinant human transcription factor A (rhTFAM) protein that can transduce cell plasma and mitochondrial membranes, bind mtDNA, and activate both mtDNA expression and replication [137].

The mitochondrial cascade hypothesis also predicts certain extensively considered therapeutic interventions may provide either no or very limited clinical benefit. Our theory predicts free radical scavengers will not prove particularly efficacious because in our scheme increased free radical production is a consequence and not a cause of declining aerobic metabolism. Antioxidants may help to a limited extent if it turns out increased mitochondrial free radical production forms part of a feedback loop that further reduces mitochondrial respiration, but according to our hypothesis this would not repair the primary problem and could possibly mitigate compensation-oriented stress signaling pathways.

Similarly, the mitochondrial cascade hypothesis sees Aβ accumulation as a downstream event and addressing Aβ accumulation by blocking production, enhancing removal, or preventing oligomerization should have limited effects [15,16]. Such interventions could perhaps help to some degree if Aβ does in fact form part of a feedback loop that reduces mitochondrial respiration in already failing mitochondria. Eliminating Aβ would not correct the primary problem, though, and would not be expected to dramatically alter clinical progression. Results from several recent anti-amyloid clinical trials are consistent with this view [138–142]. Because our hypothesis assumes sporadic, late-onset AD and autosomal dominant AD do not share a common etiology, disappointing results from late-onset AD trials may not extrapolate to autosomal dominant AD. It is still possible anti-amyloid strategies could have a profound effect in persons with autosomal dominant AD.

CONCLUSIONS

The idea that mitochondria play an important role in AD is not nearly as heretical as it once was. A recent high-profile AD review article surveying the contemporary understanding of AD pathogenesis acknowledged mitochondria are probably relevant to this disease [143].

We first proposed the mitochondrial cascade hypothesis in 2004 [15,16]. Our hypothesis assumed mitochondria initiate late-onset AD pathologies. Since then data consistent with our core assertions have been published. Our hypothesis is especially strong on several accounts. It reconciles AD research with brain aging research, provides insight into late-onset AD epidemiology and endophenotype studies, and explains biochemical and physiologic changes that occur outside the brain in AD. Right or wrong, we feel our hypothesis provides a unique perspective that will hopefully contribute to advances in the AD research field.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (AG022407 to R.H.S.; AG026374, AG029615, and K23NS058252 to J.M.B.; and EY018474 to S.M.K.). Clinical research performed in our studies was facilitated by the University of Kansas General Clinical Research Center (M01RR023940).

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=402).

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