Mitochondrial failures in Alzheimer's disease

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Abstract

Mitochondrial dysfunction and free radical-induced oxidative damage have been implicated in the pathogenesis of several different neurodegenerative diseases such as Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Alzheimer's disease (AD). The defective adenosine triphosphate (ATP) production and increased oxygen radicals may induce mitochondria-dependent cell death because damaged mitochondria are unable to maintain the energy demands of the cell. The role of vascular hypoperfusion-induced mitochondria failure in the pathogenesis of AD now has been widely accepted. However, the exact cellular mechanisms behind vascular lesions and their relation to oxidative stress markers identified by RNA oxidation, lipid peroxidation, or mitochondrial DNA (mtDNA) deletion remain unknown. Future studies comparing the spectrum of mitochondrial damage and the relationship to oxidative stress-induced damage during the aging process or, more importantly, during the maturation of AD pathology are warranted.

Key words: mitochondria, Alzheimer's disease, hypoperfusion, mitochondrial DNA deletion, amyloid cascade

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Introduction

All of the body's cells produce energy and simultaneously generate oxygen free radicals (oxyradicals). The resultant oxidative burden is the unavoidable byproduct of oxygen-based (aerobic) respiration. Therefore, mitochondria, which generate the vast majority of the adenosine triphosphate (ATP) that drives life processes, are also the major cellular "hot spots," where the bulk of oxyradicals are produced and antioxidant defenses are normally most challenged. Mitochondria are semi-independent organelles that have two to 10 molecules of their own DNA and manage the oxidative phosphorylation process. The oxidative phosphorylation system, composed of more than 80 polypeptides, is organized in five enzymatic complexes. These complexes are aggregates of enzymes that are functionally linked and distributed in groups throughout the inner membranes of the mitochondria. They occur in spatial sequences that optimize electron transfer efficiency while minimizing single electron "leakage" to molecular oxygen, which generates oxyradicals. Complexes I-IV receive electrons from suitable donors such as nicotinamide adenine dinucleotide (NADH) and sequentially transfer them throughout oxidation-reduction (redox) groups within the complexes. The final acceptor is molecular oxygen. As a result, they create an electronic potential gradient across the mitochondrial inner membrane. Complex V couples proton flow from the intermembrane space back to the matrix for the conversion of adenosine diphosphate (ADP) to ATP. However, during the transfers, single electrons occasionally escape enzymatic control and combine with oxygen to create oxygen free radicals in 1 to 2 percent of all oxygen consumed.[1] Because mitochondria use 90 percent or more of the cell's available oxygen to make ATP, they also generate 90 percent or more of the

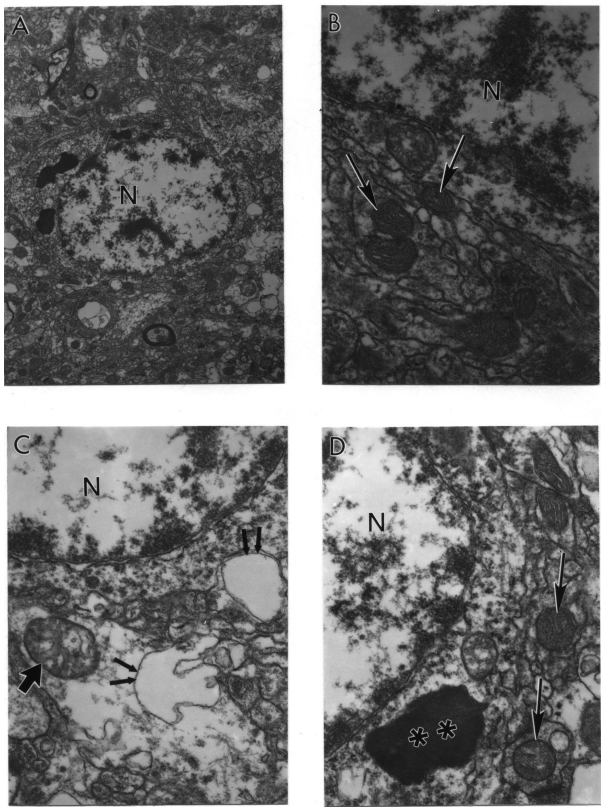

Figure 1. The ultrastructural characteristics of the neuronal mitochondria from AD brain biopsy.

A. Neurons with different degrees of ultrastructural lesions. Partially and completely damaged mitochondria are mostly located in the neuronal cell body and coexist with lipofuscin formation. (Original magnification × 5,000.)

B. Large numbers of electron-dense hypoxic mitochondria (indicated by single arrows) were present throughout the cell body and characterized the abnormal mitochondrial cristae. (Original magnification: × 20,000.)

C. Partially (indicated by single arrow) and completely damaged (double arrow) mitochondria. (Original magnification × 20,000.)

D. The neuronal cell body shows the presence of hypoxic mitochondria (indicated by single arrows) close to lipofuscin (double asterisk). (Original magnification × 20,000.)

(N = neuronal nucleus)

oxyradicals that make up the endogenous oxidative burden.[2,3] These oxyradicals are so highly reactive that they have the potential to destroy the living system. To protect against destruction by this flux of oxyradicals, the mitochondria have sophisticated antioxidant defenses by which the superoxide anion is detoxified by the mitochondrial Mn-superoxide dismutase to produce H_2O_2, which, in turn, is converted to water by glutathione peroxidase. But inevitably, a few oxyradicals slip through to attack biomolecules within and around mitochondria and cause oxidative damage to the organelles themselves and their surroundings. One of the most striking features of the human brain is its respiratory requirements: 20 to 25 percent of total body basal respiration occurs in less than 2 percent of the body's mass occupied by the brain. Within the brain, most of the oxygen is consumed by neurons. Mitochondria are essential for neuronal function because their limited glycolytic capacity make them highly dependent on aerobic oxidative phosphorylation. The total dependency of the brain on oxygen is shown by the failure of neurons to survive under ischemic conditions.

Mitochondrial abnormalities in AD

Mitochondrial dysfunction, which may be due to accumulated damage that accompanies normal aging but is amplified by disease-specific factors, is probably a key step in Alzheimer's disease (AD) progression.[4,5] Damaged mitochondria are less efficient producers of ATP and more efficient producers of reactive oxygen species (ROS), both of which characterize AD.[4,5] Indeed, damage to both the components and the structure of mitochondria are reported extensively in AD.

The most consistent defect in mitochondrial components in AD has been deficiency in several key enzymes of oxidative metabolism, including α-ketoglutarate dehydrogenase complex (KGDHC) and pyruvate dehydrogenase complex (PDHC), two enzymes in the rate-limiting step of the tricarboxylic acid cycle, and cytochrome oxidase (COX), the terminal enzyme in the mitochondrial respiratory chain that is responsible for reducing molecular oxygen.[5-11] Importantly, the degree of dementia correlates much better with reductions in KGDHC activity than with the amount of senile plaques and neurofibrillary tangles (NFTs) in the brains of ApoE4-positive AD patients.[12] Generally, the reduced activity of these key enzymes favors the aberrant production of ROS, especially in the form of superoxide. Indeed, studies using cytoplasmic hybrid technology in which mitochondria from sporadic cases of AD were

fused with other cells have demonstrated that the deficits in COX in AD platelets could be transferred to ρ^0 cells, which retain the COX deficit.[13,14] Additionally, the resulting cybrid cells showed markedly increased free radical production and impaired calcium signaling. Within the context of decreased numbers of intact mitochondria, research shows increased mitochondrial DNA (mtDNA) and COX protein in the cytoplasm and in the vacuoles associated with lipofuscin—the site of mitochondrial degradation in susceptible neurons in AD.[15] These mitochondrial components are likely damaged because hydroxynonenal adducts to lipoic acid. It is suspected that these mitochondrial components are nonfunctional because the prosthetic group of two key Krebs cycle enzymes can be found in the same vacuoles.[15] These findings indicate that vulnerable neurons in AD have increased mitochondrial degradation products, suggesting either greater turnover of mitochondria by autophagy or a reduction of proteolysis turnover by proteosome.[15] Moreover, it has been reported that mtDNA isolated from the brains of AD patients shows oxidative modifications containing 8-hydroxy-2'-deoxyguanosine (8-OHdG).[16-19] The common 5kb mtDNA deletion is increased at least three-fold[15] and mtDNA control region mutations increase significantly in AD cases as compared with controls in humans.[20] Both of these factors have a deleterious impact on mitochondrial function.

The function of mitochondria is dependent on their intact structure. The majority of the neurons closely associated with the lesioned vessels displayed a different degree of ultrastructural abnormality (Figure 1). In the neuronal cell body, the presence of partially and completely damaged mitochondria were associated with lipofuscin formation. Mitochondria appeared to be a major substrate for this process (Figure 1D). A large number of electron-dense hypoxic mitochondria were seen throughout the cell body and characterized the abnormal mitochondrial cristae (Figure 1B and 1D). In many cases, the neuronal cell body showed an absence of cellular organelles. Different stages of mitochondrial abnormality, such as the formation of mitochondria-derived lysosomes and lipofuscin, were evident in almost all of AD neurons (Figure 1). The mitochondria-derived lysosomes and lipofuscin deposits of varying density and size were the prominent, common features of the neuronal abnormality. Mitochondria lesions and lipofuscinosis were also generalized to the other cellular compartments of the brain parenchyma. Very often, glial cells at the damaged area, also characterized by the accumulation of lipofuscin and mitochondria-derived lysosomes, appeared to be a major component and source for these substrates. In addition, glial cells show the intracellular accumulation of different-sized amyloid deposits, and they are accompanied by the presence of giant-sized lipid-laden vacuoles and mitochondria-derived lysosomes. Quantitative morphometric measurements of the percentage of the different types of mitochondria (normal, partially damaged, and completely damaged) confirmed that the AD group showed a significantly lower percentage of normal mitochondria and a significantly higher percentage of the completely damaged mitochondria compared with the aged-matched control group. No significant differences between partially damaged mitochondria were seen in both groups, which indicates that aging itself induces damage to mitochondria.[15] Some disease-specific factors amplify damage and can lead to significantly more completely damaged mitochondria in AD.

Possible consequences of mitochondrial abnormalities in AD

Impaired energy metabolism in AD

Since mitochondria are the powerhouse of all human cells, damage to mitochondria will inevitably impair energy metabolism. More specifically, deficiency in the two key enzymes of rate-limiting step of the tricarboxylic acid cycle (i.e., KGDHC and PDHC) suggests defects in glucose metabolism in the AD brain.[5-11] Indeed, a large number of studies implicate metabolic defects in AD, such that a reduced rate of brain metabolism is one of the best documented abnormalities in AD.[21,22] Substantial data from positron emission tomography (PET) consistently demonstrate reduced cerebral metabolism in temporoparietal cortices in AD.[23] An increased oxidative utilization in comparison with glucose utilization in AD patients is also well documented.[24,25] Most importantly, such cerebral metabolic rate abnormalities precede rather than follow any evidence for functional impairment by neuropsychological testing or of brain atrophy by neuroimaging.[22] Notably, metabolic derangements comparable to those seen in AD (e.g., hypoxic hypoxia, hypoglycemia, vitamin deficiency) are sufficient, by themselves, to induce mental and neurological deficits similar to those in AD as evidenced by the neuropsychiatirc disorders associated with oxidative metabolism abnormalities.[26]

Oxidative imbalance in AD

As discussed above, abnormal mitochondria are more efficient producers of ROS, which therefore pose significant oxidative threat to the surroundings. Compared with other organs or tissues, the brain is more vulnerable to ROS-induced damage due to its high rate of oxygen consumption, high polyunsaturated lipid content, and relative lack of classic antioxidant enzymes.[27] Indeed, individuals affected by AD show significantly increased oxidative

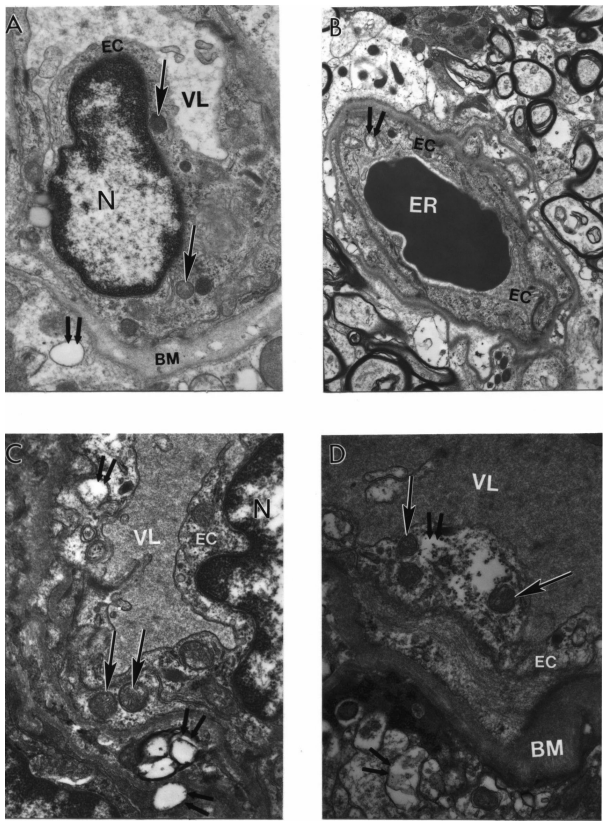

Figure 2. Ultrastructural features of the brain microvessels from AD brain characterized by heterogeneous morphology.

A. Undamaged microvessel endothelium did not show any particular changes in their ultrastructure. Mitochondrion also is intact (single arrows). However, the perivascular spaces contain large vacuolar structures (double arrow). (Original magnification × 13,000.)

B. Vascular EC shows the presence of degenerative mitochondria (double arrow). (Original magnification × 6, 600.)

C. The presence of electron-dense hypoxic mitochondria (single arrows) coexists with the formation of mitochondria derived lysosomal structure in the cytoplasmic matrix of EC and perivascular cells (indicated by double arrow). (Original magnification × 20,000.)

D. The mitochondria abnormality appeared to be permanent features of vascular endothelium and perivascular cells when damage become visible (single and double arrows indicate hypoxic and completely damaged mitochondria, respectively). (Original magnification × 20,000.)

(BM = basal membrane of endothelium; EC = endothelial cell; ER = erythrocyte; VL = vessel lumen.)

damage to every class of biological macromolecules including sugar (i.e., glycosylation and glycation), lipid (i.e., lipid peroxidation), protein (i.e., protein oxidation/nitration), and nucleic acid (i.e., DNA/RNA oxidation) in the brain.[28] That abnormal mitochondria contribute to increased oxidative stress in AD brain is evidenced by the strong positive correlation between mitochondrial abnormalities (e.g., mtDNA deletions) and the extent of oxidative damage in the cytoplasm (e.g., 8-OHG staining) ($r = 0.934$).[15] Ultrastructural analysis shows that the most 8-OHG is found in the endoplasmic reticulum with the majority of mitochondria showing little 8-OHG.[15] 8-OHG is a nucleic acid modification predominantly derived from the hydroxyl radical attack of guanosine. Since the hydroxyl radical has only a 2 nm sphere of diffusion and is unable to diffuse through the biological membrane, the source of reactive oxygen must be in close physical proximity to the damage. Therefore, 8-OHG is likely to form at the site of hydroxyl radical production within the neuronal cytoplasm. The cytosolic sites of damage seemingly excludes the mitochondria; however, we suspect a more complex relationship. It is possible that superoxide generated from the mitochondria is dismutated to freely diffusible hydrogen peroxide, which then interacts with cytosolic redox-active metals and other oxidative stress response elements to produce hydroxyl radicals and cause damage.[28] The theory is supported by the increased superoxide/catalase ratio in AD neurons and increased levels of H_2O_2 in the Tg2576 hippocampus.[29,30]

Potential causes of mitochondrial abnormalities in AD

Hypoperfusion

Hypoperfusion-induced oxidative stress in vascular abnormalities contributes to the pathogenesis of AD (Figure 2). Several studies have shown chronic cerebral hypoperfusion (CATCH) in AD.[31-36] Also, a greater fraction of oxygen is removed from the vasculature in AD patients compared with non-AD controls.[37] Mitochondria in vulnerable cells almost always show signs of damage during ischemia.[38] Importantly, chronic reductions in cerebral flow of a magnitude thought to be harmless to neurons (i.e., reduced by 25 to 50 percent) induced disorganization of the CA1 sector. Neurons in the CA1 sector showed increased lipofuscin pigments, implying mitochondrial abnormalities.[39] Therefore, it is tempting to suggest that low vascular blood flow, which is a prominent feature of the brain during chronic hypoxia/hypoperfusion, may be

one of the main initiating factors of mitochondrial abnormalities during the development of AD (Figure 2B-D).

De la Torre[40] proposed that advanced aging with a comorbid condition, such as a vascular risk factor that further decreases cerebral perfusion, promotes a critically attained threshold of CATCH. With time, CATCH induces brain capillary degeneration and suboptimal delivery of energy substrates to neuronal tissue.[40] Because glucose is the main fuel of brain cells, its impaired delivery, together with a deficient delivery of oxygen, compromises neuronal stability because the supply for aerobic glycolysis fails to meet brain tissue demand. The outcome of CATCH is a metabolic cascade that involves, among other things, mitochondrial dysfunction, increased oxidative stress, and decreased ATP production, which probably contributes to the progressive cognitive decline characteristic of patients with AD. It also produces anatomic pathology consisting of synaptic loss, senile plaques (SPs), and NFTs.

Amyloid cascade

Amyloid beta (Aß), a 39-43 amino acid peptide generated from cleavage of Aß precursor protein (AßPP), is toxic to many different cells in vitro and thought to be one of the causes of AD. AßPP was shown to be present in mitochondria where it interacted with the mitochondrial protein import motors. However, transport is not complete, which causes mitochondrial dysfunction and inhibition of ATP synthesis.[41,42] AßPP overexpression leads to decreased COX activity,[42] abnormal mitochondrial morphology, and decreased mitochondrial potential, which render these neurons more vulnerable to other stimuli in vitro.[43] Moreover, exposure of isolated mitochondria to Aß causes a decrease in mitochondrial enzyme activity, respiration, and membrane potential.[44,45] The activity of a number of mitochondrial enzymes such as KGDHC, PDHC, and COX is also decreased in cells exposed to Aß.[46,47] More recently, Aß was demonstrated to be present in mitochondria, presumably in association with Aß-binding alcohol dehydrogenase (ABAD), and promotes mitochondrial dysfunction and ROS production. This provides a direct link to Aß-induced mitochondrial toxicity.[48] In yeast artificial chromosome (YAC) AßPP mice, which develop extensive amyloid pathology, we found abnormal alterations in mitochondrial structures in the cortical neuronal cell body similar to those seen in AD.[49] Particularly, giant and ED mitochondria, along with increased lipofuscin formation, appeared to be unchanging features of the neuronal abnormality in these mice.[49] In situ hybridization analysis with mouse and human mtDNA probes found abundant deleted mtDNA in YAC AßPP compared with age-matched controls.[49] Moreover, the majority of mtDNA deletion was found in mitochondria-derived lysosomes in regions closely associated with the lipofuscin, many of which have been fused with lysosomes in YAC AßPP mice.[49] However, it is still not clear whether mitochondrial abnormalities are due to AßPP overexpression or Aß deposition.

Mitochondrial DNA mutations

As discussed above, mitochondria are semi-independent organelles that have their own DNA. The mtDNA encodes tRNA, rRNA, and 13 proteins, which are all components of the oxidative phosphorylation system.[50] The mtDNA appears to be more susceptible to accumulating oxidative damage than is nuclear DNA because of its proximity to ROS production, the short half-life of mtDNA especially in brain tissue, and a relative lack of a repair system. Indeed, during the aging process, 8-OHdG levels were higher in human brain mtDNA than nuclear DNA.[51] It is evident that mutations of mtDNA, including maternally inherited point mutations and sporadic mtDNA rearrangement mutations, have been associated with various human diseases[50] such as Kearns-Sayre syndrome (KSS), a multisystemic syndrome due to sporadic mtDNA rearrangement mutation. Interestingly, the fact that many of the over 50 identified mtDNA mutations are found in patients with neurodegenerative conditions[52] implies that mtDNA mutations may contribute to AD. Sporadic mtDNA rearrangement (i.e., the common 5-kb deletion) was significantly increased in AD patients compared with control cases, (Figure 3C).[15,53] Since deleted mtDNA are not translated because the deletions remove essential tRNAs that are required for protein synthesis, such mutations adversely affect mitochondrial replication. Moreover, several studies indicated a modestly increased frequency of A4336G mutation in $tRNA^{Gln}$ in AD patients compared with control cases,[54-56] although controversy still exists.[57] High incidence of mtDNA base changes were also found in Down's syndrome.[58] In a recent study, Coskun et al[20] determined that there were many more sporadic mutations in the mtDNA control region in AD patients compared with control cases and several mutations in the mtDNA control region (e.g., T414G, T414C, and T477C) that were unique to AD.[20] These mutations occurred at sites of known mtDNA regulatory elements; therefore, they are associated with deleterious functional consequences for mitochondrial homeostasis (e.g., COX activity) once they reach a critical mass in postmitotic cells in the brain (Figure 3D).

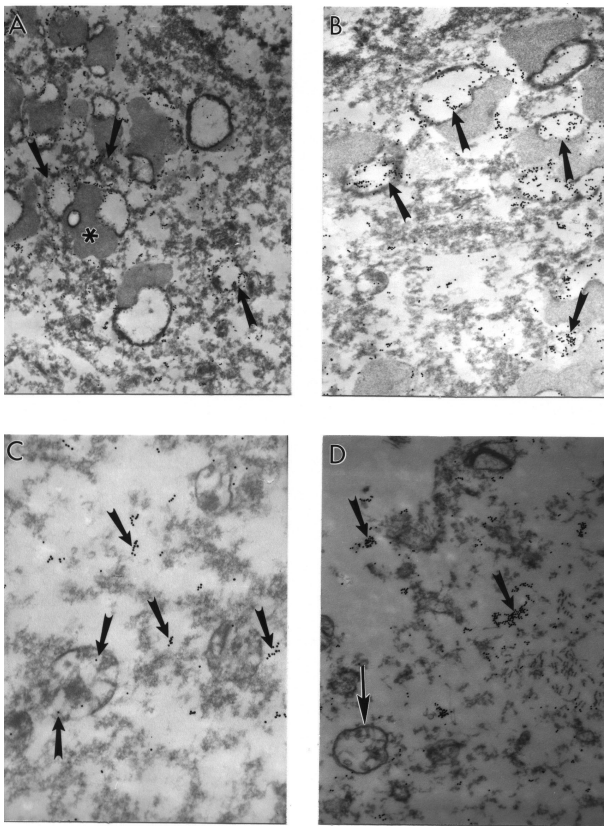

Figure 3. The features of wild, 5 kb deleted mitochondria DNA (mtDNA), and COX immunoreactivity in the hippocampus of the postmortem human AD case.

A and B. Hippocampal neuron shows wild type mtDNA containing positive signals (17 nm colloidal gold) detection were seen in the completely damaged mitochondria or mitochondria derived lysosomes (single arrows). Areas containing lipofuscin (asterisk) did not show any mtDNA containing positive signals. (Magnification X 26,000 and X 20,000, respectively).

C. 5kb deleted mtDNA containing gold particles (17 nm) were mostly located in mitochondria-derived lysosomes (single arrows). (Original magnification X 33,000.)

D. Damaged, abnormal mitochondria shows COX positive containing gold particles in the matrix (single arrows, colloidal gold, 17 nm). (Original magnification X 26,000.)

Homocysteine

Homocysteine is a key metabolic intermediate in sulfur amino acid metabolism.[59] Elevated plasma and brain homocysteine and reduced hydrogen sulfide (H_2S), both of which may be related to reduced activity of the enzyme cystathionine ß-synthase (CßS), have been reported in AD.[60] A recent study reported that elevated plasma homocysteine is a strong, independent risk factor for the development of AD.[61] However, these studies did not shed light on any relevant mechanism. Interestingly, homocysteine causes mitochondrial abnormalities that are enhanced in the presence of hydrogen peroxide[62] and homocysteine impaired energy metabolism in hippocampal prisms and reduced cytochrome-c oxidase activity.[63-65] Electron microscopic studies of the brains of folate-deprived rats which develop hyperhomocysteinemia revealed cytoplasmic swelling and mitochondrial degeneration in the endothelium, perivascular amorphous fibrosis, and pericytic degenerative appearance in the cerebrocortical microvascular wall.[66] Homocysteine promoted mitochondrial hydrogen peroxide and superoxide anion production presumably by decreasing manganese superoxide dismutase (MnSOD) and catalase activity.[67,68] It is also noteworthy, in light of homocysteine's inhibitory effect on cytochrome-c oxidase,[63-65] that down-regulation of several genes encoding mitochondrial proteins including cytochrome-c oxidase was reported in the livers of CßS-deficient mice, a murine model of hyperhomocysteinemia.[69] Therefore, homocysteine adversely affects mitochondrial function which may contribute to AD pathology.

Conclusion

There is no doubt that mitochondrial abnormalities are involved in AD, however, it is still a matter of debate whether or not such abnormalities are a cause or merely a consequence—among many—of the disease. Given the fact that impaired energy metabolism well precedes any clinical symptoms and that oxidative stress is one of the earliest features of the disease,[70,71] it is likely that mitochondria play a very proximal role in the pathogenesis of the disease. In spite of the many known factors that can contribute to mitochondrial dysfunction at some time during the disease progression, it still remains to be determined what initiates the mitochondrial abnormalities in the disease.

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