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Review

Mitochondrial involvement in Alzheimer's disease

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Abstract

The causes of most neurodegenerative diseases, including sporadic Alzheimer's disease (AD), remain enigmatic. There is, however, increasing evidence implicating mitochondrial dysfunction resulting from deafferentiation of disconnected neural circuits in the pathogenesis of energy deficit in AD. The patterns of reduced expression of both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) encoded genes is consistent with a physiological down-regulation of the mitochondrial respiratory chain in response to reduced neuronal activity. On the other hand, the role(s) of somatic cell or maternally inherited mtDNA mutations in the pathogenesis of mitochondrial dysfunction in AD are still controversial.

Keywords: Aging; Alzheimer; Deletion; Hippocampus; Mitochondrion; Mitochondrial encephalomyopathy; mtDNA; Neuritic plaque; Neurofibrillary tangle; Neuropathology; Oxidative phosphorylation; Oxidative stress; Point mutation; Reactive oxygen species; Respiratory chain

Contents

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1. Introduction

Mitochondria are the main sources of energy in the cell. They contain their own DNA (mtDNA), which encodes 13 components of the respiratory chain [1]. Mitochondria are maternally inherited, and are critical for the normal functioning of tissues that are highly dependent on aerobic metabolism, such as brain and muscle.

Alzheimer's disease (AD) is a major form of dementia, affecting 5–15% of the population over the age of 65 years [2]. Clinically, the disease is manifested by memory loss and unremitting mental deterioration. While many patients with autosomal dominant familial AD have mutations in the amyloid precursor protein (APP) or presenilin genes [3–5], most patients with AD are sporadic cases, in whom no such mutations have been identified, and etiology and pathogenic mechanisms of neuronal dysfunction remain unknown.

Recently, attention has been directed to the possible contribution of mitochondrial dysfunction and oxidative damage in late-onset neurodegenerative disorders, including the familial and sporadic forms of AD [6–8]. This review focuses on the role of mitochondria in AD, and summarizes some of the relevant neuropathological, biochemical, and molecular genetic data.

2. Neuropathology

The histopathology of the AD brain shows loss of neurons and two hallmark lesions: neurofibrillary tangles (NFT) and neuritic plaques (NP) [9]. The tangles are composed primarily of abnormally phosphorylated tau microtubule-associated protein; plaques consist mainly of aggregates of a 40–42 amino acid polypeptide derived from the proteolytic processing of APP.

The neocortex and hippocampus are both severely affected in AD, but the pathology does not affect all cell types. The pyramidal neurons in the entorhinal cortex and the CA1 and subiculum regions of the hippocampus are vulnerable to NFT formation, but the CA3 region and the granule cells in the dentate gyrus are resistant to degeneration [10]. In the neocortex, subsets of pyramidal neurons are susceptible to NFT formation and degeneration, and inhibitory interneurons do not show NFT and are resistant to degeneration [11,12]. The pyramidal neurons that form the long cortico-cortical projections are also affected in AD, but primary sensory and motor areas show only minor neuronal loss [13,14].

The most profoundly affected circuit in the cerebral cortex is the perforant pathway, which originates in layer II of the entorhinal cortex and terminates in the outer molecular layer of the dentate gyrus, thus providing the key connection between the neocortex and the hippocampus [15]. The entorhinal cortex is an area of extensive convergence of inputs from association areas of the neocortex which, in turn, transmits processed information to the dentate gyrus of the hippocampus and thereby plays a critical role in memory [16]. The perforant pathway is invariably damaged by extensive NFT formation in AD, even at early stages of the disease [17]. In addition, there is significant loss of synapses in association areas, indicating structural damage to neural circuits [13,14]. Regarding subcortical projections, the thalamic projections are spared, but the cholinergic projection from the nucleus basalis of Meynert is affected early in the course of the disease [18].

It is now well documented that neuronal degeneration in AD is extensive, but selective. Given the circuits that degenerate, it is not surprising that several spheres of higher mental functions are affected, including learning, behavior, and memory.

3. The mitochondrion and mtDNA mutations

The mitochondrial respiratory chain comprises five multisubunit proteins (Complexes I–V) located on the inner membrane, and is the most important source of superoxide radicals in aerobic cells.

All 13 proteins encoded by mtDNA are part of the respiratory chain/oxidative phosphorylation system [1].

Mutations of mitochondrial DNA, including maternally inherited point mutations and large-scale sporadic mtDNA rearrangements have been associated with a wide spectrum of human diseases [19,20]. The main disorder associated with sporadic rearrangements of mtDNA is Kearns-Sayre syndrome (KSS), a multisystemic syndrome characterized by paralysis of the extraocular muscles, pigmentary retinopathy, heart block, cerebellar ataxia, and elevated CSF protein. The mtDNA rearrangements, which are predominantly deletions (Δ-mtDNAs) are large (up to 9 kb in size) and are present at levels of up to 80% of total mtDNA (i.e. patients are heteroplasmic, with a mixture of normal and deleted mtDNAs). The size and location of the deletion, and the proportion of Δ-mtDNA, varies among patients, but one particular Δ-mtDNA, in which 4977 bp have been deleted, has been found in about 1/3 of all patients: this 5-kb deletion has therefore been called the 'common deletion' [21,22]. Deleted mtDNAs are transcribed into RNA, but are not translated, because the deletions remove essential tRNAs that are required for protein synthesis [23]. Therefore, even genes outside the deletion are not translated.

Tissues from normal aged individuals, and especially long-lived postmitotic tissues with high oxidative requirements, such as muscle and brain, contain low amounts (detectable only by the polymerase chain reaction, or PCR) of the same Δ-mtDNAs found in much greater abundance in patients with sporadic KSS [24,25]. These Δ-mtDNAs accumulate during aging. It has been shown that the 'common deletion' accumulates in muscle by a factor of 10 000 over the course of the normal human lifespan, reaching a level of approximately 0.1% of total muscle mtDNA by age 84 years [26]. Numerous other deleted species are also present in aging human muscle [27–29]. In addition, there is focal accumulation of the 'common deletion' (used as a surrogate marker to represent all possible mtDNA deletions) in regions of aging human brains [30,31]. The regions with the highest levels are the striatum (caudate and putamen), the cerebral cortex, and the substantia nigra in the midbrain; in contrast, cerebellum contains relatively low levels of deletions.

4. Oxidative stress and damage in AD

Although numerous hypotheses have been proposed for the pathogenesis of sporadic AD, the exact mechanism remains poorly understood. One hypothesis that has received considerable attention postulates the possible involvement of oxygen free radicals and hydrogen peroxide [32–35]. The oxidative stress hypothesis proposes that some as yet unknown factors cause an imbalance that favours the generation of reactive oxygen species (ROS) over antioxidant defenses, leading to oxidative damage of neuronal lipids, proteins, and DNA. Important factors that may favor oxidative stress in the brain in AD include the brain's high oxygen consumption rate, the abundant polyunsaturated fatty acid content, the short half-life of mtDNA ($\sim$30 days in rat [6]), and a relative lack of antioxidant defenses compared to other tissues [36].

During the past 5 years, there has been increasing interest in the role of free radicals in neurodegenerative diseases [7,8,36]. However, there is little direct information about ROS in the brain in AD. Smith et al. [37] and Hensley et al. [38] have suggested that increased oxidative stress in the brain in aging and in AD is reflected by increased protein oxidation. Others have shown significantly increased levels of lipid peroxidation in AD brains, indicated by elevations of thiobarbituric acid-reactive compounds in the hippocampus, pyriform cortex, and amygdala [39]. Advanced glycation end-products, which are capable of generating ROS, have also been found in NFT [40,41] and NP [42]. Protein carbonyls, indicators of protein oxidation, and peroxynitrate, a reaction product of nitric oxide and the superoxide radical, have been documented in NFT by immunohistochemical techniques [43,44]. In addition, the activities of key antioxidant enzymes, particularly catalase, was reduced, suggesting that AD brain may be vulnerable to increased ROS production [45]. In related reports, it has been shown that β-amyloid may generate free radicals in aqueous solution [46] and produce oxidative damage in hippocampal neurons [47].

There is also evidence that β-amyloid impairs the activity of the mitochondrial respiratory chain. This is best illustrated by inclusion body myositis (IBM), a common myopathy developing in patients over the

age of 50 years. Morphologically, IBM is characterized by the presence of vacuolated muscle fibers showing accumulations of β-APP and 15- to 21-nm paired helical filaments containing hyperphosphorylated tau [48]. The transfer of a cDNA encoding APP_{751} into human myoblasts produced abnormalities of mitochondrial structure and function [49]. Decrease in the histochemical staining for cytochrome *c* oxidase (COX) was observed after 24 h, becoming virtually complete in 80% of fibers at 2 weeks. Electron microscopy at 3–4 weeks showed abnormal mitochondria structure, including the presence of intramitochondrial paracrystalline inclusions.

There is also evidence of oxidative damage to both mtDNA and nDNA, documented by measuring the oxidized radical adduct 8-hydroxy-2′-deoxyguanosine (^{8}OHdG). The mtDNA appears to be more susceptible to accumulating oxidative damage than is nDNA. ^{8}OHdG levels were higher in human brain mtDNA than in nDNA, and increased with age in human muscle and brain [50]. Studies in AD brains have shown preferential accumulation of ^{8}OHdG in the mtDNA over the nuclear DNA, and significantly higher levels of ^{8}OHdG in AD than in control brains [51]. In one study, the enhanced oxidative damage was associated with increased levels of the 'common deletion' in cortical regions of AD patients who died after age 75 years [52], although we were not able to confirm these findings (unpublished data).

5. Mitochondrial dysfunction in AD

Altered brain energy metabolism is an early and prominent feature of AD [53]. As the disease progresses, significant decreases in oxygen and glucose utilization have been shown by positron emission tomography (PET) [54]. These findings are consistent with the notion that the reduction in glucose metabolism in vivo reflects reduced neuronal oxidative activity. PET has documented visually the evolution of the metabolic alterations in the AD neocortex. An early deficit in glucose metabolism is seen in the parietal and temporal regions, areas with particularly high density of NP [55]. In longitudinal studies, it has been documented that metabolic reductions in the parietal association cortex precede the impairment of such neocortical-mediated functions as language and visuospatial recognition [56].

More direct evidence for a defect of energy metabolism in AD comes from several reports of COX deficiency in AD brain. Mitochondria freshly prepared from nine entire AD hemi-brains showed a 40% decrease in complex I and a 53% reduction in COX activity [57,58]. When COX activity was measured in homogenates from AD brain, less severe, but statistically significant, decreases were found in frontal and temporal cortices [59]. In a study using both homogenates and mitochondrial preparations from 19 AD patients, COX activity (corrected for citrate synthase activity) was decreased by 27% in temporal cortex extracts and by 25–30% in mitochondrial fractions from various cortical areas [60]. Another study using tissue homogenates of frontal and parietal cortex found 34 and 38% reduction in COX activity, respectively [61].

In agreement with these biochemical observations, histochemical data by Simonian et al. [62] showed significant reduction of COX activity in the molecular layer of the dentate gyrus and in other subfields of the hippocampal formation of AD patients. Finally, in situ hybridization studies showed decreased mRNA levels of the mtDNA-encoded subunit II, but not the nDNA-encoded subunit IV, of COX [63].

Based on these observations, and to gain further insight into the pathogenesis of mitochondrial dysfunction in AD, we have initiated a study of the expression of subunits of the respiratory chain in the hippocampal formation from patients with AD and from normal controls. We studied the expression of two subunits of COX (mtDNA-encoded COX I and COX II) and of one subunit of Complex III (nDNA-encoded FeS subunit) by immunohistochemistry, using the immunoperoxidase method [64].

In normal controls, we observed immunoreaction with all antibodies in the molecular layer of the dentate gyrus (Fig. 1a,b). The cell bodies of the granule cells showed a finely punctate pattern of immunoreactivity. In the hilus, CA3, CA2, CA1, and in the subiculum (Fig. 2a,b), the immunoreaction was seen throughout the neuropil and in the perikaryon and dendrites of neurons. In the hippocampal formation of the AD patients, on the other hand, immunoreaction with all antibodies was reduced in the molecular layer of the dentate gyrus, in the cell bodies of gran-

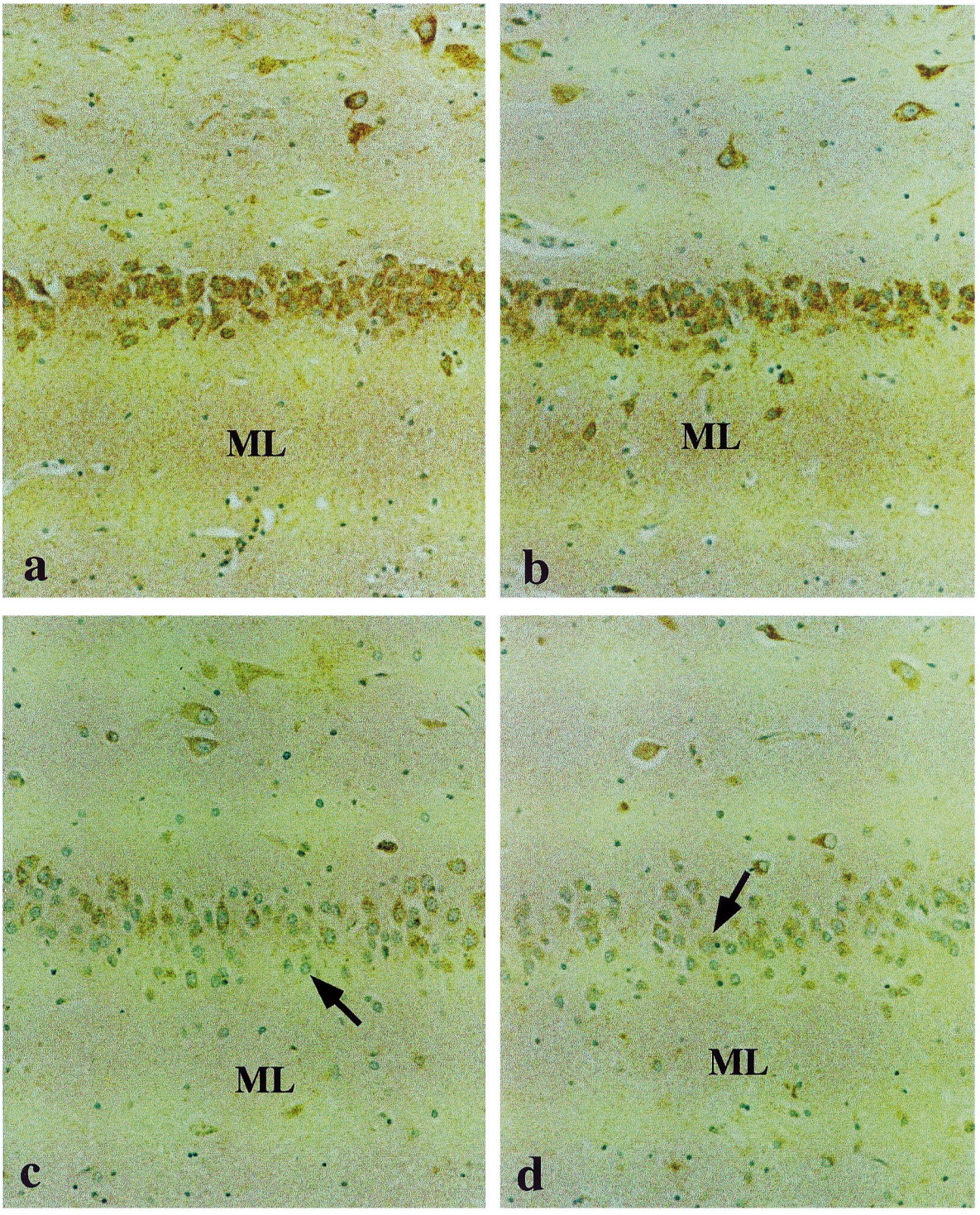

Fig. 1. Immunostaining of sections of the dentate gyrus from a normal individual (a,b) and an AD patient (c,d) for the localization of the mtDNA-encoded COX II subunit of Complex IV (a,c) and the nDNA-encoded FeS subunit of Complex III (b,d). The patient with Alzheimer's disease shows a marked decrease of immunostain for COX II and for FeS in granule cells (arrow) and in the molecular layer (ML) of the dentate gyrus.

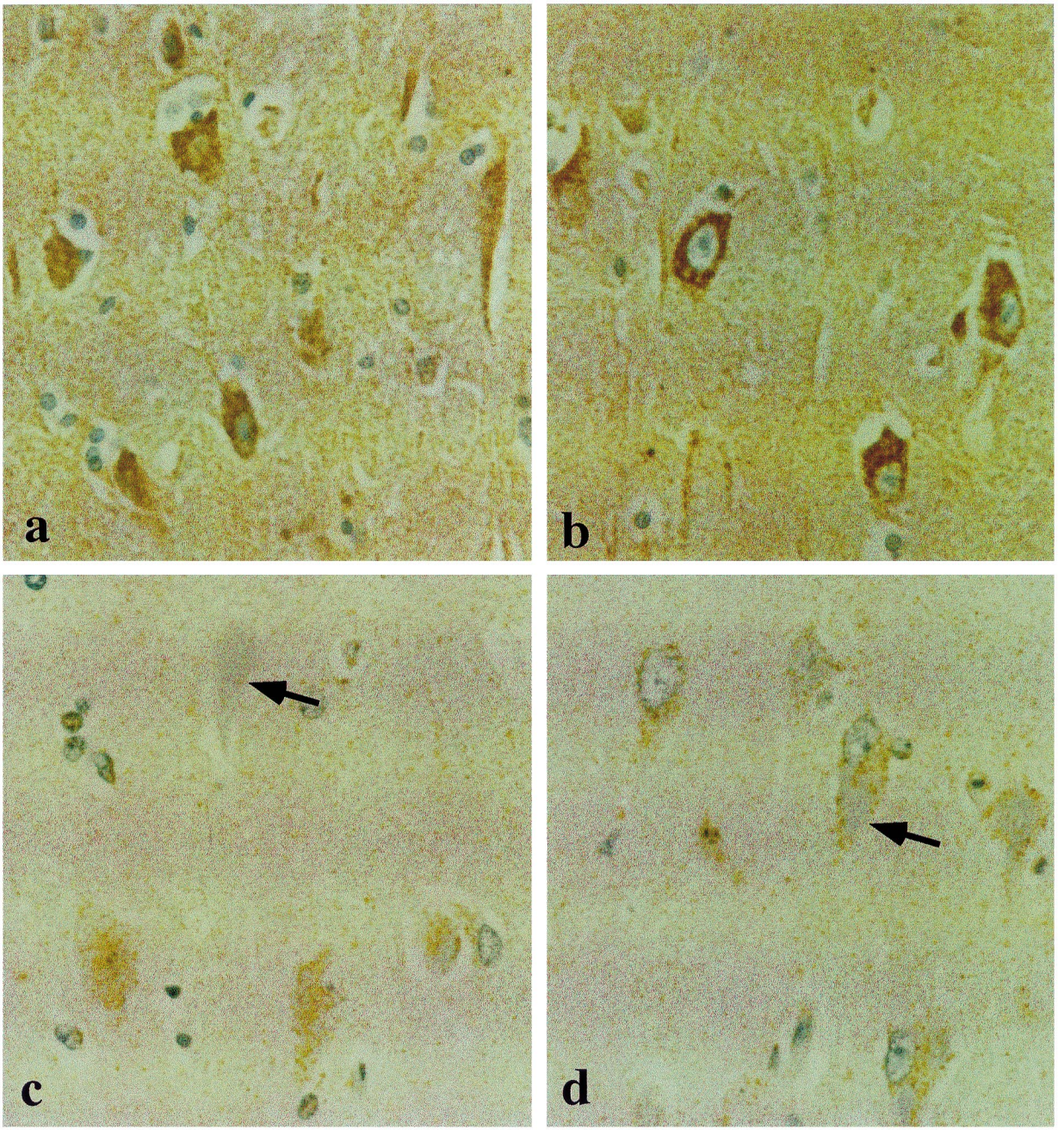

Fig. 2. Immunostaining of sections of the CA1 region of the hippocampal formation from a normal control (a,b) and from the same AD patient shown in Fig. 1 (c,d) for the localization of the mtDNA-encoded COX II subunit of Complex IV (a) and the nDNA-encoded FeS subunit of Complex III (b). Note the decreased immunostain for both COX II and FeS in the AD patient; this decrease appears to occur in the vast majority of neurons, whether they contain NFT (arrows) or not.

ule cells (Fig. 1c,d) and in all hippocampal subfields, including neurons with and without NFT formation (Fig. 2c,d).

While our results confirmed the original observations of Simonian and co-workers [62,63] that there is COX deficiency in AD hippocampus, it is noteworthy that we found a reduction not only of mtDNA-encoded COX I and COX II subunits, but

also of the nDNA-encoded FeS subunit of Complex III. The anatomic distribution of the reduced immunoreactivity for subunits of the respiratory chain was similar to the distribution observed by Simonian et al. for the reduction in COX II mRNA levels [63].

We note that both mtDNA-encoded and nDNA-encoded subunits of the respiratory chain appear to be highly sensitive to alterations in neuronal function. For instance, following monocular deprivation in monkeys, Hevner and Wong-Riley found decreased mRNA levels of both the mtDNA-encoded COX I and the nDNA-encoded COX IV subunits in specific layers of the lateral geniculate nuclei [65]. These observations suggest that alterations in COX activity as a result of deafferentation may be controlled primarily by nuclear factors regulating the expression of the respiratory chain [66]. Although our results must also represent secondary changes due to deafferentation of hippocampal subfields, they provide further evidence for mitochondrial dysfunction in AD.

Nor is evidence for mitochondrial dysfunction in AD confined to alterations in COX or to mitochondria of the hippocampal formation. For example, cortical tissue from AD brain shows decreased activity of ATP-synthase [67], pyruvate dehydrogenase [68], and α-ketoglutarate dehydrogenase [69], important enzymes in energy metabolism that are mitochondrial, but are not part of the respiratory chain. In addition, there is evidence of reduced mRNA levels of both mitochondrial and nuclear genes in the temporal cortex, but not in the primary motor cortex of AD brain. Messenger RNAs for two subunits of Complex I (ND1 and ND4), and for COX I, COX II and COX III, all of which are mtDNA-encoded genes, are also reduced in AD temporal cortex [70], but so are the mRNAs for the β-subunit of ATP synthase and for COX IV, which are nuclear genes. In contrast, mRNA for proteins that are not components of the respiratory chain, such as lactate dehydrogenase, β-actin, and mtDNA-encoded 12S rRNA, are not reduced. These observations have led to the proposal that in AD there is a global down-regulation of the expression of all subunits of the respiratory chain, but the precise mechanisms underlying this down-regulation remain to be elucidated [70–73].

6. Mitochondrial DNA mutations in AD

While there is increasing evidence that mitochondrial functions may be impaired in AD, the pathogenic role of mtDNA mutations is controversial at best. To understand the underlying issues in this controversy, it is important to distinguish somatic cell mutations from maternally inherited mtDNA mutations. An example of somatic cell mtDNA mutations is the putative increased levels of the 'common deletion' in cerebral cortex of AD patients [52], which has not been corroborated [70,74,75] and, in fact, was not confirmed by us (unpublished data). By contrast, several reports have suggested that maternally inherited point mutations of mtDNA might play a pathogenic role in AD.

The concept that maternally inherited mtDNA mutations can contribute to AD is appealing for at least three reasons. First, over 50 mtDNA point mutations have been identified, many in patients with neurodegenerative conditions [19,20], leading some investigators to hypothesize that the mitochondrial encephalomyopathies might be a 'paradigm for degenerative diseases' such as AD [6]. Second, there are at least two epidemiological reports suggesting that there may be a higher incidence of AD in mothers compared to fathers of AD probands, implying possible maternal inheritance [53,76]. Third, and perhaps most importantly, the hypothesis is testable.

The first such candidate mutation was reported in 1992 [77]. It was a G→A transition at position 5460 in ND3 gene; it was found in 10 of 19 AD brains, but was absent in all 11 controls studied. These results, however, could not be confirmed [78–81], and it appears that the G5460A mutation is almost certainly a neutral polymorphism.

The etiology ascribed to a second point mutation – an A→G transition at position 4336 in $tRNA^{Gln}$ – is less clear. Using restriction endonuclease analyses, Shoffner et al. [82] attempted to identify differences in mtDNA from 173 late-onset Caucasian patients with AD, Parkinson's disease (PD), or both. Only the A4336G polymorphism showed a modestly increased frequency in the patients (9/173, or 5.2%) compared to controls (12/1691, or 0.7%). A second group reported similar results, with a slightly higher incidence of this polymorphism in patients with AD (1/28, or 3.6%) and PD (2/23, or 8.7%) as compared

to controls (0/100) [83]. A third group also found an elevated frequency of the A4336G mutation in AD patients [84], but cladistic analysis of the mtDNA haplotypes indicated that there was a potential 'founder effect' for this mutation, implying that it is not the mutation per se that is etiologic, but rather that AD patients may harbor a mitochondrial genotype that predisposes to the disease. By contrast, a fourth study found a *lower* rate of this polymorphism in AD patients (1/155, or 0.6%) than in age-matched controls (4/105, or 4%) [85]. A fifth group found no segregation of the mutation in 100 PD patients [86].

To explore the issue of mtDNA mutations in AD in greater depth, the mitochondrial genome was sequenced in three patients with AD plus PD, and in one patient with PD alone [87]. Several mtDNA polymorphisms were identified in this group of patients, including A4336G; however, none of the polymorphisms was common to all of the patients and all could have been due to the normal variation in human mtDNA. Thus, the pathogenic role of those polymorphisms could not be established. In sum, while these results are intriguing, the A4336G polymorphism might be a marker for mtDNA involvement in some cases of AD, but it is unlikely to play a significant role in the vast majority of patients.

In a study published last year, Davis and colleagues described the identification of six novel mtDNA point mutations in both AD patients and in control individuals, and furthermore, they reported that the proportion of these polymorphisms was significantly higher in platelet-enriched DNA from the AD patients [88]. Three of the putative mutations were in the subunit I gene of COX (G6366A, C6483T, and A7146G) while the other three were in the subunit II gene of COX (C7650T, C7868T, and A8021G). However, in subsequent studies, these purported mtDNA mutations were identified as artifacts derived from PCR amplification of nuclear DNA, specifically, from nucleus-embedded mtDNA pseudogenes [89,90].

In addition to direct sequencing of mtDNA, the cybrid tissue culture system has also been utilized to identify possible mtDNA alterations in AD patients. This in vitro system uses cells devoid of mtDNA, called ρ° cells [91]. These cells can be fused with cytoplasts derived from cells harboring known or potentially pathogenic mtDNA mutations, thereby creating 'cytoplasmic hybrids' or 'cybrids.' They have been successfully used to investigate the pathogenic effects of candidate mtDNA mutations in a uniform nuclear background [92]. In one study utilizing the cybrid technology, a teratocarcinoma cell line (NT2) reported to be depleted of mtDNA [93] was fused with platelets from five AD patients and from four control individuals [94]. The cybrids derived from the AD patient platelets were found to have evidence of increased reactive oxygen species and free radical scavenging enzyme activities, with a biochemical defect in COX activity [94] and in calcium homeostasis [95] when compared to cybrids from control platelets. Unfortunately, several methodological issues have been raised regarding the appropriateness of these lines in ascribing the cause for the observed defects to authentic mtDNA mutations [96]. Thus, the interpretation of the data is unclear at present.

In short, while oxidative phosphorylation may be compromised in AD, there are no convincing data to implicate either sporadically derived mtDNA deletions or maternally inherited mtDNA point mutations in the majority of AD patients.

7. Concluding remarks

The causes of most neurodegenerative diseases, including sporadic AD, remain enigmatic. There is, however, increasing evidence implicating mitochondrial dysfunction resulting from deafferentiation of disconnected neural circuits in the pathogenesis of energy deficit in AD. The patterns of reduced expression of both mtDNA and nDNA-encoded genes are consistent with a physiological down-regulation of the mitochondrial respiratory chain in response to reduced neuronal activity. On the other hand, the roles of somatic cell or maternally inherited mtDNA mutations in the pathogenesis of mitochondrial dysfunction in AD are still controversial. It is particularly interesting that the increase in oxidative damage in mtDNA is associated with increased levels of the 'common deletion' in cortical regions of AD patients. These observations suggest that AD patients may have elevated oxidative damage, which may increase the somatic mtDNA mutation rate. It is important, however, to emphasize that this observation has not

been corroborated by other investigators, and that no direct cause-and-effect relationship has yet been established between oxidative damage to mtDNA and mtDNA mutations in AD or in aging. More work on this area is required before this hypothesis can be considered fully substantiated.

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