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REVIEW

Oxidative phosphorylation defects and Alzheimer's disease

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ABSTRACT

Abnormalities in cellular bioenergetics have been identified in patients with Alzheimer's disease (AD) as well as in patients with other neurodegenerative diseases. The most commonly reported enzyme abnormalities are in the pyruvate dehydrogenase complex, the α-ketoglutarate dehydrogenase complex, and oxidative phosphorylation (OXPHOS). Although genetic evidence supporting primary OXPHOS defects as a cause for AD is weak, functionally important reductions in OXPHOS enzyme activities appear to occur in AD and may be related to β-amyloid accumulation or other neurodegenerative processes. Since reduced neuronal ATP may enhance susceptibility to glutamate toxicity, OXPHOS defects could play an important role in the pathophysiology of AD.

Keywords: Alzheimer's disease, oxidative phosphorylation, mitochondrial DNA, cytochrome *c* oxidase, glutamate neurotoxicity, β-amyloid

INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disease in which cognitive decline is associated with the accumulation of senile plaques, neurofibrillary tangles, and occasionally amyloid angiopathy. Several neurotransmitter systems are perturbed in AD, with cholinergic deficiency being most prominent and associated with neuron loss in the nucleus basalis of Meynert (1). Over the past several years, molecular genetic studies provided important insights into pathogenetic mechanisms that cause AD. For most individuals with AD, the molecular basis of neurodegeneration is unknown. In rare autosomal dominant, early onset forms of AD gene defects have been identified. These include mutations in the amyloid precursor protein (APP) gene on chromosome 21, the gene presenilin 1 on chromosome 14, and the gene presenilin 2 on chromosome 1. Furthermore, allele ε4 of apolipoprotein E was found to be a risk factor of late onset AD (2). The role of oxidative phosphorylation (OXPHOS) defects in the pathogenesis of AD is controversial. This article reviews current concepts concerning this relationship.

OXIDATIVE PHOSPHORYLATION BIOCHEMISTRY AND GENETICS

OXPHOS consists of five protein–lipid enzyme complexes which are located in the mitochondrial inner membrane (Fig. 1). These enzymes contain flavins, coenzyme Q_{10} (ubiquinone), iron-sulfur clusters, hemes and protein-bound copper. Simplified designations for these enzymes are complex I, complex II, complex III, complex IV (cytochrome *c* oxidase) and complex V (ATP synthase). Complexes I and II collect electrons from the catabolism of fats, proteins and carbohydrates and transfer them sequentially to coenzyme Q_{10}, complex III and complex IV. Complexes I, III and IV utilize the energy in electron transfer to pump protons across the inner mitochondrial membrane, producing a proton gradient that is used by complex V to convert ADP and inorganic phosphate into ATP. The adenine nucleotide translocase (ANT) delivers ATP to the cytoplasm in exchange for ADP.

In order to assemble functional OXPHOS complexes, nuclear DNA genes and mtDNA genes are coordinately expressed. OXPHOS polypeptides coded by nuclear DNA genes are transported to the mitochondria where they join OXPHOS polypeptides coded by the mtDNA. The human mtDNA is contained within mitochondria and is a 16 569 nucleotide pair, double-stranded, circular molecule which codes for two ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA), seven complex I polypeptides, one complex III polypeptide, three

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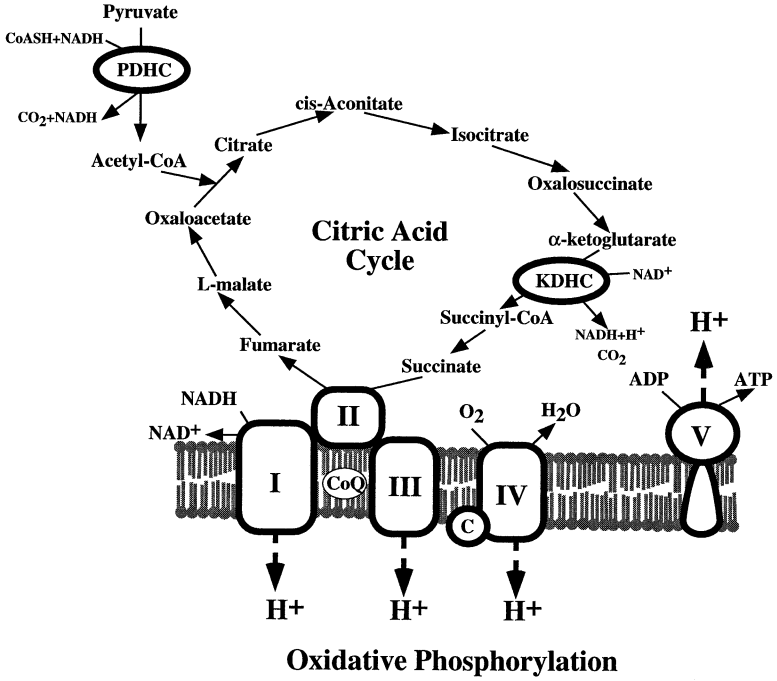

Figure 1. Abnormal CNS bioenergetics pathways in AD. The metabolic pathways shown are located within the mitochondria. PDHC, KDHC, and complexes II and IV of OXPHOS are abnormal in AD. OXPHOS complexes are designated I–V. PDHC, pyruvate dehydrogenase complex; KDHC, α-ketoglutarate dehydrogenase complex; CoQ10, coenzyme Q_{10}; c, cytochrome *c*; H^+, protons; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CoA, coenzyme A.

complex IV polypeptides, and two complex V polypeptides. Only complex II is encoded entirely by the nuclear DNA.

The clinical genetics of OXPHOS diseases are characterized by maternal and Mendelian inheritance patterns. Due to the cytoplasmic location of mtDNA within oocyte mitochondria and the lack of sperm mtDNA contribution to the human oocyte during fertilization, this genome is maternally or asexually inherited, thus accounting for the exclusive transmission of mtDNA from a mother to her children. When all mtDNAs in a tissue share a common sequence, the population of mtDNAs is referred to as *homoplasmic*. Populations of mtDNAs which contain more than one sequence are referred to as *heteroplasmic*. Pathogenic mtDNA mutations can be either homoplasmic or heteroplasmic, whereas neutral polymorphisms are generally homoplasmic. Neutral polymorphisms that are heteroplasmic typically occur within non-coding regions of the mtDNA (3,4). When heteroplasmy exists for pathogenic mutations, the normal and mutant mtDNAs segregate randomly during cytokinesis to the daughter cells. Once the mutant mtDNAs reach a critical level, cellular phenotype changes rapidly from normal to abnormal. The relationship between genotype and phenotype is more complex for pathogenic mtDNA mutations that are homoplasmic. Disease expression appears to be influenced by poorly understood genetic and environmental interactions.

The mitochondrial genome has a high mutation rate. For example, when the evolutionary rate of mammalian mitochondrial and nuclear tRNA genes was compared, the mitochondrial tRNA nucleotide substitution rate was ~25 times greater than the nuclear tRNA nucleotide substitution rate (5). This high degree of mtDNA sequence variation within human populations over time was recognized as an important tool in estimating the human common ancestor as well as aspects of geographical origins and movements of human populations (6–8). Population variants in the mtDNA occur at sites with both low and high degrees of interspecies conservation. Novel mtDNA mutations can also occur within a single generation and undergo rapid segregation between family members (3,4).

An important consequence of the high mtDNA mutation rate is that a wide variety of transmissible, pathogenic mutations are known to occur (9). Some mtDNA mutations produce systemic diseases with early ages of onset such as Leigh's disease (10,11), myoclonic epilepsy and ragged-red fiber disease (MERRF) (12), and mitochondrial encephalomyopathy with stroke-like episodes and lactic acidosis (MELAS) (13). In contrast, other mtDNA mutations produce tissue specific disease manifestations. For example, most patients with Leber's hereditary optic neuropathy (LHON) experience only the rapid onset of vision loss affecting the central visual field (14). The high mutation rate of the mtDNA is also reflected in spontaneously occurring mtDNA mutations referred to as somatic mtDNA mutations that increase with age in a variety of tissues (15,16). Free-radical mediated damage to the mtDNA is important in the formation of somatic mtDNA mutations (17).

BIOENERGETIC DEFECTS IN AD

A variety of *in vivo* and *in vitro* approaches demonstrated bioenergetic defects in AD (18). Brain imaging methods such as single photon emission computed tomography which assesses cerebral blood flow, positron emission tomography which assesses cerebral metabolism, and phosphorous nuclear magnetic resonance spectroscopy which assesses cerebral energetics by measuring the phosphocreatine/inorganic phosphate ratio demonstrated impaired cerebral blood flow, glucose

utilization, oxygen utilization, and brain energetics in early and late onset AD (19–29). AD brain biopsies demonstrated mitochondrial uncoupling, a non-specific abnormality indicative of an impairment in conversion of ADP to ATP (30). Ultrastructural studies of AD brain showed structural abnormalities of mitochondria such as increased numbers of mitochondria, laminated dense bodies, and in some cases paracrystalline inclusions (31–33). Significant increases in cerebrospinal fluid (CSF) pyruvate and mild increases in CSF lactate were observed in patients with clinically diagnosed AD (34). Although these observations are not specific for a particular metabolic defect, they are consistent with abnormal CNS energy metabolism in AD.

The most consistently reported bioenergetic defects are decreased activities in the pyruvate dehydrogenase complex (PDHC), the α-ketoglutarate dehydrogenase complex (KDHC), and OXPHOS enzymes. However, these abnormalities are not specific to AD. Reductions in the activities of these enzymes have been observed in a variety of neurodegenerative conditions such as Parkinson's disease, Huntington's disease, Friedreich's ataxia, and spinocerebellar ataxia type I (35–44). PDHC regulates pyruvate metabolism by the citrate cycle, contributes to cellular calcium homeostasis (45,46), and its activation in brain is linked to acetylcholine synthesis (47,48). PDHC abnormalities (36,48,49) and KDHC abnormalities (50–52) are present in both neuropathologically normal and abnormal areas of AD brain. Immunoblots of PDHC in AD brain suggested that the reduced activities are consistent with decreased quantities of this enzyme complex (49). In contrast to the PDHC abnormalities, it has been suggested that the reduction in KDHC activity may exceed the reduction in enzyme protein (53). Although this observation has been interpreted as evidence for an inherited genetic defect in a KDHC subunit, no pathogenic mutations have been identified.

In 1990, specific OXPHOS defects were described in mitochondria from patients with AD when W.D. Parker and colleagues reported a defect in platelet complex IV activity (54). Five of six AD patients had decreased levels of complex IV. Assays that measured complex I and complex II+III activities did not differ significantly from control values. The mean complex IV activity in these five AD patients was ~68% below control activities. The assessment of OXPHOS in platelet mitochondria is more difficult than the traditional enzymological investigations performed in tissues such as skeletal muscle. Platelet-pheresis was required to obtain adequate quantities of platelets for mitochondrial isolation and a complex isolation protocol was used to purify platelet mitochondria. The methodology was improved by Van Zulen *et al.* (55) and OXPHOS activity was investigated in platelets obtained from a 10 ml venipuncture. By this approach, Van Zulen *et al.* found no abnormalities in OXPHOS in platelets from six AD patients (55). However, in a blinded study of 19 AD patients and 17 controls which used similar methodology on 120 ml of venous blood, Parker *et al.* detected a mean complex IV activity that was ~17% lower than control activity (56). Since no abnormalities in complex II, complex III, or citrate synthase were observed, a mild defect in complex IV activity in platelets from AD patients appeared possible.

Investigations of OXPHOS in mitochondria isolated from various brain regions revealed similar results. Kish *et al.* surveyed frontal cortex (Brodmann area 10), temporal cortex (Brodmann area 21), parietal cortex (Brodmann area 7b), occipital cortex (Brodmann area 17), putamen, and hippocampus in autopsied human brain. Two regions showed a statistically significant reduction in complex IV activity, frontal cortex with a reduction of 26% and temporal cortex with a reduction of 17%. However, this study did not investigate the activities of other OXPHOS enzymes. A defect in the activities of complexes I–IV was identified by Parker *et al.* in cortical tissue from the right hemisphere of AD patients with 53% decrease in the mean complex IV activity from the control value (57). Further investigations of complex IV enzyme kinetics in AD brain revealed abnormalities in substrate binding kinetics, thus leading to the hypothesis that mutations in OXPHOS subunit genes could play a role in producing the complex IV defects (58).

Not all studies reported significant complex IV defects. Cooper *et al.* reported normal complex IV activity in AD temporal lobe mitochondria (59). OXPHOS measurements in parietal cortex, temporal cortex, entorhinal cortex, and hippocampus by Reichmann *et al.* demonstrated decreased activities of complexes I–IV (60). The most significant defects were decreases of 35–50% in the activities of the complex II and complex II+III assays measured in parietal and temporal cortex and decreases of 10% in the complex IV activities in hippocampus and 25% in the other cortical regions (60).

Little data exist that relates the magnitude of OXPHOS defects observed in human brain to clinical abnormalities, thus making it difficult to assess the consequences of these CNS OXPHOS defects. However, complex IV reductions of ~35% which were produced in rodents by the selective complex IV inhibitor, sodium azide, were associated with learning impairments (61).

OXPHOS enzymology of brain does not differentiate between primary and secondary causes of complex IV defects. Secondary causes for complex IV defects include neuronal loss, down regulation of complex IV expression as a result of decreased functional activity of neurons (62–64), decreased expression of mtDNA encoded subunits of complex IV in response to free radical mediated cell damage (65), and abnormal interactions between OXPHOS polypeptides and compounds such as β-amyloid. Deafferentation associated with decreased neuronal functional activity and decreased complex IV gene expression may be important in explaining the OXPHOS defects in AD. Complex IV histochemistry showed decreased complex IV activity in structurally intact neurons in areas prone to deafferentation in AD (66). *In situ* hybridization of AD brain sections revealed a decrease in mitochondrial mRNA for complex IV subunits (67).

β-Amyloid accumulation has a deleterious effect on OXPHOS function. Three major isoforms of β-amyloid precursor protein (APP) are produced by alternative splicing. One isoform which contains 751 amino acids (APP-751) was transfected into primary cultures of human muscle using an adenovirus vector (68). Overexpression of APP-751 produced a decrease in complex IV enzyme activity and ultrastructural abnormalities of mitochondria which included paracrystalline inclusion formation. In rat hippocampal neurons, β-amyloids have a neurodegenerative effect that is associated with a suppression of complex II activity (69). This observation raises the possibility that the abnormal complex II activity observed in AD brain could be the result of an interaction with β-amyloid (60). Therefore, biochemical investigations that show

OXPHOS abnormalities in human brain may reflect β-amyloid dependent effects on the respiratory chain.

INHERITED mtDNA MUTATIONS AND AD

The presence of mtDNA mutations in late onset AD was investigated by several laboratories. A homoplasmic A-to-G mutation in the $tRNA^{Glutamine}$ gene at position 4336 of the mtDNA ($tRNA^{Gln4336}$) was identified in patients with AD (70). This mutation occurred at the junction between the amino acid acceptor stem with the TψC stem of the $tRNA^{Glutamine}$. Analysis of various ethnic groups revealed that the $tRNA^{Gln4336}$ mutation was Caucasian specific. The $tRNA^{Gln4336}$ mutation was identified in 3.2% (2/62) AD brains and 6.8% (5/73) of brains with neuropathological changes of both AD and Parkinson's disease (PD). The overall frequency of this mutation in Caucasian controls was 0.7% (12/1691). The mtDNA was sequenced in one of the patients with AD and PD neuropathology who harbored the $tRNA^{Gln4336}$ mutation (71). No other mutations were identified that were specific for the mtDNA lineages of the AD patients. Hutchin and Cortopassi extended these initial observations by investigating the frequency of the $tRNA^{Gln4336}$ mutation in AD brains and age-matched controls (72). However, a third study did not find an increased frequency of the $tRNA^{Gln4336}$ mutation in AD (73). The $tRNA^{Gln4336}$ mutation was identified in blood from 0.6% (1/155) of patients with clinically defined late onset AD and in blood from 3.8% (4/105) of age-matched Caucasian controls. An important feature of the $tRNA^{Gln4336}$ mutation is that it is usually associated with a neutral variant in the D-Loop at position 16 304 of the mtDNA (70). Together, these two mutations define a specific mtDNA haplotype within Caucasian mtDNAs. Hence, in order to determine whether the $tRNA^{Gln4336}$ mutation is associated with AD, larger numbers of patients and controls who harbor both the $tRNA^{Gln4336}$ and the 16 304 mutations would need to be studied. Since the $tRNA^{Gln4336}$ mutation has not been shown to cause OXPHOS defects or impair mitochondrial protein synthesis and a small number of patients and controls with this mutation are identified, definitive conclusions about the role of this mutation in AD cannot be made at this time.

MtDNA mutations within the complex IV subunits I and II are reported to occur at increased frequencies in AD cases (74). Complete experimental details of the data presented in this abstract have not been published, thus preventing assessment of the relevance of these observations. Finally, a neutral variant in the E2k component of KDHC was reported to be increased in AD patients (75). However, this gene is ~1 Mb away from the presenilin 1 gene on chromosome 14 and may therefore represent a marker that is tightly linked to presenilin gene mutations or that is in an as yet unidentified gene. No mutations that are associated with AD have been described in PDHC genes.

At this time, evidence supporting a role for inherited mutations in bioenergetics genes as a cause for increased susceptibility to AD is not compelling.

SOMATIC mtDNA MUTATIONS AND AD

As individuals age, ATP production by OXPHOS declines (76–82). This decline in respiratory function is associated with an accumulation of complex IV deficient fibers in various muscle groups (83–85). The mitochondrial theory of aging was postulated to explain age-related decline in OXPHOS. This hypothesis of free radical mediated mtDNA damage has three essential elements (86). First, free radicals are continuously produced in the mitochondria by OXPHOS as well as other reactions. Second, free radical production is stimulated by inhibition of the electron transport chain representing a self-perpetuating process. Oxidative degradation of mitochondrial lipids, proteins, and mtDNAs impairs OXPHOS efficiency and stimulates more free radical accumulation. Third, the accumulation of mtDNA damage referred to as somatic mtDNA mutations blocks mitochondrial biogenesis, resulting in permanent organelle dysfunction which ultimately leads to cell death.

Heterogeneous classes of mtDNA deletions increase with age in brain (87–89), heart (15,16,90–94), skeletal muscle (95), liver (96,97), kidney (97), and various other tissues (97). Base modifications also increase in aging tissues, with 8-hydroxydeoxyguanosine showing an age related increase in human diaphragm (98) and brain (17). Although the accumulation of somatic mtDNA mutations in various tissues during aging is well established, significant increases in these mutations in AD brain have not been established. The increases in somatic mtDNA mutations reported in AD brain (99) are not convincing and have not been confirmed by other groups (100,101).

CONCLUSIONS

AD is associated with abnormal brain energy metabolism. Although the associations between mutations in bioenergetics genes and AD are weak, decreases in key bioenergetics enzymes such as PDHC, KDHC, and OXPHOS occur and may contribute to the neurodegeneration through a variety of mechanisms. The most important consequences of decreased levels of neuronal ATP may be an enhanced susceptibility of neurons to glutamate toxicity. This could occur by (a) interference with sodium-potassium adenosine triphosphatase function which would lead to abnormalities in membrane depolarization, (b) reduction of voltage dependent Mg^{2+} blockade of N-methyl-D-aspartate receptors by increases in intracellular sodium, (c) opening of voltage dependent calcium channels resulting in an influx of Ca^{2+} ions into the neurons, (d) further increases in intracellular Ca^{2+} due to a failure of mitochondria and endoplasmic reticulum to store Ca^{2+}, and (e) impaired ATP dependent uptake of glutamate by glial cells (see ref. 102 for review). These changes could activate pathways that produce oxidative stress and neuronal injury (103). Increased carbonyl modifications of proteins, increased lipid peroxidation, and increased mtDNA oxidation, provide evidence for increased oxidative stress in AD brain. However, the source for free radicals in AD is unknown. Although free radical generation during mitochondrial electron transport appears to be important in damaging mtDNA and causing somatic mtDNA mutations to accumulate within tissues, a causal relationship between somatic mtDNA mutations and OXPHOS defects in AD brain have not been established. Although further investigations are needed to determine when during the neurodegenerative process OXPHOS abnormalities occur and what the most important changes are in the neuron that lead to these

changes, neuronal deafferentation and abnormal interactions between OXPHOS enzyme subunits and β-amyloid may play a significant role in this process.

PDHC and KDHC abnormalities may contribute to the pathogenesis of AD by impairing acetylcholine synthesis and glutamate metabolism. Cholinergic neurons depend on normal mitochondrial function for the production of acetyl coenzyme A, a precursor to the synthesis of acetylcholine. Defects in PDHC decrease acetyl coenzyme A production which could decrease the availability of acetyl coenzyme A groups for cytoplasmic acetylcholine synthesis by choline acetyltransferase (18). Abnormalities in KDHC would impair glutamate removal by impairing the oxidation of α-ketoglutarate which is the product of glutamate transamination or oxidation by glutamate dehydrogenase (18).

Bioenergetics abnormalities may play a significant role in the pathogenesis of a variety of neurodegenerative diseases. Understanding the mechanisms by which these changes occur may yield new insights into treatments that could slow the progression of AD. At this time, the evidence supporting a role for inherited or somatic mtDNA mutations in AD is weak and requires further investigation before including them as susceptibility genes.

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