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Nuclear but not mitochondrial-encoded oxidative phosphorylation genes are altered in aging, mild cognitive impairment, and Alzheimer's disease

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Abstract

Introduction—We have comprehensively described the expression profiles of mitochondrial DNA and nuclear DNA genes that encode subunits of the respiratory oxidative phosphorylation (OXPHOS) complexes (I–V) in the hippocampus from young controls, age matched, mild cognitively impaired (MCI), and Alzheimer's disease (AD) subjects.

Methods—Hippocampal tissues from 44 non-AD controls (NC), 10 amnestic MCI, and 18 AD cases were analyzed on Affymetrix Hg-U133 plus 2.0 arrays.

Results—The microarray data revealed significant down regulation in OXPHOS genes in AD, particularly those encoded in the nucleus. In contrast, there was up regulation of the same gene(s) in MCI subjects compared to AD and ND cases. No significant differences were observed in mtDNA genes identified in the array between AD, ND, and MCI subjects except one *mt-ND6*.

Discussion—Our findings suggest that restoration of the expression of nuclear-encoded OXPHOS genes in aging could be a viable strategy for blunting AD progression.

Keywords

Aging; Alzheimer's disease; Mild cognitive impaired (MCI); Mitochondria; Oxidative phosphorylation-related genes expression; Microarray; Postmortem brains

1. Introduction

Alzheimer's disease (AD) is a progressive, degenerative brain disease and the most common cause of dementia [1,2]. AD is characterized by a gradual decline in cognitive function and the presence of pathologic inclusions including amyloid β (Aβ) plaques and neurofibrillary tangles [1]. The disease is preceded by a pre-symptomatic stage that can last for years during which time the clinical symptoms are undetectable. An intermediate stage between normal aging and AD has been identified as mild cognitive impairment. This disease state is characterized by problems with memory, language acquisition and processing, critical thinking, and judgment problems that are greater than normal age-related changes [2].

Bioenergetic failure and mitochondrial dysfunction in AD are well documented [2–10]. Several studies using multiple preclinical *in vitro* and *in vivo* AD models have demonstrated a decline in mitochondrial function before the development of AD pathology [2–8]. Mitochondria are key organelles that regulate a multitude of metabolic and signaling pathways including programmed cell death [11–15]. The primary function of mitochondria is to produce ATP through the process of oxidative phosphorylation (OXPHOS), which is regulated through four respiratory multi-subunit enzyme complexes (complexes I–IV) and ATP synthase (complex V), all located in the inner mitochondrial membrane (Fig. 1) [16–18]. Mammalian mitochondrial DNA (mtDNA) is a maternally inherited, double-stranded circular genome of approximately 16.6 kb [19–21]. It contains 37 genes encoding 13 protein subunits of enzymes involved in oxidative phosphorylation, two ribosomal RNAs, and 22 transfer RNAs necessary for translation of these proteins. The remaining subunits are encoded by the nuclear genome, synthesized in the cytosol, and subsequently imported into mitochondria through protein translocation machineries of the outer and inner membranes [19–21].

A critical role of mitochondrial dysfunction has been hypothesized in both aging and neurodegenerative diseases [2–10]. Numerous studies use animal models based on genetic mutations found in rare early onset familial AD cases that represent <1% of AD patients [1]. Recently, it has been demonstrated that mitochondrial bioenergetic deficits precede AD pathology in the female triple transgenic mouse model of AD (3xTgAD) [4]. Converging lines of evidence indicate that mitochondria are direct targets of Aβ [22–24], and that Aβ is directly responsible for impaired electron transport chain function [22–28]. Accumulation of Aβ in neurons is believed to be an essential step leading to Aβ-mediated mitochondrial dysfunction and contributes to energy failure, neuronal apoptosis, and production of reactive oxygen species (ROS) in AD brain tissue [25–29]. Mitochondrial dysfunction and oxidative damage occur early in the course of disease, before the onset of significant plaque pathology, and act causally in disease pathogenesis [4,30]. Normal aging and AD are both marked by prominent defects in brain metabolism and increased oxidative stress. Although inheritance of certain susceptibility genes increases the risk of the disease, aging is the most prominent risk factor for the non-Mendelian sporadic AD which affects most patients diagnosed for dementia over the age of 60 years [31]. Several studies have provided evidence of neuronal metabolic impairments at the transcript and protein level in AD brain, which was ascribed to the down regulation of mitochondrial-associated genes; in particular, oxidative phosphorylation genes [27,32–37]. Following this line of thought, we present here a

microarray-based study that focuses specifically on the role of OXPHOS-related genes in aging and in AD.

It is clear from the literature that the etiology of AD is not completely understood; hence, the use of a wide array of animal and cellular models to address the pathological process of the early onset, dominantly inherited familial form of this disease. Although these model system(s) do feature particular aspects of the disease process, no model has yet fully recapitulated the human disease. There is much evidence that late onset AD overlaps with normal aging in many clinical and pathologic features [38–43]. Therefore, the availability of human postmortem brain tissue is of great importance for biological studies on human brain aging and the progression to disease. These unique tissue specimens make it possible to investigate potential gene expression alterations before, during, and after the onset of AD. In this study, we compared expression profiles of mitochondrial genes (e.g., OXPHOS) from the hippocampus from clinically and pathologically confirmed AD cases, young controls, age-matched controls, and MCI cases.

2. Methods

Frozen unfixed hippocampus was obtained from 44 non-AD controls (age, 20–99 years), 10 amnestic mild cognitively impaired cases, and 18 sporadic AD cases (age, 74–95 years; Table 1) from seven nationally recognized brain banks. Inclusion criteria can be found in Berchtold et al. [38]. Total RNA was extracted from heterogeneous population across hippocampal subfields as described previously [38], and RNA quality was assessed using the Agilent Bio-Analyzer (Agilent Technologies, Palo Alto, CA). Average RIN for the samples was 8.29 (SD, 0.775; range, 6.7–9.6), with 93% of the cases (67 of 72) having RIN > 7. There were no significant differences in RNA integrity across groups [39]. Seventy-two hippocampal tissue samples were individually hybridized to Affymetrix HgU133 plus 2.0 arrays.

Samples were randomized across batch runs, and no batch effects were detected. Guanine-cytosine robust multi-assay analysis (GC-RMA) expression values were calculated, followed by per-chip and per-gene normalization, log-transformation of the geometric mean of expression using Gene-Spring 7.3.1 software (Agilent Technologies, Palo Alto, CA). The data underwent the following normalization steps: (1) per chip: normalize to 50th percentile, (2) per gene: normalize to median. Genes were not normalized to "housekeeping" genes. Eighty-six mitochondrial genes, 41 of 44 from complex I, four of four from complex II, eight of ten from complex III, 16 of 19 from complex IV, and 17 of 19 from complex V, were investigated for statistical significance ($P < .01$) in aging comparing young ($n = 18$, age 20–59 years, mean age 35.4 ± 10.9 years) versus aged controls ($n = 26$, age 69–99 years, mean age 82 ± 9.8 years; Table 1). AD/MCI-related gene expression changes were based on the comparison of aged controls ($n = 26$; age, 69–99 years; mean age, 82 ± 9.8 years), versus mild cognitive impairment ($n = 10$, mean age 84.5 ± 5.6 years) versus AD ($n = 18$, mean age 83 ± 8.7 years). A complete list of genes and descriptions can be found in Supplementary Table 1.

We preferentially selected those probe sets that were most specific, such that they were annotated with the smallest number of Ensembl gene IDs. After applying this criterion, if there remained multiple probe sets for any one gene, we excluded those probe sets expected to hybridize with targets in a nonspecific fashion (i.e., those with "_x_" in the Affymetrix identifier), which was the case for seven of the OX-PHOS gene. If there still remained multiple probe sets for a given gene, we took the mean of both probe sets.

2.1. Statistical analysis

Analysis between individuals, groups, and disease status were performed. All genes that did not meet the 50% present call threshold were removed by gene-spring G 7.3.1 Expression Analysis software. A two-tailed paired t test, assuming equal variance (using multiple testing corrections, by Benjamin and Hochberg False Discovery Rate) was applied to locate genes that were significant ($P = .05$ or less) in differentiating expression between AD, ND, and MCI cases. The results presented here are, of course, subject to the limitations of microarray-based approaches. These include false discovery resulting from multiple statistical tests of significance, although this source of error was limited by the use of two different analytical methods to validate the gene lists, as well as conventional validation by RT-PCR. Taqman primers (Thermo Fisher Scientific) were designed to six randomly chosen mitochondrial transcripts *MT-ND1* (Hs02596873_s1), *NDUFA6* (Hs00899690_m1), *NDUFC2* (Hs01072843_m1), *UQCRC1* (Hs00429571_g1), *ATP5D* (Hs00961521_m1), and *MT-ATP6* (Hs02596862_g1). All six transcripts showed the same pattern of expression identified in the array.

3. Results

3.1. Aging and AD-related changes in gene expression levels of OXPHOS-related genes from hippocampal autopsy tissue

Aging is the most salient risk factor for cognitive decline in the elderly and the predominant risk factor for late-onset of AD. Thus, in this study, an aging cohort was included for a better understanding of age-related effects on the expression of OXPHOS-related genes. Young controls (20–59 years) and age-matched controls (69–99 years) were included as shown in the demographic details (Table 1). Our microarray data revealed age-related alterations of the OXPHOS-related genes which encode mitochondrial respiratory chain complexes I, II, III, IV, and V. Ninety-five percent of the identified genes were down regulated in aging and 100 percent of genes in AD, particularly those which were nuclear encoded (Fig. 2). These data indicate that aging may be a contributing factor for the decline in OXPHOS-related gene observed in AD. This is in agreement with the previous finding that aging resulted in down-regulation of genes in the respiratory chain complexes I, III, IV, and V in the brains of aged C57/BL6 mice [38]. In the context of aging, our results (Fig. 2), a general trend can be observed in complexes I, III, IV, and V; as age increases the mean expression of OXPHOS-related genes decrease, a phenomenon which is exacerbated in AD. Correspondingly, in these same complexes (I, III, IV, and V), MCI cases demonstrate an unequivocal increase in these same OXPHOS-related genes, suggesting a compensatory-like mechanism at the early stages of disease. An exception to this general trend is observed in complex II where only a modest difference can be observed across groups. To determine whether a significant

correlation was present between Braak stage and a decrease in OXPHOS-related transcripts in AD vs. aged controls, we ran a Pearson's correlation test. Accordingly, a significant (P<. 001, r = .78) correlation was observed between Braak stage and a decrease in OXPHOS-related transcripts, indicating that disease-related pathology may account, to some extent, for the decrease in OXPHOS-related transcripts in AD.

Relative gene expression profiles of OXPHOS-related genes from hippocampal tissue of young, MCI, AD, and age-matched controls were subdivided into two groups: nuclear-encoded genes and mitochondrial-encoded genes (Fig. 3). Seventy of the 73 nuclear-encoded genes and six of the seven mitochondrial-encoded genes identified in the Affymetrix array showed a robust correspondence in the overall direction in the expression levels (e.g., up/down regulation) within groups (Fig. 3A–C). As such, in complex I (3a), AD cases showed significantly less expression in all 37 OXPHOS-related genes, compared to control, where MCI cases showed a significant increase in all 37 genes. In contrast, *MT-ND6* gene (see Fig. 3A) was the only mitochondrial-encoded transcript identified in the array that was significantly upregulated in MCI and down regulated in AD, reflecting the same trend as nuclear-encoded OXPHOS genes. Interestingly, all other OXPHOS mitochondrial-encoded genes identified proved to be equivalent to control and appear to be unaffected in the disease progression. Similar findings were observed in complexes II–IV (Fig. 3B) and complex V (Fig. 3C). This finding suggests that, in AD, aging is also an important contributing factor in disease progression, consistent with previous reports [39–43]. The proportion of significantly expressed OXPHOS-related genes in the hippocampal sampled tissue for the different groups in this study is shown in Supplementary Table 2. The three nuclear-encoded and one mitochondrial-encoded transcript not included in the analysis were removed because they were found to hybridize to gene targets in a nonspecific fashion (i.e., those with "_x_" in the Affymetrix identifier).

4. Discussion

Evidence has accumulated over decades that mitochondria play a role in the metabolic deficits of AD. Indeed, studies using the same data set revealed a global decrease in many mitochondrial transcripts in AD [39]. A recent PubMed search of "Mitochondria and Alzheimer's" yielded 1908 hits. Among the significant proponents of the "mitochondrial cascade hypothesis" have been RH Swerdlow [44] and MF Beal [45]. More recent work has shown that mitochondrial dysfunction is, in fact, an early event in AD that is detectable in "preclinical" AD CSF [46] and in imaging data [47]. Recently, a nonamyloid driven hypothesis known as the "inverse Warburg effect" has drawn significant attention in the field. The theory behind the inverse Warburg effect is that AD is the result of a cascade of three events—mitochondrial dysregulation, metabolic reprogramming (the Inverse Warburg effect), and natural selection [48,49]. Although these papers among others make valid arguments in proving there hypothesis, none are absolute. The one absolute finding among all these articles are that mitochondria are affected in the progression of AD, and here, we provide evidence of mitochondrial gene expression changes as a function of age and complement some studies and argue against others.

Herein, we present microarray data in brain that demonstrate significant down regulation of nuclear-encoded mitochondrial genes within respiratory chain complexes in aging, and in AD, but not in mitochondrial-encoded genes. Additionally, we show that these same genes are over-expressed in amnestic MCI cases; which is suggestive of a compensatory-like mechanism in these subjects. Differential expression of OXPHOS-related transcripts could have deleterious effects on ATP production, providing further insight into preclinical strategies for therapy.

4.1. Early AD is related to selected cellular phenomena

Deficits in several cellular events have been shown to occur early in the course of AD cellular pathology. These include metabolic deficits [7,50], altered synaptic structure and function [51], altered phosphorylation and configuration of tau [52], and alterations in the endocytic/autophagy system [53]. Relatively recently, protein misfolding has received more attention [54,55]. In addition, we have recently shown that intracellular redistribution of the key epigenetic molecule, H3K4me3, is also an early event in intracellular AD pathology, indicating that epigenetic regulation of gene expression may be an early event in AD, capable of modulating expression of a wide variety of the molecules attributed to early AD, notably synapses [56].

4.2. Prospective causal mechanism for the decrease in nuclear-encoded OXPHOS genes in AD

We have previously proposed a model of AD pathophysiology in which oligomeric Aβ disrupts the exchange of molecules between the cell nucleus and the cytoplasm [57]. Disruption of this exchange then jeopardizes critical functions of the cell leading to selected phenotypes of AD [58]. The present data showing the effect of AD on expression of nuclear-encoded but not mitochondrial-encoded OXPHOS genes identifies another example of nucleocytoplasmic dysfunction in AD. Because nuclear-encoded mitochondrial proteins require transport molecules for delivery, and mitochondrial-encoded proteins do not, this may implicate the exchange process from the cell nucleus to the mitochondria in AD.

Although other OXPHOS expression changes and causal mechanism have been reported [36], we believe our data set is more comprehensive and addresses the need to analyze the entire mitochondrial transcriptome. Although our data contradicts work by Chandrasekaran et al. [36], we stress the importance of sample size and more importantly brain regions. It was reported that a 50%–60% reduction in mRNA levels of nDNA-encoded subunit IV of COX (COX411) and β-subunit of the F_0F_1-ATP synthase (*ATP5B*) in the mid temporal cortex in AD compared to control brains. A single mt-DNA-encoded gene was used in their study (*mt-ND1*) and showed a similar reduction (50%) in mRNA levels in AD brain tissue when compared to control. It is very difficult to compare the two analysis when only two nDNA-OXPHOS-encoded genes and one mt-DNA-encoded gene were used in their study. Whereas, in our study, 98% of OXPHOS encoded genes and 65% of mt-DNA genes were used for analysis. The cause for the discrepancy in the mt-DNA-encoded gene is unclear but may reflect the different brain regions studied, the small sample size, and the different method used. We know for example that the hippocampus is particularly vulnerable in AD [59], it may be that the amount of pathology is directly correlated to mitochondrial gene

expression levels, which we found in our study as a strong correlate ($P < .001$, r = 0.78). We expect, however, that other, particularly less-affected brain regions would yield different results.

4.3. Compensatory mechanisms are initiated in mild cognitively impaired subjects

Compensatory mechanisms are not new to the field of aging or age-related diseases [33,40,60,61]. It has been postulated that compensatory mechanisms like the one we show (e.g., up regulation of mitochondrial genes in MCI) are largely the product of survival and protection from disease progression. These processes are innately activated due to various brain insults (e.g., oligomeric amyloid β [OAβ], inflammation, ROS production, among others).

Severe metabolic deficit has been shown to be a prominent feature of AD in human brain [44,62], as well as *in vitro* [63] and animal models [4,64]. *In vitro* [65], mouse model [6,7] and human studies [5] reveal that Aβ and OAβ are both toxic to mitochondria, suggesting that Aβ may be responsible for the metabolic deficit observed in AD. Although they do not represent the exclusive source of cellular ATP, mitochondria are the primary source of ATP in brain cells and provide the energy required to perform metabolic processes from cell signaling to calcium homeostasis [66]. Deficiencies in energy metabolism have been shown to be one of the earliest events in AD pathobiology [2,33]. Although a number pathogenic pathways have the ability to affect mitochondrial function, the amyloid precursor protein and Aβ are at the forefront [67]. Progressive accumulation of Aβ in the mitochondria of AD neurons is believed to be an essential step leading to Aβ-mediated mitochondrial dysfunction, and several studies provide substantial evidence that mitochondria contain Aβ in AD [21–23,28,68], and that Aβ is directly responsible for impaired respiratory chain function [69–71]. It is interesting that, although complex I inhibition is believed to result from direct interaction with Aβ, impairment of complex IV function results primarily from ROS production at complex I [63]. The recent emergence of a putatively more toxic form of Aβ, oligomeric Aβ (OAβ), has prompted several groups to evaluate mitochondrial function in the presence of OAβ. Collectively, they have found that OAβ permeated mitochondrial membranes [71], increased reactive oxygen species levels [72], and impaired ATP production [73]. These data reinforce the toxicity of OAβ on mitochondrial function.

4.4. Potential significance of the only affected mitochondrially encoded transcript identified in the Affymetrix array

NADH: ubiquinone oxidoreductase core subunit 6 (*mt-ND6*) was the only of the seven identified mitochondrially encoded transcripts affected in AD. The reason for this alteration in *mt-ND6* (5% decrease in AD) is unclear but quite intriguing. It is important to note that the *mt-ND6* is the only protein-encoding gene present in the L strand of mtDNA, whereas the rest of the 13 protein-encoding mitochondrial genes all reside in the H strand. Furthermore, the mtDNA-encoded subunits are transcribed in a polycistronic fashion and are regulated by the same promoter (excluding *mt-ND6*) [74]. Recently, it has been found that the myocyte enhancer factor 2 (*MEF2*) family member (nuclear transcription factor), exclusively regulates expression of the *mt-ND6* gene without significant effects on several other protein-encoding mtDNA genes [75]. It is important to note that the full biological

significance of this added layer of regulation of *mt-ND6* gene expression by *MEF2D* requires further exploration.

Although select papers make a distinction between the amyloid hypothesis and hypotheses emphasizing bioenergetics (e.g. Demetrius et al., 2015), we believe that our data presented here and in other papers references suggest a blending of the two classes of hypotheses in which an age-related decline in bioenergetics is exacerbated by OAβ. Our data in this and other articles suggest a model in which actions of OAβ lead to early events in the cascade of AD pathologies which directly impedes mitochondrial function. Thus, these findings suggest that restoration of mitochondrial function could blunt disease progression, and that a focused molecular strategy may be applicable to a range of neurodegenerative diseases, notably AD. A novel type of catalytic antioxidant is suggested for possible therapeutic application, and its mechanism(s) for blunting neurodegeneration and cell death should be explored. As such, we have recently described the ability of novel co-enzyme Q_{10} analogs to reverse the effect of OAβ-induced mitochondrial dysfunction [76]. We show that treatment with these novel compounds also leads to restoration of mitochondrial function as observed in nonaffected middle aged or younger individuals. Although enhancing ATP production would likely be beneficial to the aging population in general, other significant factors such as mitochondria half-life and abundance and changes in protein expression may or may not result in functional alterations at the protein level. Detailed analysis of compensatory mechanisms as shown here might be a starting point for further treatment and diagnostic strategies to combat AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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RESEARCH IN CONTEXT

1. Systemic review: Alzheimer's disease (AD) is a complex disease with a prolonged trajectory of etiopathogenic changes in brain bioenergetics decades before the onset of measureable cognitive deficits.

2. Interpretation: Increasing evidence suggests that alterations in energy metabolism are among the earliest events that occur in AD. Previous studies, including our own, have identified expression changes in OX-PHOS genes in AD, but our detailed expression array study identifies deficits in nuclear not mitochondrial-encoded transcripts.

3. Future directions: To elucidate the specific mechanism(s) by which OAβ interferes with the transport of nuclear-derived mitochondrial mRNAs, and to demonstrate that these mechanisms are necessary and sufficient to explain the mitochondrial dysfunction observed in aging and AD.
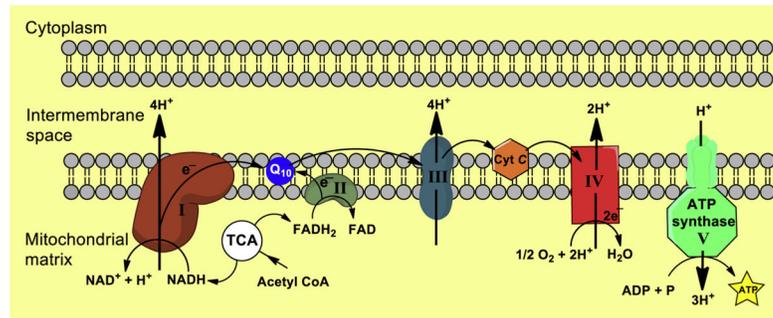
Fig. 1.
Mitochondrial oxidative phosphorylation system (OXPHOS) shown: The five respiratory chain complexes with the corresponding electron transport chain numbers (I–V). In the bottom part, these are indicated by the subunits of each respiratory chain complex gene numbers, which were selected by microarray analysis. Among the ~80 polypeptides constituting the electron transport chain, 13 are encoded by mtDNA, and the rest are all encoded by nDNA, synthesized in the cytosol, and translocated to the mitochondria. TCA, trichloroacetic acid; Cyt C, cytochrome C; ADP, adenosine diphosphate; ATP, adenosine triphosphate.
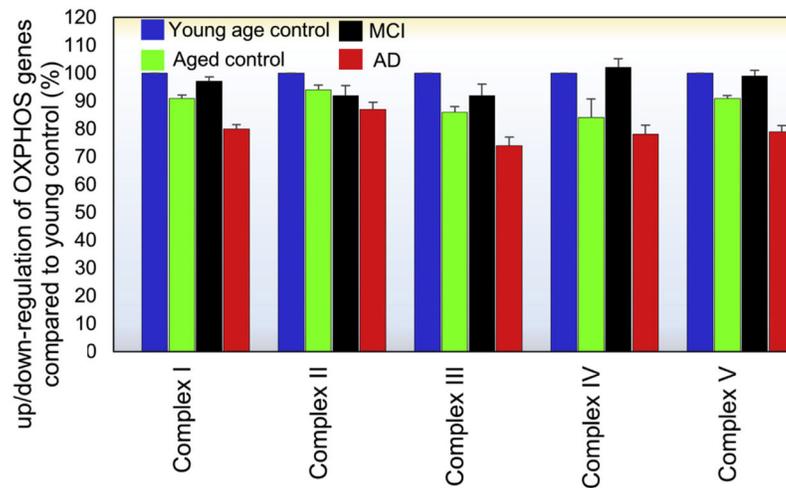
Fig. 2.
Mean expression of oxidative phosphorylation system (OXPHOS)–related genes within complexes I–V in the hippocampus compared to young controls (20–52 years). As a function of age, a general trend can be observed in complexes I, III, IV, and V; as one's age increases, the mean expression of OXPHOS-related genes decreases, a phenomenon which is exacerbated in AD. Correspondingly, in these same complexes (I, III, IV, and V), MCI cases demonstrate an unequivocal increase in these same OXPHOS-related genes, suggesting a compensatory-like mechanism at the early stages of disease. An exception to this general trend is observed in complex II where only a modest difference can be observed across groups. Error bars generated using standard error of the mean. MCI, mild cognitively impaired; AD, Alzheimer's disease.
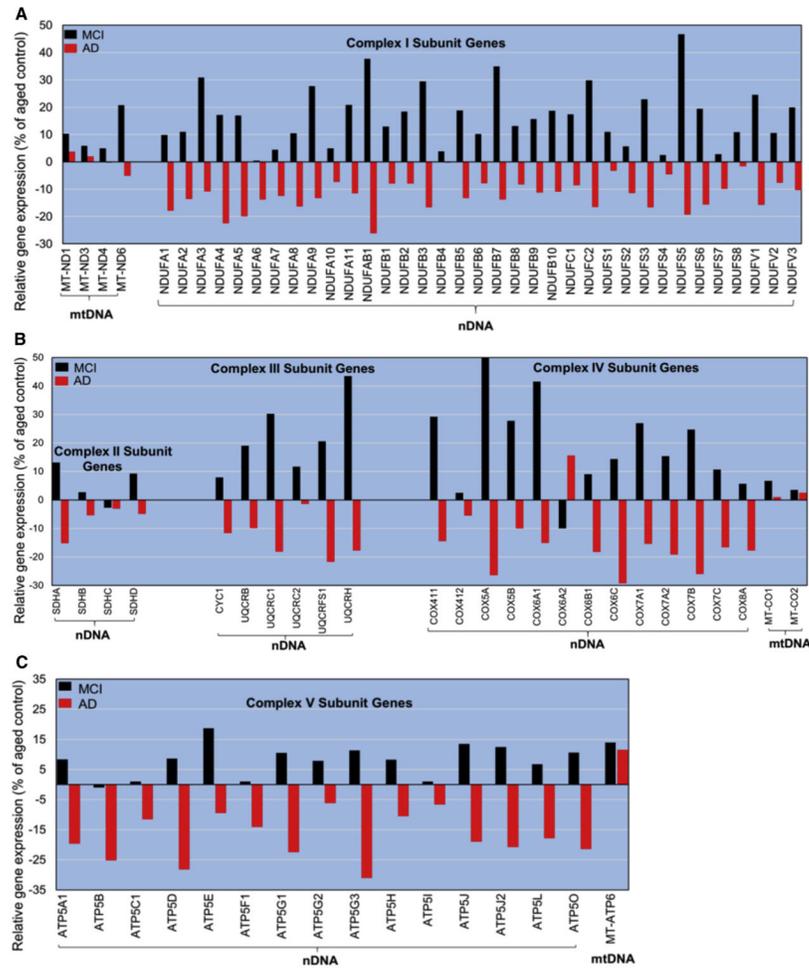
Fig. 3.

Relative gene expression profiles of oxidative phosphorylation system (OXPHOS)–related genes from hippocampal tissue are shown in, and grouped by, OXPHOS complexes, compared to age-matched controls (62–99 years). Up regulation/down regulation of OXPHOS-related gene(s) within complex I relative to aged controls (A). Up regulation/ down regulation of OXPHOS-related gene(s) within complex II–IV relative to aged controls (B). Up regulation/down regulation of OXPHOS-related gene(s) within complex V relative to aged controls (C). The nDNA-encoded OXPHOS-related genes profile was significantly reduced in AD compared to CTL for all complexes, where MCI cases show a significant increase in all 37 genes (a significant P value of <.05 (two-tailed student t test), graphs a, b, and c). Mitochondrial-encoded transcript showed no significant difference from control for all complexes with one exception (MT-ND6). MCI, mild cognitively impaired; Alzheimer's disease; MT-ND1, mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 1; MT-ND3, NADH dehydrogenase, subunit 3 (complex I); MT-ND4, Homo sapiens DC24 mRNA, complete cds.; MT-ND6, NADH dehydrogenase, subunit 6 (complex I); NDUFA, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex; NDUFAB1, NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex; NDUFB, NADH dehydrogenase (ubiquinone) 1 beta subcomplex; NDUFC, NADH dehydrogenase (ubiquinone) 1,

subcomplex unknown; NDUFS, NADH dehydrogenase (ubiquinone) Fe-S protein; NDUFV, NADH dehydrogenase (ubiquinone) flavoprotein; SDH, succinate dehydrogenase; CYC1, cytochrome c-1; UQCR, ubiquinol-cytochrome c reductase; COX, cytochrome c oxidase; MTC, mitochondrial electron transport chain; ATP, adenosine triphosphate.

Table 1

Description of subjects studied

Group# ()*S	Sex ()*S	Braak ()*S	Age ()*S
Young control	Male	unknown	20
Young control	Male	unknown	20
Young control	Male	unknown	20
Young control	Male	unknown	22
Young control	Female	unknown	26
Young control	Male	unknown	28
Young control	Female	unknown	30
Young control	Male	unknown	33
Young control	Female	unknown	34
Young control	Female	unknown	37
Young control	Male	unknown	42
Young control	Female	unknown	44
Young control	Male	unknown	45
Young control	Female	unknown	45
Young control	Male	unknown	45
Young control	Female	unknown	47
Young control	Female	unknown	48
Young control	Male	unknown	52
Age match control	Female	0	64
Age match control	Male	2.5	69
Age match control	Male	1	69
Age match control	Male	1	69
Age match control	Female	1	70
Age match control	Female	2	74
Age match control	Female	2	74
Age match control	Female	0	74
Age match control	Male	2	75
Age match control	Male	2	80
Age match control	Male	3	82
Age match control	Female	3	82
Age match control	Female	2	82
Age match control	Male	2	83
Age match control	Female	2	83
Age match control	Male	2	84
Age match control	Male	2	85
Age match control	Male	1	86
Age match control	Female	3	87
Age match control	Male	unknown	91
Age match control	Female	unknown	91

Group# ()*S	Sex ()*S	Braak ()*S	Age ()*S
Age match control	Female	2	91
Age match control	Male	1	95
Age match control	Female	4	96
Age match control	Male	2	97
Age match control	Female	unknown	99
MCI	Female	4	74
MCI	Male	4	75
MCI	Female	1.5	83
MCI	Male	2	85
MCI	Male	3	86
MCI	Female	3	88
MCI	Female	3	88
MCI	Male	3	88
MCI	Male	4	89
MCI	Female	4	89
AD	Female	6	60
AD	Female	6	74
AD	Male	5	76
AD	Female	5	76
AD	Male	3	76
AD	Male	6	79
AD	Female	6	79
AD	Male	unknown	80
AD	Male	4	85
AD	Male	3	85
AD	Male	4	86
AD	Male	5	87
AD	Female	6	90
AD	Female	4	90
AD	Female	3	90
AD	Female	5.5	91
AD	Male	6	94
AD	Male	4	94

Abbreviations: MCI, mild cognitively impaired; AD, Alzheimer's disease.