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Reduced Mitochondria Cytochrome Oxidase Activity in Adult Children of Mothers with Alzheimer's Disease

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Abstract

Biomarker studies demonstrate inheritance of glucose hypometabolism and increased amyloid- β deposition in adult offspring of mothers, but not fathers, affected by late-onset Alzheimer's disease (LOAD). The underlying genetic mechanisms are unknown. We investigated whether cognitively normal (NL) individuals with a maternal history of LOAD (MH) have reduced platelet mitochondrial cytochrome oxidase activity (COX, electron transport chain complex IV) compared to those with paternal (PH) or negative family history (NH). Thirty-six consecutive NL individuals (age 55–71 y, range 27–71 y, 56% female, CDR = 0, MMSE $\geq$ 28, 28% APOE-4 carriers), including 12 NH, 12 PH, and 12 MH, received $\pm$ a blood draw to measure platelet mitochondrial COX activity. Citrate synthase activity (CS) was measured as a reference. Groups were comparable for clinical and neuropsychological measures. We found that after correcting for CS, COX activity was reduced by 29% in MH compared to NH, and by 30% in MH compared to PH ($p \leq 0.006$). Results remained significant controlling for age, gender, education, and APOE. No differences were found between PH and NH. COX measures discriminated MH from the other groups with accuracy $\geq 75\%$, and relative risk ≥ 3 ($p \leq 0.005$). Among NL with LOAD-parents, only those with MH showed reduced COX activity in platelet mitochondria compared to PH and NH. The association between maternal history of LOAD and systemic COX reductions suggests transmission via mitochondrial DNA, which is exclusively maternally inherited in humans.

Keywords

Early diagnosis; electron transport complex IV; late onset Alzheimer's disease; mitochondria

INTRODUCTION

While the rare early-onset forms of AD have autosomal dominant genetic inheritance, the risk for developing late-onset AD (LOAD), which comprises over 99% of the AD population after the age of 60, is influenced by several genetic and non-genetic factors. Although LOAD does not show recognizable Mendelian inheritance, risk is to some extent genetically determined, as shown by the familial aggregation of many LOAD cases. Having

a 1st degree family history of LOAD, especially when a parent is affected, is a major risk factor for developing the disease among cognitively normal (NL) individuals [1, 2].

Recent biomarker studies reveal a maternal contribution to LOAD. NL individuals whose mothers had LOAD manifest AD-endophenotypes including reductions of brain glucose hypometabolism and increases in amyloid- β ($A\beta$) pathology, oxidative stress, and brain atrophy [3–8]. In contrast, NL with LOAD-affected fathers are far less likely to show biomarker abnormalities [3–8].

Maternal inheritance of oxidative dysmetabolism suggests genetic transmission that may be mediated by mitochondrial DNA (mtDNA) [9], since mtDNA is maternally inherited in humans [10]. Mitochondrial dysfunction is observed in LOAD and includes the reduced activity of cytochrome oxidase (COX, Complex IV of mitochondria electron transport chain, ETC) in brain tissue, fibroblasts, and platelets [11]. While COX is encoded by both mitochondrial and nuclear genomes, its three most catalytically important subunits are on mtDNA [12].

This study shows that NL with LOAD-mothers have reduced platelet mitochondria COX activity compared to NL with LOAD-fathers and to NL with unaffected parents, suggesting a role for mtDNA in maternal transmission of LOAD.

MATERIAL AND METHODS

Subject

This study examined a cohort of 36 consecutive clinically and cognitively normal (NL) individuals enrolled at the Center for Brain Health at New York University School of Medicine (NYU), divided into 3 groups of 12 subjects each according to their parental history of LOAD (see below). All subjects signed informed consent to participate in this institutional review board approved study, and received thorough medical, psychiatric, neuropsychological, and clinical MRI exams, and a blood draw to examine platelet COX activity within a three-month window. Individuals with medical conditions or history of significant conditions that may affect brain structure or function (i.e., stroke, diabetes, head trauma, neurodegenerative diseases, depression, MRI evidence of hydrocephalus, intracranial mass, and infarcts), and those using psychoactive medications were excluded.

As previously described, subjects were 27–71 years of age, had education ≥ 12 years, Clinical Dementia Rating (CDR) score = 0, Global Deterioration Scale (GDS) scores ≤ 2 , Mini Mental State Examination (MMSE) scores ≥ 28 , Modified Hachinski Ischemia Scale normal cognitive test performance scores < 4 , and relative to appropriate normative values [3–5]. APOE genotype was determined using standard polymerase chain reaction-based procedures.

A family history of AD that included at least one 1st degree relative whose AD onset was after age 60 was elicited by using standardized family history questionnaires [3, 5]. Participants were asked to fill in names, dates of birth, age at death, cause of death, and clinical information of all family members. The information was confirmed with other family members by interview with the examining neurologist. Only NL subjects whose parents' diagnosis of LOAD or no dementia was reportedly clinician certified, and whose parents had lived to at least age 65 were included in this study and divided into 3 groups: MH (i.e., only the mother had LOAD), PH (i.e., only the father had LOAD), and NH (i.e., neither parent had AD or another dementia). An AD postmortem diagnosis was established for the parents of 3 subjects (2 MH and 1 PH).

COX analysis

Fifty ml of blood were collected in tubes containing acid-citrate-dextrose. Blood samples were obtained at NYU and sent overnight to the University of Kansas. Upon receipt, platelets were isolated by centrifugation and enriched mitochondrial fractions were prepared using previously described methods [13, 14]. The protein concentrations of the enriched mitochondrial fractions were measured using a DC protein assay kit (BioRad, Hercules, CA). Cytochrome c oxidase Vmax activity (COX, Complex IV, $\text{sec}^{-1}/\text{mg}$) was determined as a pseudo first order-rate constant by measuring the oxidation of reduced cytochrome c at 550 nm. Citrate synthase (CS) Vmax activity ($\text{nmol}/\text{min}/\text{mg}$) was determined spectrophotometrically following the formation of 5-thio-2-nitrobenzoate (412 nm) following the addition of 100 μM oxaloacetate at 30°C. In addition to referencing COX Vmax activity to total protein, to correct for potential inter-sample differences in mitochondrial mass, the COX activity for each sample was also referenced to its corresponding CS activity. CS activity is reportedly comparable between NL and AD patients, with variations as little as 0.5–2%, and does not show age effects [13, 14].

Statistical analysis

Statistical analyses were done with SPSS 12.0 (SPSS inc., Chicago, IL). Differences in clinical, demographical, and COX measures across groups were examined with χ^2 tests and the General Linear model (GLM) with post-hoc LSD tests. The total-protein COX Vmax was examined as absolute values and after adjustment for CS, which was examined both as a covariate and as a denominator (COX/CS). Analyses were repeated controlling for age, gender, education, and APOE status by including these variables as covariates in the GLM.

Stepwise forward logistic regressions and ROC curves were used to examine COX and COX/CS as predictors of group membership, and to calculate associated relative risk and 95% confidence intervals. Analyses were repeated controlling for age, gender, education, and APOE as confounds. This was done by entering covariates in the model as fixed effects at the first step, and entering regional predictors at the second step using a forward conditional method.

Although limited by the small number of APOE-4 carriers within each family history group (Table 1), we tested for interactions between APOE and family history status on COX activity using a 2×2 GLM, with and the GLM without controlling for CS. Additionally, was used to compare family history groups within the group of APOE-4 non-carriers. Results were confirmed with non-parametric Mann Whitney U tests. All results were examined at $p < 0.05$.

RESULTS

Groups were comparable for age, gender, education, neuropsychological measures, and APOE status (Table 1).

COX measures by family history groups are found in Table 2. There was a significant main effect of family history on total protein-referenced COX Vmax activity ($F_{[2,33]} = 7.0, p = 0.003$). On post-hoc examination, COX Vmax activity was reduced in the MH group as compared to the NH (28%, $p = 0.02$) and PH (36%, $p < 0.001$) groups. Likewise, correcting for CS, COX activity was reduced by 29% in MH compared to NH and by 30% in MH compared to PH ($p \leq 0.006$). There were no differences between PH and NH with or without correcting for CS as a covariate (Fig. 1).

Similar results were obtained using the ratio of COX to CS. There was a significant main effect of family history on COX/CS ($F_{[2,33]} = 5.4, p = 0.009$), which on post-hoc

examination was due to the MH group having lower COX/CS compared to NH and to PH (34% and 26%, respectively, $p \leq 0.03$; Table 2). There were no differences between PH and NH.

When age, gender, education, and APOE were included as covariates in the GLM, there were no significant associations between these variables and total protein-referenced COX Vmax activity, with or without correcting for CS, while there remained a significant effect of family history status ($p \leq 0.005$). Post-hoc results remained unchanged by further controlling for these variables as covariates ($p \leq 0.03$, Table 2).

Examination of interactions between APOE genotype and family history status showed no significant effects of APOE status ($F_{(1, 34)} = 1.69$, $p = 0.39$, n.s.) and no significant APOE by family history interactions on COX activity ($F_{(2, 30)} = 0.25$, $p = 0.78$, n.s., Fig. 2). Results remained unchanged controlling for CS as a covariate or denominator (Fig. 2). Within the group of APOE-4 non-carriers, there was a significant main effect of family history ($F_{[2,23]} = 4.0$, $p = 0.03$), which on post-hoc examination was due to the MH group having lower COX compared to NH and to PH (32% and 35%, respectively, $p \leq 0.02$). Likewise, MH had 39% and 31% lower COX/CS than NH and PH, respectively ($p < 0.05$). These results were confirmed with non-parametric tests (Mann-Whitney U Exact sig. MH versus NH: $p \leq 0.027$; MH versus PH: $p \leq 0.05$). There were no differences between PH and NH with or without controlling for CS.

Results from logistic regressions are summarized in Table 3 and shown in Fig. 3. COX activity discriminated MH from NH with 79% accuracy ($X^2_{(1)} = 7.9$, $p = 0.005$), MH from PH with 83% accuracy ($X^2_{(1)} = 10.0$, $p = 0.001$), and MH from the other two groups combined with 81% accuracy ($X^2_{(1)} = 11.6$, $p < 0.001$), yielding relative risk ≥ 3.0 for all contrasts $p \leq 0.005$, Table 3). Similar estimates of discrimination accuracy $\geq 75\%$ were obtained for COX/CS measures (Table 3). Neither COX nor COX/CS discriminated PH from NH. Results remained unchanged including age, gender, education, and APOE in the model ($p \leq 0.05$).

DISCUSSION

This study shows reduced COX activity in platelet mitochondria from NL MH compared to PH and to controls. These differences remained significant controlling for CS, an index of mitochondrial mass, and were independent of other possible risk factors for LOAD, such as age, gender, education, and APOE genotype. There were no differences in COX activity between PH and controls.

There is extensive evidence for altered oxidative metabolism and reduced COX activity in AD brain tissue, fibroblasts and blood platelets [11, 14–20]. COX is the mitochondrial enzyme responsible for the consumption of oxygen during aerobic respiration and is critically tied to ATP production [12]. As such, it is tightly linked to the regulation of glucose metabolism in the synapses, and therefore supports neuronal transmission. Multiple in vivo studies report reduced platelet mitochondria COX activity in patients with AD and with mild cognitive impairment (MCI), which is frequently an AD prodrome, while the other ETC complexes are generally not impaired [11, 14–17, 19]. Additionally, COX abnormalities are specific to AD as compared to other neurological disorders such as Parkinson's disease [11, 21].

In light of previous findings, our results of reduced platelet mitochondrial COX activity in NL MH suggest that these individuals may have a greater risk of developing AD than PH and NH subjects. This observation is consistent with epidemiological studies showing a main role for maternal transmission in LOAD. First, maternal transmission is more frequent

than parental transmission [9]. In LOAD subjects with an AD-affected parent the affected parent is, even after correcting for enhanced female longevity, more commonly the mother [22]. Second, maternal transmission is associated with a more predictable age of onset and lower performance on cognitive testing in the offspring [22–24]. A recent study has shown that, among NL APOE-4 carriers, only those with MH have reduced memory test performance [24]. Additionally, maternally-inherited LOAD endophenotypes are increasingly recognized, which include altered brain glucose utilization on FDG-PET, increased A β deposition on PIB-PET, increased atrophy on MRI, decreased A β_{42} /A β_{40} and increased oxidative stress in cerebrospinal fluid [3–7]. Our findings of reduced mitochondrial COX activity in NL MH add a biochemical parameter to these previous biomarker data, and further suggest that a maternally inherited genetic factor contributes to the development of LOAD.

Reduced COX activity in NL MH could potentially arise as a consequence of or independent of its genetic encoding. According to the amyloid cascade hypothesis [25], mitochondrial dysfunction arises downstream of A β pathology, either in fibrillar or oligomeric form [26]. Cell culture experiments and studies of transgenic mice that express mutant human amyloid- β protein precursor (A β PP) showed that A β inhibits COX and alters COX gene expression [27–29]. As platelets express A β PP, it remains to be established whether platelet A β or A β PP influenced our data. However, while the amyloid cascade hypothesis is particularly attractive for the rare, autosomal dominant AD cases that associate with autosomal dominant genetic mutations, it remains to be clarified whether A β is the initiating event in LOAD, a multifactorial disease in which many genetic and environmental risk factors play a role [2].

Alternatively, reduced COX activity in NL MH may reflect a mainstream mechanism in MH that is primarily determined through genetic encoding. Evidence for a connection between mtDNA and COX activity in AD is demonstrated by cytoplasmic hybrid (cybrid) studies. Cybrid cells transplanted with platelet mitochondria, and therefore mtDNA from AD patients, display persistently reduced COX activity, increased ROS, and increased A β production as compared to cells transplanted with mitochondria from healthy controls [11, 30–32]. Our finding that COX Vmax activity in NL MH platelet mitochondria is lower than that of PH and NH is consistent with the cybrid literature and suggests that altered COX in NL MH may be at least partly determined by mtDNA, maternally transmitted from one generation to the next. COX structure is additionally perturbed in AD, which is also more consistent with a genetic etiology [11, 33]. Moreover, since platelets are a peripheral tissue, our data suggest that COX abnormalities may not simply be a secondary consequence of neurodegeneration but instead represent a systemic deficit in maternally inherited LOAD.

From a mechanistic perspective, perturbed COX function may promote the processing of A β PP to amyloidogenic derivatives or A β itself by increasing free radical production [34, 35]. A deficient energy metabolism resulting from defective mitochondrial function and increased oxidative damage may change the overall oxidative microenvironment during progression of AD, disposing neurons to dysfunction and degeneration [10]. This could account for biomarker studies showing LOAD-endophenotypes in asymptomatic MH, and is consistent with the “mitochondrial cascade hypothesis” of LOAD [27]. The relationship of AD to aging, a process that closely associates with various aspects of mitochondrial function and integrity, has been used to argue that, at least in LOAD, mitochondria may constitute the apex of biochemical cascades in AD [27]. The core assumptions of this model are that a person's genes determine baseline mitochondrial function and durability, this durability determines how mitochondria change with advancing age, and critical changes in mitochondrial function initiate other pathologies characteristic of AD [27]. While placing mitochondria at the apex of an AD cascade remains controversial, it is increasingly recognized that mitochondria play an important role in LOAD. Our findings indicate that

mitochondria may be of particular relevance to the maternally inherited form of LOAD, which accounts for approximately 20% of all LOAD cases (for review see [9]).

While X-linked inheritance or genetic imprinting could potentially explain our findings, the fact that COX is partly mtDNA-encoded and that reduced COX activity segregates with MH status argues that mtDNA more likely represents the putative maternal-inherited genetic factor underlying our findings. The question of whether assumed mtDNA abnormalities are inherited or acquired in maternally inherited LOAD, as in LOAD in general, is debated [10, 11, 25, 36, 37]. Our current study is more consistent with an inherited origin, at least in NL MH. It is important to note, however, that while specific mtDNA polymorphisms, haplotypes, and low-abundance mutations reportedly associate with AD (for review, see [9, 11]) at this time no specific mtDNA change is universally accepted as a signature LOAD feature. The role of mitochondrial genome changes in the pathogenesis of aging and LOAD needs further clarification, and maternally-inherited endophenotypes may be helpful for such investigations.

While it was beyond the scope of the present study to perform analysis of mtDNA genes, present findings of reduced COX activity in NL MH compared to PH and to controls set the stage for future investigations of mtDNA haplogroups and mtDNA direct sequencing with special attention to mtDNA COX sub units in this population. However, negative sequencing or haplogrouping results would not exclude there being differences between the mtDNA of the MH and non-MH subjects, as standard sequencing would not reveal low abundance heteroplasmies [11]. Moreover, as shown in Lu and colleagues [38], while differences in mtDNA COX gene sequences between groups are likely to be found, these changes will likely also vary from subject to subject, making it difficult to conclude or exclude causality. Overall, future studies with larger samples are warranted to establish or exclude actual mtDNA sequence changes in NL MH.

Postmortem studies of NL APOE-4 carriers have shown reduced COX activity in the posterior cingulate cortex, which is one of the first brain regions to show A β deposits and neuronal dysfunction in AD [20]. It was recently reported that APOE-4 carriers have reduced COX activity in brain tissue as relatively young adults [39]. There are currently no published in vivo studies showing reduced platelet COX activity in NL APOE-4 carriers. In our study, reduced COX activity in NL MH was independent of APOE genotype, and only 25% of our MH subjects were APOE-4 carriers, indicating that other factors contribute to the etiology of their endophenotype. Although limited by the relatively small sample, our results did not show a significant interaction between family history and APOE status. Other studies with larger samples are needed to confirm these findings.

Steps were taken to ensure that the AD diagnosis in the subjects' parents was accurate. All affected parents of the subjects in this study had a documented, clinician certified diagnosis of AD, including 3 cases confirmed at postmortem. Questionnaires used to elicit FH information are known to have good agreement with clinical and neuropathological findings [40], which reduces potential for misclassification. Nonetheless, in the absence of postmortem confirmation, our cohort may have included subjects whose parents did not have AD but rather another dementia. This would lead to inclusion of subjects with decreased risk for AD in the MH and PH groups, conservatively reducing power for detecting differences.

In conclusion, an individual's risk of developing LOAD is influenced by whether either of the parents had LOAD, with the mother's status having a greater impact. The present study shows reduced platelet mitochondrial COX activity in NL MH compared to PH and NH, suggesting COX abnormalities reflect a maternal inheritance-dependent LOAD

endophenotype, and possibly reflecting mtDNA involvement. Larger samples, longitudinal follow-ups, and population-based comparisons are necessary to test COX as a risk factor for LOAD and for investigations of potential susceptibility genes.

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Fig. 1.
COX and COX/CS measures by family history groups. Horizontal bars indicate mean values. Abbreviations: see legend to Table 2.

Fig. 2. COX and COX/CS measures by family history (NH = circles, PH = triangles, MH = diamonds) and APOE status (E4 non carriers, E4- = white, E4 carriers, E4+ = black). Abbreviations: see legend to Table 2.

Fig. 3.
ROC curves showing group separation as predicted by COX (blue) and COX/CS (green lines).

Table 1

Clinical and demographical measures by family history group

	NH	PH	MH
<i>n</i>	12	12	12
Age	56 (18)	56 (12)	55 (14)
Gender (male/female)	7/5	6/6	3/9
Education	18 (2)	18 (3)	17 (2)
APOE status (APOE4 carriers/non-carriers)	2/10	5/7	3/9
<i>Neuropsychological measures</i>			
Mini Mental State Examination	29.4 (0.7)	29.8 (0.4)	29.4 (0.7)
Digit symbol substitution	57.5 (8.2)	62.4 (10.0)	59.3 (13.0)
Paired associates recall, delayed	6.6 (2.5)	5.9 (3.0)	4.7 (2.0)
Paragraph recall, immediate	7.2 (1.0)	7.9 (1.8)	6.8 (2.0)
Paragraph recall, delayed	9.9 (3.1)	11.2 (2.9)	8.9 (2.5)
Designs	7.6 (2.2)	6.3 (2.2)	7.4 (1.9)
Object naming	53.8 (2.3)	57.7 (1.9)	57.9 (1.8)
Visual recognition	23.2 (2.2)	23.6 (1.9)	23.0 (2.4)
WAIS-vocabulary	69.7 (7.8)	68.3 (5.2)	68.9 (4.6)
WAIS-digit span backward	5.5 (1.6)	5.6 (1.9)	5.7 (1.5)

Values are means (standard deviations). Abbreviations: MH = maternal history of LOAD, NH = negative history of LOAD, PH = paternal history of LOAD.

Table 2

COX and COX/CS measures by family history group

	NH	PH	MH
<i>n</i>	12	12	12
CS (nmol/min/mg)	171.4 (33.8)	200.0 (47.3)	178.6 (22.5)
COX (sec ⁻¹ /mg)	60.4 (13.6)	68.0 (20.2)	43.3 (15.1) ^{*#}
Adjusted by CS	63.1 (15.9)	63.8 (16.6)	43.4 (15.6) ^{*#}
Adjusted by age, gender, and education	64.2 (17.7)	63.0 (17.6)	44.5 (16.5) ^{*#}
Adjusted by ApoE	63.6 (16.0)	63.2 (16.7)	44.9 (15.6) ^{*#}
COX/CS (ratio, unitless)	0.37 (0.10)	0.35 (0.09)	0.24 (0.08) ^{*#}
Adjusted by age, gender, and education	0.38 (0.09)	0.33 (0.09)	0.23 (0.10) ^{*#}
Adjusted by ApoE	0.37 (0.09)	0.33 (0.09)	0.24 (0.10) ^{*#}

Values are means (standard deviations). Abbreviations: CS = citrate synthaseVmax activity (nmol/min/mg), COX = total-protein referenced cytochrome oxidaseVmax activity (sec⁻¹/mg), MH = maternal history of LOAD, NH = negative history of LOAD, PH = paternal history of LOAD

* MH ≠ NH

MH ≠ PH, *P* < 0.05.

Table 3

Group discrimination as predicted by COX and COX/CS

	Acc	SS	SP	RR	95% C.I.
MH vs NH					
COX (<i>cutoff</i> = 53.1 mg)	79%	75%	83%	4.5	1.5–14.9
COX/CS (<i>cutoff</i> = 0.32)	83%	83%	83%	5.0	1.8–13.9
MH vs PH					
COX (<i>cutoff</i> = 59.7 mg)	83%	83%	83%	5.0	1.8–13.9
COX/CS (<i>cutoff</i> = 0.30)	75%	75%	75%	3.0	1.3–7.3
MH vs NH and PH					
COX (<i>cutoff</i> = 53.2 mg)	81%	75%	83%	4.5	1.9–9.3
COX/CS (<i>cutoff</i> = 0.30)	78%	75%	79%	3.6	1.6–6.6
PH vs NH					
COX	n.s.				
COX/CS	n.s.				

Abbreviations: Acc = accuracy, CI = 95% confidence interval, CS = citrate synthase, COX = cytochrome oxidase, MH = maternal history of LOAD, NH = negative history of LOAD, n.s. = not significant, PH = paternal history of LOAD, RR = relative risk, SP = specificity, SS = sensitivity.