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Mitochondrial genes are altered in blood early in Alzheimer's disease

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ABSTRACT

Although mitochondrial dysfunction is a consistent feature of Alzheimer's disease in the brain and blood, the molecular mechanisms behind these phenomena are unknown. Here we have replicated our previous findings demonstrating reduced expression of nuclear-encoded oxidative phosphorylation (OXPHOS) subunits and subunits required for the translation of mitochondrial-encoded OXPHOS genes in blood from people with Alzheimer's disease and mild cognitive impairment. Interestingly this was accompanied by increased expression of some mitochondrial-encoded OXPHOS genes, namely those residing closest to the transcription start site of the polycistronic heavy chain mitochondrial transcript (*MT-ND1*, *MT-ND2*, *MT-ATP6*, *MT-CO1*, *MT-CO2*, *MT-C03*) and *MT-ND6* transcribed from the light chain. Further we show that mitochondrial DNA copy number was unchanged suggesting no change in steady-state numbers of mitochondria. We suggest that an imbalance in nuclear and mitochondrial genome-encoded OXPHOS transcripts may drive a negative feedback loop reducing mitochondrial translation and compromising OXPHOS efficiency, which is likely to generate damaging reactive oxygen species.

1. Introduction

There are an estimated 35.6 million cases of dementia worldwide which is likely to treble by 2050 due to an increasingly aging population (Prince and Jackson, 2009). Alzheimer's disease (AD), the most common form of dementia, is characterized by slow progressive loss of cognition and development of behavioral and personality problems associated with neuronal cell loss. Within the brain, there is an accumulation of insoluble extracellular plaques consisting of aggregated amyloid-β (Aβ) and intracellular neurofibrillary tangles of hyperphosphorylated tau. Their generation is believed to lead to the disruption of calcium homeostasis (LaFerla, 2002), collapse of neuronal synapses and loss of connectivity (Terry et al., 1991), increased production of reactive oxygen species (ROS), oxidative damage (Nunomura et al., 2001) and a damaging inflammatory response (Hanisch and Kettenmann, 2007) in vulnerable brain regions. Although much progress has been made we still lack a full understanding of the molecular pathology of AD, thus the treatments currently available only temporarily alleviate some symptoms and do not modify the underlying causes.

Mitochondria are key providers of energy to the cell in the form of adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS). OXPHOS requires 97 proteins to assemble in 5 multiprotein complexes in the correct stoichiometry for a functioning supramolecular complex (Chaban et al., 2014). Eighty-four

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OXPHOS genes are encoded by the nuclear genome, whereas an additional 13 (encoding for proteins in complexes I, III, IV, and V) are expressed as polycistronic RNAs from 3 mitochondrial DNA (mtDNA) promoter regions (HSP1, HSP2 and, LSP1) (Kyriakouli et al., 2008). Mitochondrial gene expression is tightly controlled.

OXPHOS dysfunction can produce ROS and oxidative stress leading to neuronal cell death in aging and in AD brain (Devi et al., 2006). Complex IV appears to be particularly vulnerable in AD, with reduced levels of many subunits within this complex leading to a reduction in overall complex activity (Bosetti et al., 2002; Kish et al., 1992; Maurer et al., 2000; Mutisya et al., 1994; Valla et al., 2001). Amyloid precursor protein, amyloid-β, and apolipoprotein E have all been shown to accumulate in neuronal mitochondrial membranes (Devi et al., 2006; Manczak et al., 2004) and either through direct binding to OXPHOS proteins or indirect mechanisms have been shown to perturb mitochondrial energy balance (Manczak et al., 2006). Even in the early stages of disease, prior to a clinical diagnosis of AD, many of the nuclear genes encoding subunits involved in OXPHOS are downregulated in the brains of people with mild cognitive impairment (MCI) particularly in those brain regions most vulnerable to AD pathology such as the hippocampus and cortex (Liang et al., 2008; Manczak et al., 2004). People with MCI are considered to be in the symptomatic predementia phase of AD, displaying cognitive impairment beyond what is expected for their age, but not severe enough to affect their function and are thus not considered to have dementia at that point in time. Many people with MCI will progress to AD, particularly those with high levels of AD pathology markers (Jack et al., 2016).

Similar OXPHOS changes and markers of oxidative damage in AD brain appear to be mirrored in the periphery including in platelets (Bosetti et al., 2002; Cardoso et al., 2004; Parker et al., 1990; Valla et al., 2006) and white blood cells from AD patients (Feldhaus et al., 2011; Lunnon et al., 2012, 2013; Mecocci et al., 1998, 2002; Sultana et al., 2011, 2013; Wang et al., 2006). We previously observed a significant reduction in OXPHOS gene expression in white blood cells, even in subjects with MCI, many of whom were subsequently found to have prodromal AD (Lunnon et al., 2012). Some of these changes were capable of distinguishing AD and MCI subjects from elderly controls as part of a biomarker panel (Booij et al., 2011; Lunnon et al., 2013). In the current study, we have sought to replicate these findings and establish if they represent a decrease in steady-state numbers of mitochondria in AD, or may lead to an alteration in OXPHOS activity, in a step to understanding the mechanism behind these changes and thus the context in which they could be used as a biomarker for testing the efficacy of drugs targeting AD.

First, we found that nuclear genome-encoded OXPHOS transcripts are downregulated in MCI and AD blood. Second, we analyzed mitochondrial genome-encoded OXPHOS subunits to see if they were also decreased in a similar way to the nuclear-genome OXPHOS subunits, which might point to a change in mitochondrial biogenesis or mitophagy. Finally we measured the relative abundance of mtDNA to nuclear DNA to establish if there was an alteration in mitochondrial steady-state levels or whether the changes we observed were more likely to represent a reduction in cellular respiratory chain activity.

2. Materials and methods

2.1. Subjects and samples

Blood samples for DNA and RNA analyses were taken from subjects participating in 2 biomarker studies coordinated from the Institute of Psychiatry, Psychology and Neuroscience, King's College London; The AddNeuroMed study and the Maudsley Biomedical Research Center Dementia Case Register curated by the National Institute for Health Research Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London. Full details on sample collection and assessment are supplied in the Supplementary Methods. Subject characteristics are summarized in Table 1.

2.2. RNA extraction

Whole blood samples were collected in PAXgene tubes (BD Diagnostics) and stored at −80 °C until RNA extraction. Total RNA was extracted, quantified and quality assessed as previously described (Lunnon et al., 2012).

2.3. Analysis of nuclear-encoded OXPHOS genes using BeadArrays

Total RNA was converted to cDNA (200 ng) and then biotinylated cRNA according to the protocol supplied with the Illumina TotalPrep-96 RNA Amplification Kit (Ambion). Previously we studied disease pathway changes in AD, MCI, and control subjects by hybridizing blood RNA to Illumina HT-12 V3 (Lunnon et al., 2012), which is deposited in the Gene Expression Omnibus (GEO) (batch 1, GEO accession number GSE63060). For the current study we used an independent set of subject samples that were hybridized to Illumina HT-12 V4 according to the manufacturer's protocol (batch 2, GEO accession number GSE63061). Gene expression values were obtained using Genome Studio (Illumina).

Table 1
Subject characteristics of individuals used in the study

	Illumina HT-12 V4 arrays (batch 2)			qRT-CR			Protein			mtDNA		
	Control	MCI	AD	Control	MCI	AD	Control	MCI	AD	Control	MCI	AD
Samples analyzed	129	109	132	177	168	164	27	19	24	28	31	28
Gender (M/F)	52/77	48/61	50/82	73/104	77/91	55/109	12/15	9/10	11/13	12/16	13/18	11/17
Age in years (Mean ± SD)	75.2 (5.8)	78.5 (7.7)	77.8 (6.7)	73.6 (7.0)	74.7 (6.4)	76.8 (6.5)	82.4 (2.7)	82.2 (1.2)	82.0 (2.5)	77.5 (7.7)	77.0 (6.9)	80.3 (4.6)
MMSE (Mean ± SD)	28.3 (3.8)	26.6 (3.5)	20.2 (5.9)	28.9 (1.3)	27.1 (1.9)	20.8 (4.5)	28.3 (1.6)	26.7 (1.9)	20.2 (4.5)	29.1 (1.0)	27.3 (1.8)	20.1 (4.6)
CDR sum of boxes (Mean ± SD)	0.03 (0.12)	0.45 (0.15)	1.03 (0.53)	0.03 (0.12)	0.50 (0.06)	1.10 (0.52)	0.04 (0.13)	0.50 (0.00)	1.19 (0.44)	0.04 (0.13)	0.50 (0.00)	1.18 (0.51)

In total, 370 individuals had genome-wide expression data generated in leukocytes using the Illumina HT-12 V4 expression BeadArray. For the purposes of the current manuscript only the 240 nuclear-genome expressed probes relating to mitochondrial function were analyzed. Quantitative Real-Time PCR (qRT-PCR) was used to measure gene expression levels of 12 mitochondrial-genome expressed transcripts in 509 individuals. This included 181 of the 370 individuals for whom genome-wide expression data are presented in this manuscript, and an additional 272 of the 329 individuals for whom we previously published genome-wide expression data (Lunnon et al., 2012, 2013, Batch 1). Luminex was used to quantify levels of functional electron transport chain proteins in a subset of 70 individuals for whom both BeadArray and qRT-PCR data were generated. Finally qRT-PCR was used to assess mitochondrial DNA copy number in 87 individuals, which also had BeadArray and qRT-PCR data generated.
Key: CDR, Clinical Dementia Rating scale; MMSE, Mini Mental State Examination; SD, standard deviation.