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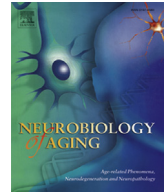
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## Mitochondrial genes are altered in blood early in Alzheimer's disease



Katie Lunnon<sup>a,b,1</sup>, Aoife Keohane<sup>a,1</sup>, Ruth Pidsley<sup>a,2</sup>, Stephen Newhouse<sup>a</sup>, Joanna Riddoch-Contreras<sup>a,3</sup>, Elisabeth B. Thubron<sup>c</sup>, Matthew Devall<sup>b</sup>, Hikka Soininen<sup>d</sup>, Iwona Kłoszewska<sup>e</sup>, Patrizia Mecocci<sup>f</sup>, Magda Tsolaki<sup>g</sup>, Bruno Vellas<sup>h</sup>, Leonard Schalkwyk<sup>a,4</sup>, Richard Dobson<sup>a</sup>, Afshan N. Malik<sup>c</sup>, John Powell<sup>a</sup>, Simon Lovestone<sup>a,5</sup>, Angela Hodges<sup>a,\*</sup>, on behalf of the AddNeuroMed Consortium

<sup>a</sup> Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK

<sup>b</sup> University of Exeter Medical School, University of Exeter, Devon, UK

<sup>c</sup> Diabetes Research Group, Faculty of Life Sciences and Medicine, King's College London, London, UK

<sup>d</sup> Department of Neurology, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland

<sup>e</sup> Medical University of Lodz, Lodz, Poland

<sup>f</sup> Institute of Gerontology and Geriatrics, University of Perugia, Perugia, Italy

<sup>g</sup> 3rd Department of Neurology, Aristotle University of Thessaloniki, Thessaloniki, Greece

<sup>h</sup> INSERM U 558, University of Toulouse, Toulouse, France

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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-CO3*) 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.

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### 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- $\beta$  (A $\beta$ ) 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

\* Corresponding author at: King's College London, Institute of Psychiatry, Psychology and Neuroscience, De Crespigny Park, London, UK. Tel.: (+44) 207 848 0772; fax: (+44) 207 848 0632.

E-mail address: [angela.k.hodges@kcl.ac.uk](mailto:angela.k.hodges@kcl.ac.uk) (A. Hodges).

<sup>1</sup> Equal contributors.

<sup>2</sup> Present address: Garvan Institute of Medical Research, Sydney, NSW, Australia.

<sup>3</sup> Present address: Queen Mary, University of London, London, UK.

<sup>4</sup> Present address: School of Biological Sciences, University of Essex, Essex, UK.

<sup>5</sup> Present address: Department of Psychiatry, Oxford University, Oxford, UK.

OXPPOS 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.

OXPPOS 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- $\beta$ , 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 OXPPOS 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 OXPPOS 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 prodromal 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 OXPPOS 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 OXPPOS 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 OXPPOS 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 OXPPOS transcripts are downregulated in MCI and AD blood. Second, we analyzed mitochondrial genome-encoded OXPPOS subunits to see if they were also decreased in a similar way to the nuclear-genome OXPPOS 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  $-80^{\circ}\text{C}$  until RNA extraction. Total RNA was extracted, quantified and quality assessed as previously described (Lunnon et al., 2012).

### 2.3. Analysis of nuclear-encoded OXPPOS 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-PCR			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 $\pm$ 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 $\pm$ 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 $\pm$ 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.

Preprocessing and analysis of data quality including background correction and normalization were performed in R using the Bioconductor packages, Lumi (Gonzalez de Aguilar et al., 2008), MBCB (Allen et al., 2009), and SVA (Leek et al., 2012). BeadChips with a very low detection rate (<80%), or a discrepancy in XIST and/or EIF1AY gene expression with recorded sex were removed from further analyses, leaving 370 batch 2 samples available for analysis. Two hundred and forty probes on the array corresponded to genes coding for OXPHOS-related proteins: 110 OXPHOS protein subunits, 10 probes encoding genes required for mitochondrial transcription and 120 mitochondrial ribosome protein subunits (MRP) involved in mitochondrial translation. Of these, 225 probes passed quality control within the lumi package and were carried forward to analysis (Table 2).

The effects of age, sex, collection site, and RNA integrity number were regressed out of the data and the corresponding residuals were compared between diagnostic groups (control, MCI, and AD) using linear models followed by post hoc *t* tests. In line with our previous BeadChip data (Lunnon et al., 2012), probes were deemed to be differentially expressed if false discovery rate (FDR)  $q < 0.01$  (Benjamini and Hochberg, 1995). A Fisher's exact test was used to compare the number of probes within each OXPHOS complex reaching  $q < 0.01$  between our previously published data set (Lunnon et al., 2012) (batch 1) and data from the new validation cohort (batch 2), while a  $\chi^2$  test was used to compare the number of significant probes between OXPHOS complexes.

#### 2.4. Analysis of mitochondrial genome-encoded OXPHOS transcripts using qRT-PCR

Genes from the mitochondrial genome are not assayed on Illumina HT-12 V4 Expression BeadArrays. Therefore, specific primers targeting the 13 mitochondrial OXPHOS genes were designed for use in qRT-PCR (Table S1). cDNA was synthesized from 250 ng of total RNA using the QuantiTect Reverse Transcription Kit (Qiagen) and diluted 5-fold for polymerase chain reaction (PCR). Real-time PCR was performed with 5× HOT FIREPol EvaGreenqPCR Mix Plus (ROX) (SolisBioscience). The copy number of each sample was generated from comparison to a standard curve which was further normalized using the geometric mean of the housekeeping genes ATP5B and SF3A1 (Primer Design Ltd, UK), which we identified as the most stable of 12 routine housekeeping genes, using the Normfinder application. Data were transformed to achieve a parametric distribution and the effects of age, sex, collection site, and RNA integrity number were regressed out of the data within R, and residuals compared between diagnostic groups using linear models, with *p*-values adjusted for multiple testing (Benjamini and Hochberg, 1995). Genes were deemed to be differentially expressed if FDR  $q < 0.01$ . Further details are provided in the Supplementary Methods.

#### 2.5. Analysis of OXPHOS protein subunits using Luminex

Briefly, buffy coat samples were lysed using red cell lysis buffer, followed by centrifugation and removal of the supernatant. The pellet was resuspended in 300  $\mu$ L of Cell/Mitochondria Lysis Buffer (Human Oxidative Phosphorylation Magnetic Bead Panel), pre-mixed with an EDTA-free Protease Inhibitor Cocktail (Roche). Following mixing on ice for 30 minutes, the lysate was centrifuged at 14,000 g for 20 minutes at 4 °C. The concentration of protein in the supernatant was measured using the Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific).

The MILLIPLEX MAP Kit Human OXPHOS Magnetic Bead Panel was used according to the manufacturer's protocol (EMD, Millipore). This 6-plex immunoassay measures key

proprietary subunits in complexes I–V. Data from each sample were normalized using nicotinamide nucleotide transhydrogenase measured in the same assay. Data were log transformed and technical outliers >2 standard deviations from the mean were removed. The effects of age, sex, and collection site were regressed out of the data within R, and residuals were compared between diagnostic groups using linear models, with post hoc *t* tests. Further details are provided in the Supplementary Methods.

#### 2.6. Analysis of mtDNA copy number using qRT-PCR

Total genomic DNA was prepared (Qiagen blood DNA kit) from 100  $\mu$ L of whole blood collected in EDTA-coated vacutainer tubes. It was pretreated by sonication prior to extraction and DNA extracted according to the manufacturer's protocol (Qiagen blood DNA kit). Samples were assayed in triplicate using the QuantiTect SYBRgreen PCR kit (Qiagen), using primers complementary to unique regions and genes of the mitochondrial genome (thus not amplifying nuclear mitochondrial DNA sequences) and the single copy nuclear gene beta 2 microglobulin in the presence of reference standards, as previously described (Malik et al., 2009). Mitochondrial DNA content was quantified as the ratio of mitochondrial genome to nuclear genome. Data were log transformed and the effects of age, sex, and collection site regressed out within R. Corresponding residuals were compared between diagnostic groups (control, MCI, and AD) using linear models, with post hoc *t* tests.

### 3. Results

#### 3.1. Reduced expression of nuclear-encoded OXPHOS genes

We previously demonstrated reduced expression of a significant number of nuclear OXPHOS genes and mitochondrial ribosome protein subunits (MRP genes) in MCI and AD blood compared to controls (Lunnon et al., 2012) (batch 1). We have replicated and extended these findings in a further independent group of 370 individuals in the current study (batch 2; Table 2). There was a significantly high degree of overlap in the genes found to be differentially expressed and their direction of change between control and MCI/AD subjects between the 2 cohorts (FDR  $q < 0.01$ ) (Table S2) with 34/99 complex I to complex IV probe sets and 24/117 MRP subunits significantly altered in disease compared to 44/99 and 26/118, previously. As expected, the majority of these genes had lower expression in MCI/AD relative to age-matched controls. The number of probes reaching  $q < 0.01$  within each OXPHOS complex was similar between the 2 data sets (Table S3). Decreased expression was not biased to any particular complex in either this data set ( $\chi^2$  [5.044, 4],  $p = 0.228$ ), or the previously published data set ( $\chi^2$  [4.195, 4],  $p = 0.380$ ).

#### 3.2. Increased expression of mitochondrial-encoded OXPHOS genes

Having shown further evidence for decreased expression of many of the nuclear-encoded OXPHOS genes and protein subunits of the mitochondrial ribosome required for translation of OXPHOS genes from the mitochondrial genome in AD and MCI, we were interested to see if this impacted on OXPHOS subunits expressed from the mitochondrial genome required for complex I, III, IV, and V. Of the 13 OXPHOS genes encoded by the mitochondria, we were able to successfully quantify 12 using qRT-PCR (Fig. 1, Table 3, Figure S1). Unlike nuclear-encoded genes, the mitochondrial-encoded genes displayed significantly increased levels of expression in MCI and AD blood relative to controls in one of the 7 mtDNA complex I genes, mitochondrial transcript (MT) MT-ND1 ( $F$  [12.9, 504],  $p = 3.61 \times 10^{-4}$ ,  $q = 1.08 \times 10^{-3}$ ), all of the mtDNA complex IV genes, MT-CO1

**Table 2**

Expression of many nuclear genome-encoded OXPHOS genes, mitochondrial ribosomal protein (MRP) genes, and mitochondrial transcriptional regulator genes are decreased in Alzheimer's disease

OXPHOS complex	Gene symbol	Illumina probe ID	Ref. seq ID	C'Some	ANOVA			Control-MCI		Control-AD		MCI-AD		
					F value	p value	FDR corrected p value	log2 FC	p value	log2 FC	p value	log2 FC	p value	
OXPHOS complex I	<b>NDUFA1</b>	ILMN_1784286	NM_004541.2	Xq24c	54.77	9.29E-13	1.73E-10	-0.27	1.50E-05	-0.42	9.40E-10	-0.15	1.20E-02	
	<b>NDUFA2</b>	ILMN_3243890	NM_002488.3	5q31.3b	53.63	1.54E-12	1.73E-10	-0.17	5.30E-11	-0.18	7.10E-10	n.s.	n.s.	
	NDUFA3	ILMN_1784641	NM_004542.2	19q13.42a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFA4	ILMN_1751258	NM_002489.2	7p21.3b	11.12	9.41E-04	4.41E-03	-0.16	3.51E-02	-0.24	9.50E-04	n.s.	n.s.	
	NDUFA5	ILMN_1759973	NM_005000.2	7q31.32b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFA6	ILMN_3238269	NM_002490.3	22q13.2b	10.11	1.60E-03	6.91E-03	-0.07	1.30E-02	-0.08	1.60E-03	n.s.	n.s.	
	NDUFA7	ILMN_1675239	NM_005001.2	19p13.2d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFA8	ILMN_1759729	NM_014222.2	9q33.2b	11.19	9.08E-04	4.35E-03	-0.06	1.02E-02	-0.07	9.10E-04	n.s.	n.s.	
	<b>NDUFA9</b>	ILMN_1760741	NM_005002.3	12p13.32a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFA10</b>	ILMN_2225698	NM_004544.2	2q37.3e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFA10</b>	ILMN_1791119	NM_004544.2	2q37.3e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFA11</b>	ILMN_2175712	NM_175614.2	19p13.3b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFA12</b>	ILMN_1737738	NM_018838.3	12q22c-q22d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFA13	ILMN_1767139	NM_015965.4	19p13.1a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFAB1	ILMN_2179018	NM_005003.2	16p12.1c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFB1	ILMN_1724367	NM_004545.3	14q32.12b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFB2	ILMN_2117330	NM_004546.2	7q34c-q34d	14.17	1.94E-04	1.20E-03	-0.16	2.27E-03	-0.18	1.90E-04	n.s.	n.s.	
	<b>NDUFB3</b>	ILMN_2119945	NM_002491.1	2q33.1e	14.21	1.90E-04	1.20E-03	-0.18	1.49E-02	-0.25	1.90E-04	n.s.	n.s.	
	NDUFB3	ILMN_2119937	NM_002491.1	2q33.1e	19.05	1.66E-05	1.70E-04	-0.13	1.80E-02	-0.22	1.70E-05	n.s.	n.s.	
	NDUFB4	ILMN_1770589	NM_004547.4	3q13.33b	—	—	—	—	—	—	—	—	—	
	NDUFB5	ILMN_1807397	NM_002492.2	3q26.33a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFB6	ILMN_2369924	NM_002493.3	9p21.1a	19.65	1.23E-05	1.46E-04	-0.12	9.60E-07	-0.10	9.80E-06	n.s.	n.s.	
	NDUFB6	ILMN_1763147	NM_002493.3	9p21.1a	16.75	5.26E-05	4.55E-04	-0.14	2.80E-06	-0.11	4.40E-05	n.s.	n.s.	
	NDUFB7	ILMN_1813604	NM_004146.4	19p13.12c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFB8	ILMN_1661170	NM_005004.2	10q24.31a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFB9	ILMN_3243859	NM_005005.2	8q24.13d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFB10	ILMN_1811754	NM_004548.1	16p13.3e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFB11	ILMN_1749709	NM_019056.3	Xp11.3a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFC1	ILMN_1733603	NM_002494.2	4q31.1c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFC2	ILMN_1694274	NM_004549.3	11q14.1a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFS1</b>	ILMN_1728810	NM_005006.5	2q33.3b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFS2</b>	ILMN_1789342	NM_004550.3	1q23.3a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFS3</b>	ILMN_1756355	NM_004551.1	11p11.2b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFS4</b>	ILMN_1812312	NM_002495.1	5q11.2c	11.80	6.59E-04	3.29E-03	n.s.	n.s.	-0.16	6.70E-04	n.s.	n.s.	
	NDUFS5	ILMN_1776104	NM_004552.1	1p34.3a	52.23	2.87E-12	2.15E-10	-0.22	4.60E-05	-0.36	3.00E-12	-0.14	9.20E-03	
	<b>NDUFS6</b>	ILMN_1794303	NM_004553.2	5p15.33c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFS7</b>	ILMN_1669966	NM_024407.3	19p13.3i	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFS8</b>	ILMN_1794132	NM_002496.1	11q13.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFV1</b>	ILMN_1786718	NM_007103.2	11q13.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	<b>NDUFV2</b>	ILMN_2086417	NM_021074.1	18p11.22c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFV3	ILMN_2387731	NM_001001503.1	21q22.3b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	NDUFV3	ILMN_1765500	NM_021075.3	21q22.3b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	OXPHOS complex II	<b>SDHA</b>	ILMN_1744210	NM_004168.1	5p15.33e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		SDHA	ILMN_2051232	NM_004168.1	5p15.33e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
SDHB		ILMN_1667257	NM_003000.2	1p36.13e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
SDHC		ILMN_2323366	NM_001035513.1	1q23.3a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
SDHC		ILMN_1746241	NM_003001.2	1q23.3a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
SDHD		ILMN_1698487	NM_003002.1	11q23.1c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
OXPHOS complex III	CYC1	ILMN_1815115	NM_001916.3	8q24.3g	9.96	1.73E-03	7.33E-03	n.s.	n.s.	0.06	1.70E-03	0.05	2.68E-02	
	UQCRI0 (UCR)	ILMN_1781986	NM_001003684.1	22q12.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	UQCRI0 (UCR)	ILMN_2366714	NM_013387.3	22q12.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	UQCRI0 (UCR)	ILMN_2366710	NM_013387.3	22q12.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	UQCRI1 (UQC)	ILMN_1745049	NM_006830.2	19p13.3h	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

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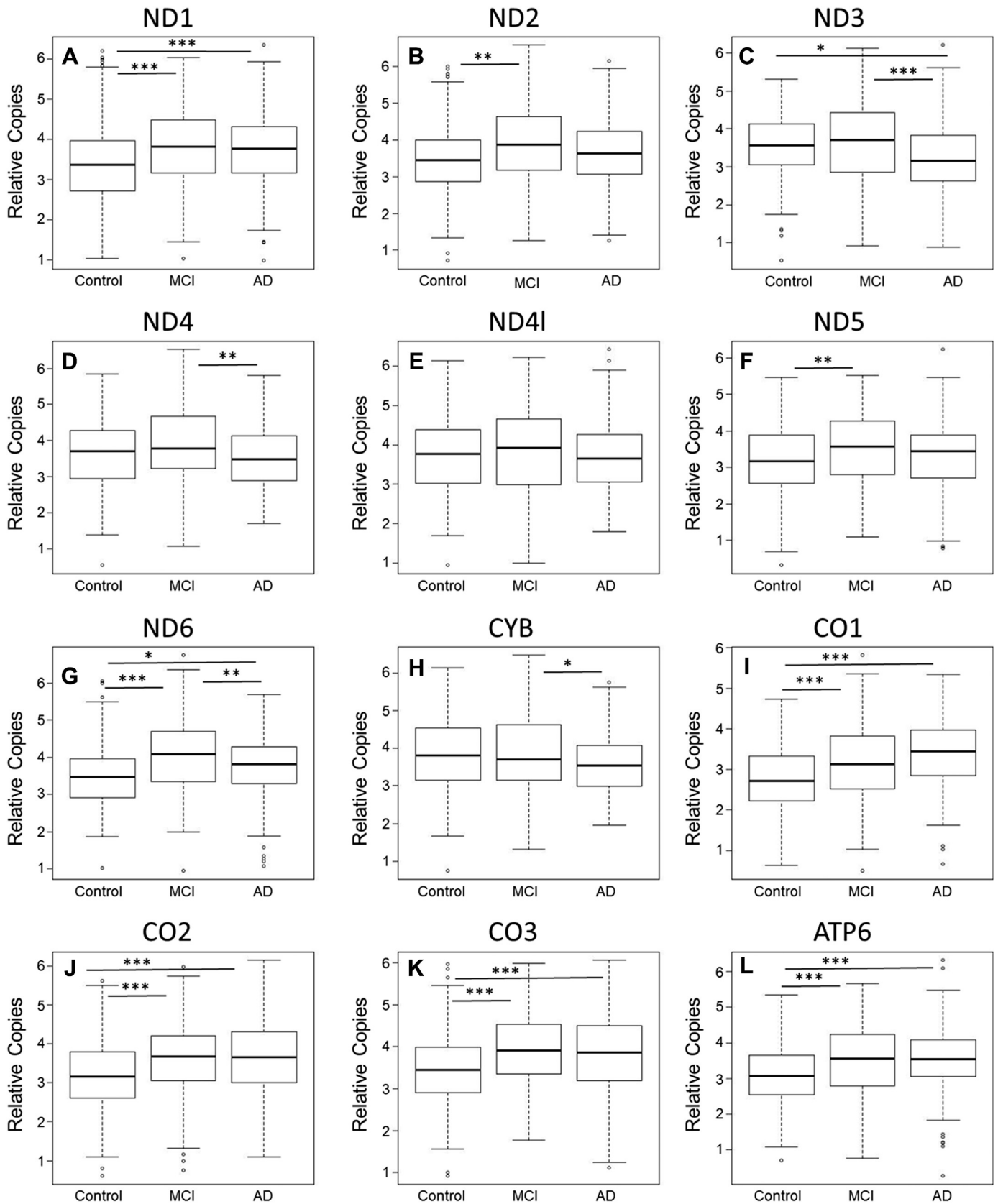
Mitochondrial	TFAM	ILMN_1715661	NM_003201.1	10q21.1e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DNA transcription	TFB1M	ILMN_1733562	NM_016020.1	6q25.3a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	TFB2M	ILMN_2067708	NM_022366.1	1q44d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	TFB2M	ILMN_2067709	NM_022366.1	1q44d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MTERFD1	ILMN_2044617	NM_015942.3	8q22.1d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MTERFD1	ILMN_1782504	NM_015942.3	8q22.1d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MTERFD2	ILMN_1689899	NM_182501.2	2q37.3f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MTERFD3	ILMN_2388517	NM_025198.3	12q23.3c	—	—	—	—	—	—	—	—	—
	MTERFD3	ILMN_1680150	NM_001033050.1	12q23.3c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	POLRMT	ILMN_1770356	NM_005035.3	19p13.3j	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mitochondrial	MRP63	ILMN_2203807	NM_024026.4	13q12.11b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ribosome proteins	MRP63	ILMN_1774312	NM_024026.4	13q12.11b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL1	ILMN_2076658	NM_020236.2	4q21.1c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL2	ILMN_1763264	NM_015950.3	6p21.1d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	<b>MRPL3</b>	ILMN_2230592	NM_007208.2	3q22.1b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL3	ILMN_1713143	NM_007208.2	3q22.1b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL4	ILMN_1804207	NM_015956.2	19p13.2c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL4	ILMN_1681230	NM_146388.1	19p13.2c	—	—	—	—	—	—	—	—	—
	MRPL4	ILMN_2352042	NM_146387.1	19p13.2c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL9	ILMN_1773716	NM_031420.2	1q21.3a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL10	ILMN_1695472	NM_148887.1	17q21.32b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL10	ILMN_2396002	NM_145255.2	17q21.32b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL11	ILMN_2316540	NM_016050.2	11q13.1e	12.12	5.58E-04	2.85E-03	-0.07	5.80E-03	-0.08	5.60E-04	n.s.	n.s.
	MRPL11	ILMN_1676458	NM_170739.1	11q13.1e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL11	ILMN_1690371	NM_170738.1	11q13.1e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL12	ILMN_1699603	NM_002949.2	17q25.3f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL13	ILMN_1671158	NM_014078.4	8q24.12c	12.77	3.99E-04	2.19E-03	-0.06	4.96E-02	-0.10	4.10E-04	n.s.	n.s.
	MRPL14	ILMN_2072603	NM_032111.2	6p21.1b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL15	ILMN_2103720	NM_014175.2	8q11.23d	10.50	1.30E-03	5.87E-03	n.s.	n.s.	-0.07	1.00E-03	n.s.	n.s.
	MRPL16	ILMN_1730685	NM_017840.2	11q12.1d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL17	ILMN_1797933	NM_022061.2	11p15.4c	22.26	3.38E-06	4.75E-05	-0.10	1.10E-04	-0.11	3.20E-06	n.s.	n.s.
	MRPL18	ILMN_1804479	NM_014161.2	6q25.3f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL18	ILMN_2230672	NM_014161.2	6q25.3f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL19	ILMN_1771149	NM_014763.3	2p12i	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL20	ILMN_2189424	NM_017971.2	1p36.33a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL20	ILMN_1693352	NM_017971.2	1p36.33a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL21	ILMN_1744835	NM_181515.1	11q13.2b	10.74	1.15E-03	5.27E-03	-0.09	2.40E-03	-0.09	1.10E-03	n.s.	n.s.
	MRPL21	ILMN_1654250	NM_181512.1	11q13.2b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL21	ILMN_2348050	NM_181514.1	11q13.2b	14.18	1.93E-04	1.20E-03	-0.06	3.77E-03	-0.07	1.90E-04	n.s.	n.s.
	MRPL22	ILMN_1663220	NM_014180.2	5q33.2b	14.68	1.50E-04	1.05E-03	-0.05	3.91E-02	-0.09	1.50E-04	n.s.	n.s.
	MRPL22	ILMN_1748819	NM_001014990.1	5q33.2b	15.20	1.15E-04	8.38E-04	-0.10	6.03E-03	-0.13	1.20E-04	n.s.	n.s.
	MRPL23	ILMN_1806123	NM_021134.2	11p15.5b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL24	ILMN_1695576	NM_145729.1	1q23.1a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL24	ILMN_2398995	NM_024540.2	1q23.1a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL27	ILMN_1753976	NM_148570.1	17q21.33b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL27	ILMN_1727558	NM_148571.1	17q21.33b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL27	ILMN_1811327	NM_148571.1	17q21.33b	16.06	7.44E-05	5.77E-04	-0.05	1.00E-02	-0.07	7.60E-05	n.s.	n.s.
	MRPL28	ILMN_1694950	NM_006428.3	16p13.3f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL30	ILMN_1766154	NM_145212.2	2q11.2c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL30	ILMN_1661039	NM_145212.2	2q11.2c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL32	ILMN_1749432	NM_031903.1	7p14.1a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL33	ILMN_1706326	NM_145330.2	2p23.2b	24.91	9.30E-07	1.49E-05	-0.19	1.20E-08	-0.15	6.00E-07	n.s.	n.s.
	MRPL33	ILMN_1726417	NM_004891.2	2p23.2b	25.06	8.62E-07	1.49E-05	-0.10	1.70E-04	-0.13	8.30E-07	n.s.	n.s.
	MRPL34	ILMN_1783681	NM_023937.2	19p13.11e	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL35	ILMN_1812777	NM_016622.2	2p11.2f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL35	ILMN_1753016	NM_016622.2	2p11.2f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL35	ILMN_2341952	NM_145644.1	2p11.2f	16.55	5.81E-05	4.67E-04	-0.10	2.80E-03	-0.12	5.80E-05	n.s.	n.s.
	MRPL36	ILMN_1800197	NM_032479.2	5p15.33c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL37	ILMN_2041327	NM_016491.2	1p32.3b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

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Table 2 (continued)

OXPHOS complex	Gene symbol	Illumina probe ID	Ref. seq ID	C'Some	ANOVA			Control-MCI		Control-AD		MCI-AD	
					F value	p value	FDR corrected p value	log2 FC	p value	log2 FC	p value	log2 FC	p value
													n.s.
	MRPL38	ILMN_1719656	NM_032478.2	17q25.1d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL39	ILMN_1778730	NM_080794.2	21q21.3a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL39	ILMN_1726391	NM_017446.3	21q21.3a	14.14	1.97E-04	1.20E-03	-0.05	2.01E-02	-0.08	2.00E-04	n.s.	n.s.
	MRPL40	ILMN_1687403	NM_003776.2	22q11.21c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL41	ILMN_1705464	NM_032477.1	9q34.3f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL42	ILMN_2356890	NM_172177.1	12q22b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL42	ILMN_2356895	NM_172177.1	12q22b	9.41	2.31E-03	9.29E-03	n.s.	n.s.	-0.04	2.00E-03	-0.03	2.15E-02
	MRPL42P5	ILMN_2042343	NR_002208.1	15q15.1b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL43	ILMN_1700477	NM_032112.2	10q24.31a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL43	ILMN_2258774	NM_032112.2	10q24.31a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL43	ILMN_1652147	NM_176792.1	10q24.31a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL44	ILMN_1671452	NM_022915.2	2q36.1d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL44	ILMN_2141523	NM_022915.2	2q36.1d	—	—	—	—	—	—	—	—	—
	MRPL45	ILMN_1748650	NM_032351.3	17q12c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL45	ILMN_1808301	NM_032351.3	17q12c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL46	ILMN_1722838	NM_022163.2	15q25.3d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL47	ILMN_2391522	NM_177988.1	3q26.33a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL47	ILMN_1687036	NM_020409.2	3q26.33a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL48	ILMN_1773369	NM_016055.4	11q13.4b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL49	ILMN_1681324	NM_004927.2	11q13.1c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL50	ILMN_1664833	NM_019051.1	9q31.1b	13.02	3.52E-04	1.98E-03	n.s.	n.s.	-0.11	3.60E-04	n.s.	n.s.
	MRPL51	ILMN_2097421	NM_016497.2	12p13.31d	50.85	5.32E-12	2.99E-10	-0.15	6.80E-05	-0.25	5.50E-12	-0.10	9.10E-03
	MRPL52	ILMN_2311041	NM_181306.1	14q11.2f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL52	ILMN_1713966	NM_180981.1	14q11.2f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL53	ILMN_1813682	NM_053050.2	2p13.1b	5.77	1.68E-02	4.71E-02	-0.05	2.50E-02	-0.05	0.0170	n.s.	n.s.
	MRPL54	ILMN_1658486	NM_172251.1	19p13.3e	8.65	3.47E-03	1.28E-02	-0.07	1.11E-02	-0.07	3.50E-03	n.s.	n.s.
	MRPL55	ILMN_1799289	NM_181462.1	1q42.13c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL55	ILMN_2348090	NM_181463.1	1q42.13c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPL55	ILMN_1813817	NM_181454.1	1q42.13c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS2	ILMN_1815043	NM_016034.2	9q34.3b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS5	ILMN_1760441	NM_031902.3	2q11.1c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS6	ILMN_1723874	NM_032476.2	21q22.11c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS7	ILMN_1813389	NM_015971.2	17q25.1c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS9	ILMN_1813207	NM_182640.1	2q12.1d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS10	ILMN_1663664	NM_018141.2	6p21.1f	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS11	ILMN_2405915	NM_176805.1	15q25.3d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS11	ILMN_1722905	NM_176805.1	15q25.3d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS12	ILMN_2371964	NM_033363.1	19q13.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS12	ILMN_1754860	NM_021107.1	19q13.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS12	ILMN_1714515	NM_033362.2	19q13.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS12	ILMN_1810866	NM_033362.2	19q13.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS14	ILMN_1779423	NM_022100.1	1q25.1b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS15	ILMN_1680703	NM_031280.2	1p34.3d	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	<b>MRPS16</b>	ILMN_1775744	NM_016065.3	10q22.2a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS17	ILMN_1804851	NM_015969.2	7p11.2b	28.96	1.32E-07	2.70E-06	-0.05	2.00E-03	-0.09	1.40E-07	n.s.	n.s.
	MRPS18A	ILMN_1730391	NM_018135.2	6p21.1c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS18B	ILMN_1721337	NM_014046.2	6p21.33b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS18B	ILMN_2121282	NM_014046.2	6p21.33b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS18C	ILMN_1658416	NM_016067.1	4q21.23a	20.47	8.18E-06	1.08E-04	-0.10	2.10E-02	-0.18	8.40E-06	n.s.	n.s.
	MRPS18C	ILMN_2085903	NM_016067.1	4q21.23a	—	—	—	—	—	—	—	—	—
	MRPS21	ILMN_1660292	NM_018997.1	1q21.2b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MRPS21	ILMN_1655765	NM_018997.1	1q21.2b	29.93	8.30E-08	1.87E-06	-0.14	1.00E-03	-0.21	8.50E-08	n.s.	n.s.
	<b>MRPS22</b>	ILMN_1655377	NM_020191.2	3q23a	12.38	4.87E-04	2.55E-03	-0.08	1.58E-02	-0.11	4.90E-04	n.s.	n.s.





**Fig. 1.** Differences in expression of mitochondrial genome genes in circulating white blood cells in control (CTL,  $n = 177$ ), MCI ( $n = 168$ ) and AD ( $n = 164$ ). qRT-PCR was used to determine the difference in the expression levels of MT-ND1 (A), MT-ND2 (B), MT-ND3 (C), MT-ND4 (D), MT-ND4L (E), MT-ND5 (F), MT-ND6 (G), MT-CYB (H), MT-CO1 (I), MT-CO2 (J), MT-CO3 (K), and MT-ATP6 (L) in control (CTL), MCI and AD samples. Linear regression demonstrated between group differences for ND1 ( $F = 12.90$ ;  $p = 3.61 \times 10^{-4}$ ;  $q = 1.08 \times 10^{-3}$ ), ND3 ( $F = 5.41$ ;  $p = 0.0204$ ;  $q = 0.0396$ ), ND6 ( $F = 5.19$ ;  $p = 0.0231$ ;  $q = 0.0396$ ), CO1 ( $F = 36.45$ ;  $p = 3.05 \times 10^{-9}$ ;  $q = 3.66 \times 10^{-8}$ ), CO2 ( $F = 21.04$ ;  $p = 5.69 \times 10^{-5}$ ;  $q = 2.28 \times 10^{-5}$ ), CO3 ( $F = 9.479$ ;  $p = 2.19 \times 10^{-3}$ ;  $q = 5.26 \times 10^{-3}$ ), and ATP6 ( $F = 21.88$ ;  $p = 3.73 \times 10^{-6}$ ;  $q = 2.24 \times 10^{-5}$ ). Regression  $p$  values and post hoc  $t$  tests were corrected for multiple

**Table 3**

qRT-PCR was used to determine the differences in expression of genes expressed from the mitochondrial genome in blood samples from control, MCI and Alzheimer's disease

Complex	Gene	ANOVA			Control-MCI			Control-AD			MCI-AD			
		F value	df	p value	Q value	Expression change	p value	Q value	Expression change	p value	BH p value	Expression change	p value	BH p value
Complex I	MT-ND1	<b>1.29E+01</b>	<b>504</b>	<b>3.61E-04</b>	<b>1.08E-03</b>	↑	<b>2.40E-05</b>	<b>9.60E-05</b>	↑	<b>4.10E-04</b>	<b>1.48E-03</b>	↓	5.08E-01	6.12E-01
	MT-ND2	2.18E+00	502	1.40E-01	1.87E-01	↑	<b>5.10E-04</b>	<b>1.67E-03</b>	↑	1.53E-01	2.21E-01	↓	4.35E-02	7.45E-02
	MT-ND3	5.41E+00	489	2.04E-02	3.96E-02	↑	4.72E-01	6.07E-01	↓	1.84E-02	3.90E-02	↓	<b>2.30E-03</b>	<b>6.43E-03</b>
	MT-ND4	3.30E-01	491	5.66E-01	6.17E-01	↓	4.07E-02	7.45E-02	↑	5.27E-01	6.12E-01	↑	8.60E-03	1.94E-02
	MT-ND4L	1.20E-01	495	7.32E-01	7.32E-01	↑	7.60E-01	8.29E-01	↓	7.20E-01	8.10E-01	↓	5.70E-01	6.12E-01
	MT-ND5	2.01E+00	494	1.57E-01	1.88E-01	↑	<b>2.40E-03</b>	<b>6.43E-03</b>	↑	1.70E-01	2.36E-01	↓	1.02E-01	1.59E-01
Complex III	MT-CYB	5.19E+00	505	2.31E-02	3.96E-02	↑	<b>0.00E+00</b>	<b>9.00E-06</b>	↑	2.68E-02	5.36E-02	↓	<b>3.40E-03</b>	<b>8.16E-03</b>
	MT-CO1	3.21E+00	484	7.40E-02	1.11E-01	↓	0.836	0.874286	↑	6.70E-02	1.10E-01	↑	4.30E-02	7.45E-02
Complex IV	MT-CO1	<b>3.65E+01</b>	<b>501</b>	<b>0.00E+00</b>	<b>3.66E-08</b>	↓	<b>9.00E-06</b>	<b>4.60E-05</b>	↑	<b>4.00E-06</b>	<b>4.05E-05</b>	↑	1.30E-01	1.95E-01
	MT-CO2	<b>2.10E+01</b>	<b>501</b>	<b>5.70E-05</b>	<b>2.28E-05</b>	↑	<b>4.00E-06</b>	<b>4.10E-05</b>	↑	<b>7.00E-06</b>	<b>4.63E-05</b>	↓	9.60E-01	9.60E-01
Complex V	MT-CO3	<b>9.48E+00</b>	<b>505</b>	<b>2.19E-03</b>	<b>5.26E-03</b>	↑	<b>1.80E-05</b>	<b>8.10E-05</b>	↑	<b>2.50E-03</b>	<b>6.43E-03</b>	↓	2.15E-01	2.86E-01
	MT-ATP6	<b>2.19E+01</b>	<b>500</b>	<b>4.00E-06</b>	<b>2.24E-05</b>	↑	<b>9.00E-06</b>	<b>4.60E-05</b>	↑	<b>5.00E-06</b>	<b>4.05E-05</b>	↑	8.50E-01	8.74E-01
	MT-ATP8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Data are shown graphically in Fig. 1. Regression *p* values and post hoc *t* tests were corrected for multiple testing using the Benjamini-Hochberg method (correcting for 12 ANOVA tests and subsequently 36 *t* tests). ↓ denotes decreased expression and ↑ denotes increased expression. Data shown in bold denotes significant difference between groups after correcting for multiple testing.

Key: ANOVA, analysis of variance; BH, Benjamini-Hochberg; MCI, mild cognitive impairment; MT, mitochondrial transcript; n.d., not determined; qRT-PCR, quantitative real-time PCR.

nominal significance after correction for multiple testing, possibly because far fewer samples were available for protein analysis compared to that for gene expression. Although complex IV and V had the greatest number of subunits with altered expression, this did not translate in to a change at the protein level in those with disease. Few studies have explored the association between OXPHOS gene expression and functional read-outs. However, in at least one study there is a modest association between levels of OXPHOS gene expression and mitochondrial membrane potential, cell viability, ATP, and ROS (Wagner et al., 2008). It appears that this relationship can become disassociated under certain conditions, which makes precise prediction of functional outcomes from changes in gene expression difficult. Further work, beyond the scope of the current study will be required to further verify what, if any, the functional significance of the changes we have found at the level of gene expression are, although they do not appear to simply reflect a simple alteration in the number of steady-state mitochondria due to altered biogenesis or mitophagy. We also can't rule out the possibility that increased/decreased biogenesis and mitophagy together may result in no net change in steady-state levels of mitochondria, which could explain these results.

Downregulation of the OXPHOS system in blood leukocytes is usually only associated with their migration to the site of tissue damage or pathogen invasion accompanied by their differentiation and activation during acute inflammation (Saeed et al., 2014). However, recent data suggest in chronic re-exposure to stimuli, downregulation of OXPHOS may be also observed in circulating cells such as monocytes as a result of epigenetic reprogramming or trained immunity which enable them to respond more readily to restimulation (Netea et al., 2016; Saeed et al., 2014). Such cells activate a metabolic switch to glycolysis that not only provides substrates for biosynthetic programs, maintains mitochondrial membrane potential, and provides energy in the form of ATP to the cell in oxygen depleted environments during an inflammatory response, but also generates significant ROS which has important bactericidal benefits (O'Neill and Hardie, 2013). Our results may reflect a systemic and general response to a chronic proinflammatory environment in the brain found in many

neurodegenerative diseases, including AD. The changes we observed, particularly in complex I and IV, are expected to lead to inefficiencies in the OXPHOS system resulting in an excess of electrons available to react with dioxygen which then produce unstable and damaging free radicals (ROS) (Sena and Chandel, 2012). Unchecked ROS can damage biomolecules in their vicinity and for this reason complex I, III, and IV are particularly vulnerable (Musatov and Robinson, 2012). Complexes I and IV have previously been shown to have lower subunit expression or activity in AD brain (Kish et al., 1992; Leutner et al., 2005; Maurer et al., 2000; Mutisya et al., 1994) and in peripheral blood cells in AD (Bosetti et al., 2002; Parker et al., 1990). Changes in complex IV are believed to have significant knock-on effects on complex I, which not only produces the majority of ROS, but is itself particularly vulnerable to ROS (Musatov and Robinson, 2012). These changes may also contribute to the oxidative inactivation of m-aconitase observed in blood and brain which is a source of Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> and believed to be a contributing factor to neurotoxicity in AD (Mangialasche et al., 2015).

We did not find an increase in mitochondrial steady-state levels to explain the changes in gene expression we observed. The selective increase in levels of OXPHOS subunits from the mitochondrial genome were more likely to result from a block at the level of translation as large numbers of nuclear-encoded mitochondrial ribosome genes (MRP genes) required for their translation were also downregulated in MCI and AD, whereas there were no changes in nuclear-encoded genes required for their transcription. Furthermore, we observed selective increases in adjacent genes, particularly those which lay close to the mtDNA transcriptional start site and/or are posttranscriptionally processed together following initial transcription as a single polycistronic heavy chain transcript (Figure S1). OXPHOS genes from the mitochondria can only be transcribed on one of two polycistronic transcripts. The changes appear therefore to have arisen after transcription and probably represent selective transcript turnover, perhaps in response to a translational block contributed by the lower expression of MRP genes from the nuclear genome. Further work will be needed to establish if this is the case.

testing using the Benjamini-Hochberg method (correcting for 12 ANOVA tests and subsequently 36 *t* tests). Full data are provided in Table 3. Open circles denote potential outliers. These are datapoints that fall either above the upper quartile plus 1.5 times the interquartile distance (IQD), or below the lower quartile minus 1.5 times the IQD. \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.005 in post hoc *t* tests after Benjamini-Hochberg correction; Abbreviations: AD, Alzheimer's Disease; ANOVA, analysis of variance; MCI, Mild Cognitive Impairment; MT, mitochondrial transcript; qRT-PCR, Quantitative Real-Time PCR.

Recessive mutations in 16 of the 84 nuclear and 13 mitochondrial OXPHOS genes are associated with brain disorders characterized by symptoms of dementia, including many of the genes altered in the present study (Fattal et al., 2006; Koopman et al., 2013). Interestingly complex II is the only OXPHOS complex where all subunits are synthesized by the nuclear genome and where we did not see any difference in MCI or AD blood. A study investigating regional differences in the expression of nuclear-encoded OXPHOS genes in AD brain showed this was the only complex to show no decrease in expression in the entorhinal cortex, a region of the brain affected relatively early in disease (Liang et al., 2008). This complex has however been shown to be reduced in Parkinson's disease white blood cells (Yoshino et al., 1992) suggesting there may be disease specific changes.

The overall process of OXPHOS is tightly controlled by transcriptional regulation at the level of DNA and RNA, by substrate feedback inhibition and by posttranslational modifications including phosphorylation and acetylation. Inefficient electron transfer through complexes I–IV leads to various human abnormalities, which is due in part to a loss of energy metabolism and a deregulation of critical enzymes, such as complexes I, II, and III. Without proper regulation, the production of ROS is known to increase. Some diseases associated with impaired OXPHOS include diabetes, Parkinson's disease, AD, cancer, and the aging process itself. Many diverse classes of drugs inhibit OXPHOS and induce mitochondrial toxicity. Not surprisingly, the ability to monitor the expression levels of the OXPHOS complexes is a key element in the study of many diseases and to the process of drug safety evaluation and to monitor drugs which may have beneficial effects in AD.

Overall, our findings suggest a change in OXPHOS in mitochondria from people with MCI and AD, particularly in complexes I, IV, and V, which appear to be independent of mitochondrial biogenesis or mitophagy. The decreased expression of nuclear-encoded OXPHOS and MRP genes in MCI and AD was accompanied by an increase in mitochondrial-encoded OXPHOS genes in a pattern suggesting a selective block in their translation. These changes in OXPHOS genes are expected to drive a negative feedback loop that further reduces mitochondrial translation (Nolden et al., 2005).

## 5. Conclusions

Changes in OXPHOS appear before a clinical diagnosis of AD in blood. It isn't clear whether they lead to maladaptive functional consequences, or merely reflect changes occurring in AD brain and have no functional consequences of their own. Our findings, which mirror some of the changes occurring in the brain, could support efforts to identify compounds or genes upstream which may coordinately regulate OXPHOS gene expression in efforts to mitigate potentially harmful downstream ROS production. Further work would be needed to confirm and refine these pathways in the brain and blood, but ultimately our results could be used for assessing drug efficacy for target development and for monitoring effects using *ex vivo* blood cell assays or analysis of blood *in vivo*.

## Disclosure statement

The authors have no actual or potential conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2016.12.029>.

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