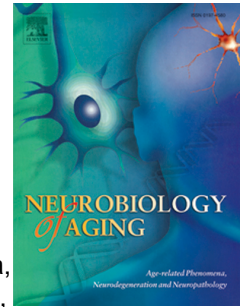


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Mitochondrial genes are altered in blood early in Alzheimer's disease

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ABSTRACT

Although mitochondrial dysfunction is a consistent feature of Alzheimer's disease (AD) 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 AD and mild cognitive impairment (MCI). 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 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 (ROS).

Keywords: Mitochondria, Alzheimer's disease, AD, gene expression, blood, biomarker, mild cognitive impairment, MCI, oxidative phosphorylation, OXPHOS

ABBREVIATIONS: AD (Alzheimer's Disease), ANM (AddNeuroMed cohort), ATP (adenosine triphosphate), DCR (Dementia Care Register cohort), ETC (electron transport chain), GEO (Gene Expression Omnibus), MCI (Mild Cognitive Impairment), MRP (mitochondrial ribosome protein), mtDNA (mitochondrial DNA), NADH (reduced nicotinamide adenine dinucleotide), ncDNA (nuclear DNA), (Nicotinamide nucleotide transhydrogenase), OXPHOS (Oxidative phosphorylation), ROS (Reactive oxygen species)

1.0 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 (A β) 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 ATP through oxidative phosphorylation (OXPHOS). OXPHOS requires 97 proteins to assemble in five multi-protein complexes in the correct stoichiometry for a functioning supramolecular complex (Chaban *et al.*, 2014). 84 OXPHOS genes are encoded by the nuclear genome, whilst an additional 13 (complexes I, III, IV and V) are expressed as polycistronic RNAs from three 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). APP, A β and APOE 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 down-regulated 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 pre-dementia 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; Lunnon *et al.*, 2013; Mecocci *et al.*, 2002; Mecocci *et al.*, 1998; Sultana *et al.*, 2013; Sultana *et al.*, 2011; 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 down-regulated 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.

2.0 MATERIALS AND METHODS

2.1 Subjects and Samples

Blood samples for DNA and RNA analyses were taken from subjects participating in two biomarker studies coordinated from the Institute of Psychiatry, Psychology & Neuroscience, King's College London; The AddNeuroMed (ANM) study and the Maudsley Biomedical Research Center (BRC) Dementia Case Register (DCR) curated by the National Institute for Health Research (NIHR) 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 (200ng) 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). Pre-processing 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 gender were removed from further analyses, leaving 370 Batch 2 samples available for analysis. 240 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, gender, collection site and RIN 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 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 dataset (Lunnon *et al.*, 2012) (Batch 1) and data from the new validation cohort (Batch 2), while a chi-squared 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 250ng of total RNA using the QuantiTect Reverse Transcription Kit (Qiagen) and diluted five-fold for PCR. Real time PCR was performed with 5x HOT FIREPol® EvaGreen®qPCR Mix Plus (ROX) (SolisBiodyne). 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 twelve routine housekeeping genes, using the Normfinder application. Data was transformed to achieve a parametric distribution and the effects of age, gender, collection site and RIN 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 μ 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 min, the lysate was centrifuged at 14,000 g

for 20 min 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 Oxidative Phosphorylation (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 to V. Data from each sample were normalized using Nicotinamide nucleotide transhydrogenase (NNT) measured in the same assay. Data was log transformed and technical outliers >2 standard deviations from the mean were removed. The effects of age, gender 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 mitochondrial DNA (mtDNA) copy number using qRT-PCR

Total genomic DNA was prepared (Qiagen blood DNA kit) from 100 µl of whole blood collected in EDTA-coated vacutainer tubes. It was pretreated by sonication prior to extraction and DNA extracted according to the manufacturers 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 *NUMTs*) and the single copy nuclear gene Beta 2 microglobin (B2M) 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 was log transformed and the effects of age, gender 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.

3.0 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 two cohorts (FDR $q < 0.01$) (Table S2) with 34/99 complex I to complex IV probesets and 24/117 mitochondria ribosome protein 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 two datasets (Table S3). Decreased expression was not biased to any particular complex in either this dataset (X^2 (5.044, 4), $p = 0.228$), or the previously published dataset (X^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 (Figure 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 seven mtDNA complex I genes, 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 (F (36.45, 501), $p=3.05 \times 10^{-9}$, $q=3.66 \times 10^{-8}$), MT-CO2 (F (21.04, 501), $p=5.69 \times 10^{-5}$, $q=2.28 \times 10^{-5}$) and MT-CO3 (F (9.48, 505), $p=2.19 \times 10^{-3}$, $q=5.26 \times 10^{-3}$), and, the only mtDNA complex V gene assayed, MT-ATP6 (F (21.88, 500), $p=3.73 \times 10^{-6}$, $q=2.24 \times 10^{-5}$). We also observed significantly increased expression of MT-ND2 ($p=5.1 \times 10^{-4}$; $q=1.67 \times 10^{-3}$), MT-ND5 ($p=2.4 \times 10^{-3}$; $q=6.43 \times 10^{-3}$) and MT-ND6 ($p=2.40 \times 10^{-7}$; $q=8.64 \times 10^{-6}$) from complex 1 in MCI subjects, following a post hoc t -test.

3.3 Changes in gene expression do not reflect altered mtDNA copy number

Having identified an overall increase in the level of expression in many of the mitochondrial genes, we were keen to establish whether the net effect of the changes we observed are likely to represent altered mitochondrial biogenesis or mitophagy, or altered OXPHOS efficiency at the individual mitochondrial level. No difference in the copy number of mtDNA was found between control, MCI and AD groups for those subjects where blood was available for assessment (Figure S2) (F (1.857), $p=0.1766$) on 1 and 85 degrees of freedom, with between group t -tests (corrected for false discovery rate (FDR) (Benjamini and Hochberg, 1995)): control versus MCI $p = 0.27$; control versus AD $p = 0.27$; MCI versus AD $p = 0.94$.

3.4 Changes in gene expression are not associated with alteration in protein markers of OXPHOS complexes I to V

Having identified a significant decrease in many nuclear-encoded OXPHOS genes alongside an increase in many of the mitochondrial-encoded OXPHOS genes, we wanted to assess the potential impact on the profiles of different OXPHOS complexes. The results of a Human OXPHOS Magnetic Bead Panel showed a significant decrease in the level of Complex I between groups ($p=0.045$), reflecting lower levels in AD cases compared to controls ($p=0.043$) (Figure S3; Table S4). However, this did not pass

nominal significance once multiple testing correction was applied ($q=0.224$ and $q=0.570$, respectively). There were no differences in the levels of other complexes with disease.

4.0 DISCUSSION

Our results have confirmed and extended previous findings of a perturbed mitochondrial OXPHOS system in blood cells from people with MCI and in AD platelets (Bosetti *et al.*, 2002; Cardoso *et al.*, 2004; Feldhaus *et al.*, 2011; Lunnon *et al.*, 2012; Lunnon *et al.*, 2013; Mecocci *et al.*, 2002; Mecocci *et al.*, 1998; Parker *et al.*, 1990; Sultana *et al.*, 2013; Sultana *et al.*, 2011; Valla *et al.*, 2006; Wang *et al.*, 2006). Complexes I, IV and V appear to be particularly vulnerable with ~25%, ~30% and ~50%, of nuclear genes, respectively, having reduced expression while ~14%, 100% and 100%, of mitochondrial genome genes, respectively, have increased expression in MCI or AD blood relative to controls, which is predicted to be due to a block in their translation. The changes we report are already evident in people with MCI, many of whom are expected to have prodromal disease (Gauthier *et al.*, 2006) and significant AD pathology (Albert, 2011). The magnitude of differential expression in OXPHOS genes was greater in people with an AD diagnosis compared to those with MCI, which either reflects the relative mildness of pathology in MCI, or that not all people with MCI have AD pathology. We are unable to establish from the present study which of the different AD pathological biomarkers these changes may be most closely associated with.

Some of these changes appear to have culminated in changes at the protein level; there was a trend towards lower abundance of the protein marker highly correlated with the functional activity of Complex I in AD blood. However, this modest change failed to reach 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, at least in one study there is modest association between levels of OXPHOS gene expression and mitochondrial membrane potential, viability, ATP, MTT 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, 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.

Down-regulation 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 suggests in chronic re-exposure to stimuli, down-regulation of OXPHOS may be also observed in circulating cells such as monocytes as a result of epigenetic re-programming or trained immunity which enable them to respond more readily to re-stimulation (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 pro-inflammatory 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^{2+} and H_2O_2 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 down-regulated in MCI and AD, whilst 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 post-transcriptionally 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.

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 synthesised 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 (PD) white blood cells (Yoshino *et al.*, 1992) suggesting there may be disease specific changes.

The overall process of oxidative phosphorylation is tightly controlled by transcriptional regulation at the level of DNA and RNA, by substrate feedback inhibition and by post-translational 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, PD, 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).

5.0 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 co-ordinately 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*.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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Table 1: Subject characteristics of individuals used in the study. 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 analysed. 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 is 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; Lunnon et al, 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 was generated. Finally qRT-PCR was used to assess mitochondrial DNA copy number in 87 individuals, that also had beadarray and qRT-PCR data generated. MMSE: Mini Mental State Examination; CDR: Clinical Dementia Rating scale.

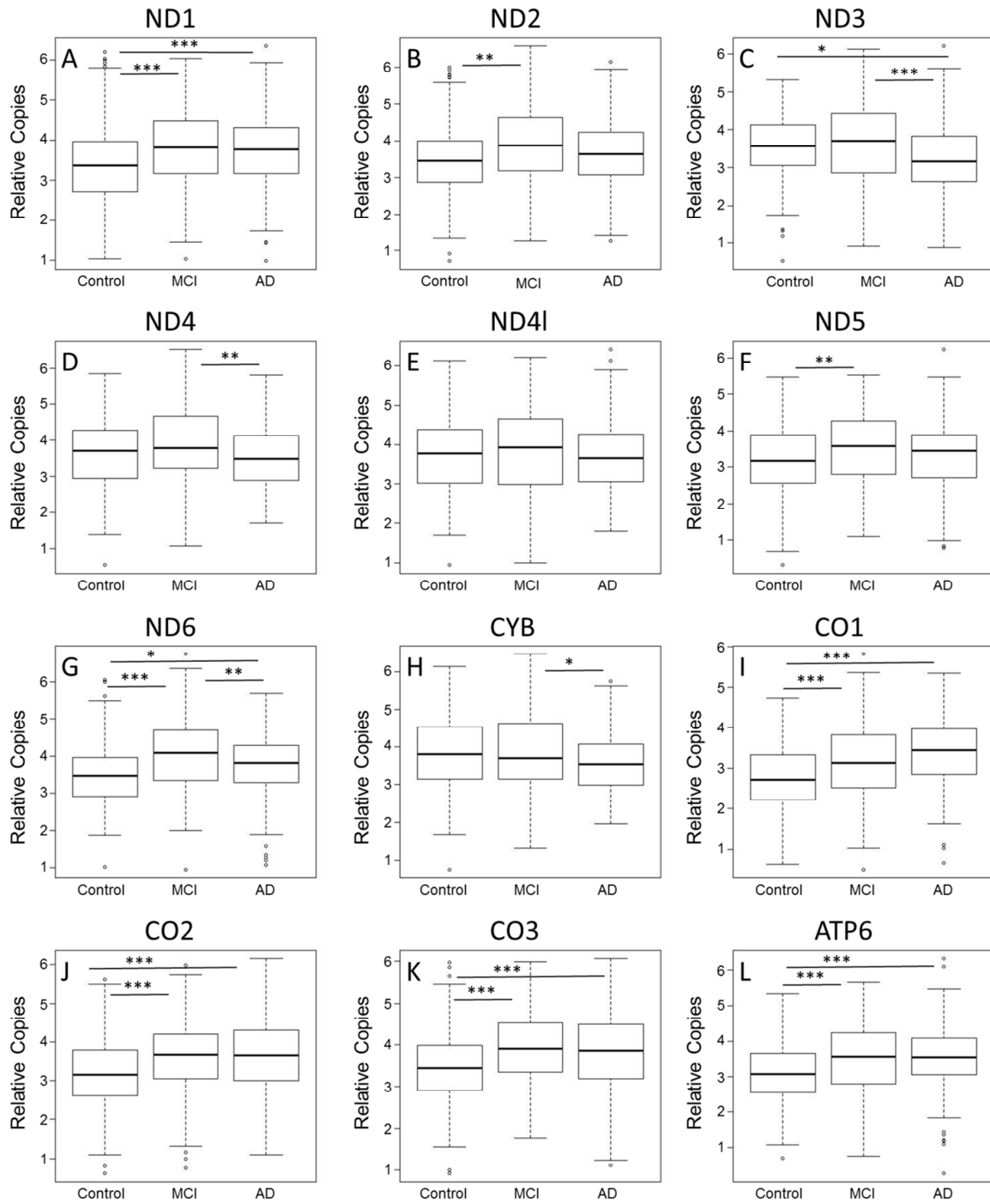
	Illumina HT-12 V4 Arrays (batch 2)			qRT-CR			Protein			mtDNA		
	Control	MCI	AD	Control	MCI	AD	Control	MCI	AD	Control	MCI	AD
Samples analysed	129	109	132	177	168	164	27	19	24	28	31	28
Gender (M/F)	52/77	48/61	50/82	73/104	77/91	55/109	12/15	9/10	11/13	12/16	13/18	11/17
Age in years (Mean±SD)	75.2 (5.8)	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)

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. The 240 probes on the Illumina HT-12 V4 expression beadarray corresponding to OXPHOS genes, MRP genes and mitochondrial transcription were selected for analysis. Data was adjusted for co-variables such as age, sex, centre and RIN and subsequently analysed using one-way ANOVA to assess the relationship between disease group and expression levels. Data was corrected using the Benjamini Hochberg method for multiple testing and only data with an FDR $q < 0.01$ was deemed significant. For data with $q < 0.01$ post-hoc t-tests were performed to assess differences between groups. Genes highlighted in bold have previously been shown to contain recessive mutations known to cause disease with symptoms of dementia.

Gene Symbol	Illumina Probe ID	RefSeq 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
NDUFA1	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
NDUFA2	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.
NDUFA9	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.
NDUFA10	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.
NDUFA10	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.
NDUFA11	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.
NDUFA12	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	19p13.11a	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.
NDUFB3	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.
NDUFS1	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.

OXPHOS Complex I

Figure 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 testing using the Benjamini-Hochberg method (correcting for 12 ANOVA tests and subsequently 36 t-tests). Full data is provided in Table 3. Key: $*=p<0.05$; $**=p<0.01$; $***=p<0.005$ in post-hoc t-tests after Benjamini-Hochberg correction.

Figure 1

Highlights

- Decreased OXPHOS and mitochondria ribosome nuclear genes in Alzheimer's disease blood
- Increased OXPHOS mitochondrial genes in Alzheimer's disease blood
- Changes appear to be independent of mitochondrial biogenesis or mitophagy
- OXPHOS efficiency may be compromised leading to damaging reactive oxygen species