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Exploring mitochondrial blood-based and genetic markers in older adults with mild cognitive impairment and remitted major depressive disorder

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Mild cognitive impairment (MCI) is a prodromal stage in aging to possible progression to Alzheimer's disease and related dementia (ADRD), where co-occurrence of major depressive disorder (MDD) accelerates the progression. Metabolic and mitochondrial abnormalities in ADRD and other neurodegenerative disorders have been widely suggested, while possible mitochondrial dysfunction has been associated with etiopathology of both MCI and MDD. Hence, investigation of mitochondrial markers in MCI, MDD, and presence of both conditions is warranted. In total, 332 older adult participants were included: 168 with MCI, 108 with MCI plus remitted MDD (rMDD), and 56 with rMDD but without MCI. We measured plasma circulating mitochondrial DNA (ccf-mtDNA), lactate, and extracted nuclear mitochondrial encoded (NMt) single-nucleotide variants (SNVs) ($n = 312$). Non-parametric statistical tests on ccf-mtDNA and lactate levels were performed on the diagnosis, clinical and cardiometabolic variables. Binary sequence kernel association test (SKAT-O) and burden test were performed on NMt-SNV, adjusted for age, race, gender, type II diabetes, and APOE genotype. Lower level of lactate was observed in MCI (KW $\chi^2 = 14.8$, $P = 0.0024$), more specifically, significant differences of lower plasma lactate between MCI only and rMDD, but not between MCI+rMDD and MCI were found, suggesting potential roles in MCI driving lactate lower levels. While higher levels of ccf-mtDNA were observed in APOE-ε4 carrier ($\chi^2 = 5.04$, $P = 0.05$). This relationship was present only in MCI ($P = 0.043$) and MCI+rMDD groups ($P = 0.023$). No significant nuclear-encoded mitochondrial gene associations were observed with MCI or MDD. The results suggest decreased level of plasma lactate in individuals with MCI and MCI+rMDD, with inverse correlation with ccf-mtDNA, in addition to effect of APOE-ε4 in further increasing ccf-mtDNA specifically in participants with cognitive impairment. These findings contribute to a deeper understanding of the mitochondrial markers in MCI and MDD, warranting further research to explore the precise roles of mitochondrial abnormalities in the development and progression of MCI.

Translational Psychiatry (2024)14:457 ; https://doi.org/10.1038/s41398-024-03155-9

INTRODUCTION

Mild cognitive impairment (MCI) is a prodromal stage of dementia affecting up to 20% of older adults, where 10–20% of individuals with MCI progress to dementia annually [1]. While the progression of MCI to dementia is influenced by a range of factors, lifetime major depressive disorder (MDD) is one of most prevalent and influential clinical risk factors of developing late onset Alzheimer's disease and related dementia (ADRD) [2]. Lifetime history of MDD has been suggested to increase the risk of developing ADRD by twofold [3, 4]. Furthermore, both remitted and active late-life depression has been reported to accelerate the progression from MCI to AD by 20–40%, respectively [5, 6]. Lifetime occurrence of MDD is estimated up to 20%, while MCI affects up to 20% of older adults—together, individuals with MCI and MDD represents a large subgroup of patients with pre-dementia sharing neuropsychiatric considerations [7–9].

Despite the identification of risk factors for developing sporadic forms of ADRD including genetic, biological, and environmental factors in recent years, understanding of the contribution and interactions of each factor to etiopathology remain largely elusive [10]. Previous investigations of MDD as a risk factor for dementia predominantly focused on symptomatology and active depression [3, 11]. Yet, careful considerations are required in the investigation and interpretation of depressive symptom as a neuropsychiatric feature of cognitive impairment, or rather, exacerbated impairment affected by pre-existing early to midlife MDD [12–14]. Thus, further investigations on co-existing risk conditions and its interactions are required, while considering and controlling for

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Received: 26 February 2024 Revised: 6 October 2024 Accepted: 9 October 2024
Published online: 29 October 2024

semi-modifiable factors such as remission status and onset of depression.

A common biological pathway disruption that has been implicated across the etiopathologies of MDD, MCI, and ADRD is mitochondrial dysfunction [15–17]. While mitochondrial and metabolic abnormalities of AD patients were first documented several decades ago, age-related cumulative damages of the mitochondria have been suggested as a possible mechanism of ADRD pathophysiology [18–20]. More recently, evidence of cellular mitochondrial dysfunction in the periphery has been associated with MCI [21]. Similarly, mitochondrial dysfunction in both the brain and the periphery has been associated with MDD and late-life depression [22–26]. Hence, it is plausible that the co-occurrence of MCI and MDD may involve additive mitochondrial stress leading to metabolic alterations and damage that contribute to acceleration in cognitive decline in dementia. However, there has been no research yet investigating how the simultaneous presence of MCI and MDD affects molecular changes in metabolic and mitochondrial functions. Thus, the investigation of mitochondrial markers in individuals with MCI and MDD may be valuable in exploring the biological differences across each clinical group, and possible contributions to etiology of dementia progression.

In this study, we investigate several mitochondrial markers in participants with MCI, remitted MDD (rMDD), and both (MCI +rMDD) to explore possible differences between each clinical group. We examine the two blood-based mitochondrial markers, lactate and circulating cell-free mitochondrial DNA (ccf-mtDNA) as potential markers of metabolism and cellular damage of the mitochondria [26–29]. Previous studies have suggested increased resting blood lactate as a risk factor for MCI, which may robustly represent reduced oxidative metabolism at baseline [30, 31]. Moreover, increased ccf-mtDNA has been reported in older adults with late-life depression and frailty [32]. Furthermore, increased ccf-mtDNA has been suggested as a longitudinal predictor of both physical and cognitive decline in older adults, suggesting the evidence for ccf-mtDNA as a marker associated with increased apoptotic stress and chronic inflammation in progressive cognitive decline [33]. As MCI and MDD have been associated with mitochondrial dysfunction and oxidative stress, we hypothesize an increased level of both ccf-mtDNA and lactate with additive mitochondrial dysfunction, in comorbid MCI and rMDD group, compared to single MCI or rMDD group. As an exploratory analysis, we study nuclear-encoded mitochondrial (NMt) single-nucleotide variants (SNV) and their role on MCI and/or rMDD. We use SNV-wise logistic regression, along with other grouping methods, to investigate if there are any notable differences in the genetic impact of these mitochondrial-related genes.

METHODS

Participants

Participants' clinical, demographic, genetic data and plasma samples were obtained from subset of participants enrolled in Prevention of Alzheimer's dementia with Cognitive remediation plus Transcranial direct current stimulation in Mild cognitive impairment and Depression (PACt-MD) study at baseline (ClinicalTrials.gov Identifier: NCT02386670) [34]. The diagnoses of MCI (both amnestic and non-amnestic) and MDD were made in accordance with Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V). Detailed description of the clinical trial, inclusion criteria, and assessments is provided elsewhere [2, 34, 35]. A total of 332 older adult participants at high-risk for ADRD were included in the current study: 168 with MCI, 56 with rMDD, and 108 with MCI+rMDD.

Blood collection

Blood was collected in EDTA tubes via venipuncture in the morning, and overnight fasting was not required for participants. The plasma was separated immediately after collection and aliquots were stored at −80 °C.

Measurement of lactate in plasma

The plasma concentration of plasma lactate was measured using Cayman L-Lactate kit (Cayman Chemical Company, MI, USA) according to manufacturer's protocol. This fluorescent-based method detects lactate dehydrogenase catalyzed-oxidation of lactate to pyruvate, where concomitant reduction of NADH further reacts with the fluorescent substrate that has an excitation wavelength of 540 nm and emission wavelength of 585–595 nm. In each well, 5 µL of plasma was used with technical duplicates. The final plasma concentration of L-Lactate was calculated using standard curves. Experimenters were blinded prior to final analysis. The inter-plate coefficient of variability (%CV) was 13.2% across 15 plates assayed, and intra-plate %CV was 2.7%.

DNA isolation and measurement of circulating-cell free mitochondrial DNA in plasma

Using 50 µL of plasma, mitochondrial DNA extraction was performed via QiaAMP DNA mini kit (Cat# 51304; Qiagen, Hilden, Germany) following manufacturer's protocol. DNA was eluted with 100 µL Ultra-Pure distilled water free from DNAse and RNAse (Cat# 10977015; Invitrogen, MA, USA). The absolute concentration (copies/µL) of ccf-mtDNA was quantified using extracted HEK DNA of known concentration, pre-determined by digital PCR, diluted in half-logs of up to 12 points. Mitochondrially encoded NADH dehydrogenase 1 (*ND1*) and 4 (*ND4*) were used to represent the major and minor arc of the mitochondrial genome, respectively, and *B2M* and *PPIA* were used as nuclear controls. qPCR was run as a duplex reaction using a 20 µL TaqMan™ qPCR mixture which contains 10 µL of TaqMan™ Fast Advanced Master Mix (Cat# 4444556; ThermoFisher Scientific, MA, USA), 4 µL DNA, 1 µL each of Forward and Reverse primers, and 1 µL TaqMan™ probe (IDT Technologies, IA, USA) for each gene. Using BioRad's C1000 Thermal cycle CFX384 Real Time System (Rio-Rad Laboratories, CA, USA), qPCR cycling conditions, described by TaqMan™ manufacturer, were as follows: 50 °C for 2 min, 95 °C for 20 s, 40 cycles of 95 °C for 3 s, and 60 °C for 30 s, followed by a fluorescent read per cycle. The absolute concentration is estimated against the standard curve using a linear equation. The inter-plate %CV was 6.5%, and intra-plate %CV was 4.2%.

Genotyping

Genotyping was performed in CAMH Biobank and Molecular Core Facility (Toronto, ON, Canada) according to standardized genotyping protocols. The total genomic DNA was extracted from collected buffy coat using Qiagen QIAamp DNA mini kit (Qiagen Inc, Hilden, Germany). The extracted DNA integrity was assessed using agarose gel electrophoresis (1% agarose, 1× TBE) against a 1 kB ladder (FroggaBio, ON, Canada), and concentration and purity was characterized by nanodrop via Nandrop-8000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). The custom Psych arrays with added Neuro content (Illumina Inc, San Diego, CA) were run as per manufacturer's directions for HTS array processing.

QC and Nuclear-encoded mitochondrial gene extraction

Quality control and gene set SNV extraction was performed on plink v1.9. In the unpruned/clump dataset, non-SNV and trimorphic variants were removed, thresholds of MAF < 0.005, HWE *P* value < 1-e06, genotyping rate >0.99 were used, and variants on mitochondrial, X and Y chromosomes were removed. The remaining SNVs were extracted (--extract-range) by GRCh37.hg19 genomic coordinates of MitoCarta 3.0 (https://www.broadinstitute.org/mitocarta/mitocarta30-inventory-mammalian-mitochondrial-proteins-and-pathways, accessed 2023) for nuclear-encoded mitochondrial (NMt) genes [36]. Furthermore, rs7414 and rs429358 loci were extracted for Apolipoprotein E (*ApoE*)–Ɛ2,3,4 genotypes. The region extracted binary file was minor allele encoded for analysis input.

Statistical analysis

All statistical analysis was performed in *PLINK v1.90b*, and *R programming language for statistical computing v. 4.2.0*. SNV-wise logistic regression analysis on mitochondrial gene sets, LD-pruning and principal component analysis (PCA) were conducted in *PLINK v1.90b*. The parameters of the genome-wide LD-pruning have been set to 50 kb, 10 base pair steps, at $r^2 < 0.2$. The mitochondrial SNV sets were unpruned.

Normality of the markers were inspected via Shapiro–Wilk test and visual methods, and the quality of ccf-mtDNA was checked using Pearson's correlation test between plasma copy numbers of ND1 and ND4 genes, which natural-log-transformed ND1 was used for later

analysis. Mean and standard deviation were used for reporting demographics, and tests for homogeneity tests such as Fisher's exact test and Chi squared tests were performed. Non-parametric statistical tests including Mann–Whitney *U*, unpaired Wilcoxon's signed rank, Spearman correlation, and Kruskal–Wallis tests were used to compare levels of markers across diagnostic groups and clinical variables, and appropriate statistical tests were applied in accordance with distribution and residual variance such as ANOVA and regression. The *P* values of univariate tests were adjusted using Benjamini-Hochberg method, and a threshold of $P \geq 0.05$ was used to determine statistical significance. For multivariate and stratified analyses lineaging from univariate tests, we report the unadjusted *P* values.

To examine the association of ccf-mtDNA and lactate with cardiovascular metabolism, we examined total cholesterol to high density lipoprotein (TC/HDL) ratio, and heart age calculated via Framingham risk score, described previously in PACt-MD participants [35, 37]. Furthermore, we assessed the association of the markers with psychiatric comorbidities, evaluated by Cumulative Illness Rating Scale-Geriatric (CIRS-G), described previously by Diniz et al. in PACt-MD participants [2, 38].

The number of SNVs in the NMt regions (NMt SNV load) was quantified as an average minor allele carried per successfully genotyped loci. Two aggregation methods, burden test and sequence kernel association test (SKAT-O), were used to investigate the NMt SNVs as MCI and rMDD as binary traits. The analyses were performed using SKAT package v. 2.2.5 [39]. In both tests, Madsen-Browning weighting was used for SNV-adaptive weighting [40, 41]. Covariates including age, gender, type II diabetes (T2D), ccf-mtDNA, lactate, and principal components (PCs) representing ethnicities were sequentially added for statistical modeling.

RESULTS

Demographics

Breakdown of the participant demographics for each diagnostic group can be found in Table 1. No statistical differences between age, T2D, smoking status, self-reported ethnicity (White/other) and gender were found among the groups. However, statistical differences in self-reported race in proportions across diagnostic groups were found ($\chi^2 = 17.7$, $P = 0.024$).

Plasma circulating cell-free mitochondrial DNA

The median plasma concentration of ccf-mtDNA across the three diagnostic groups were $e^{9.41}$ copies/μL (IQR: $e^{0.76}$ copies/μL) for MCI, $e^{9.55}$ copies/μL (IQR: $e^{0.77}$ copies/μL) for MCI + rMDD, and 9.47 (IQR: $e^{0.90}$ copies/μL) for rMDD only (Table 2, Fig. 1A, $\chi^2 = 3.50$, $P = 0.21$). A significant negative correlation between ccf-mtDNA and age (Spearman's $\rho = -0.13$, $P = 0.048$, Table 2) was found. The level of ccf-mtDNA was different among gender in pooled analysis ($P = 0.049$), however, it was not statistically significant after stratifying for diagnostic groups (Fig. 1B). The level of ccf-mtDNA was not associated with MCI, smoking, rMDD, cholesterol/HDL ratio, amnesia, heart age, and T2D. Nominal association of ccf-mtDNA was found with presence of neuropsychiatric comorbidities in MCI + rMDD group only (raw $P = 0.038$, Table 2). The levels of ccf-mtDNA were significantly higher in APOE-ε4 carriers compared to non-carriers ($\chi^2 = 5.04$, $P = 0.05$, Table 2).

Plasma lactate levels

The median plasma concentration of lactate across the three diagnostic groups were 544 μM (IQR: 306 μM) for MCI only, 549 μM (IQR: 510 μM) for MCI + rMDD, and 715 μM (IQR: 506 μM) for rMDD only (Table 2 and Fig. 2A). There was a statistically significant difference among the medians (Kruskal–Wallis $\chi^2 = 14.8$, $P = 0.0024$), and post-hoc Dunn's test identified higher plasma lactate in MCI only and MCI+rMDD compared to rMDD only group ($Z = -3.82$, $P = 0.0001$; $Z = -2.35$, $P = 0.0094$, respectively), but no difference was found between MCI + rMDD and MCI without rMDD ($Z = -1.64$, $P = 0.0503$) after adjusting for multiple testing. Furthermore, plasma lactate was increased in individuals with T2D ($W = 3443$, $P = 0.0017$; Table 2), which remained significant after adjusting for MCI and rMDD ($F_{T2D} = 15.1$, $P_{T2D} = 0.00013$; $F_{diagnosis} = 8.7$, $P_{diagnosis} = 0.00021$). The proportion of participants with T2D taking metformin had higher lactate levels, however, was not statistically significant ($t = 1.35$, $P = 0.194$ univariate; $F = 1.715$, $P = 0.200$ adjusted for diagnosis). Plasma lactate was not associated with age, smoking, amnesia, and race.

While plasma lactate and ccf-mtDNA were negatively correlated ($\rho = -0.21$, $P = 0.00008$), when explored by each diagnostic groups, the negative correlations were observed in d MCI ($\rho = -0.2$, raw $P = 0.0082$; Fig. 3A) and MCI+rMDD group ($\rho = -0.32$, raw $P = 0.00076$; Fig. 3A) while no significant correlation was shown in rMDD only group ($\rho = -0.096$, raw $P = 0.48$; Fig. 3A). We further investigated the relationship between lactate and cardiovascular metabolic measures (TC/HDL: $\rho = 0.14$, $P = 0.078$; heart age: $\rho = 0.16$, $P = 0.054$; Table 2), where no significant correlation of plasma lactate was found when stratified per diagnostic groups (Fig. 3B, C).

While higher levels of ccf-mtDNA were observed in APOE-ε4 carrier, there were nominally significant increases of ccf-mtDNA in MCI ($P_{raw} = 0.043$) and MCI+rMDD groups ($P_{raw} = 0.023$) but not rMDD ($P = 0.35$). Plasma lactate, stratified into diagnostic groups, was increased in ApoE-ε4 carriers among rMDD+MCI group ($P_{raw} = 0.045$), but not MCI only and rMDD only groups (Fig. 4).

Table 1. Demographics.

		MCI	MCI + rMDD	rMDD
N		168	108	56
Age (mean, SD)		72 (7.5)	71.1 (4.6)	69.8 (4.5)
Gender (F/M)		96 F/72 M	66 F/42 M	37 F/19 M
Genotyped (*n*)		162	100	50
Self-reported race (*n*, %)	White	83 (83%)	111 (68%)	46 (92%)
	African	6 (6%)	11 (7%)	1 (2%)
	Asian	5 (5%)	23 (17%)	1 (2%)
	Other	6 (6%)	17 (10%)	2 (4%)
	N/A	8	6	6
T2D (*n*, %)		18 (11%)	12 (11%)	8 (14%)
Clinically significant psychiatric comorbidity (%)		2 (1%)	56 (42%)	25 (44%)
Smoking (%)		4 (3%)	5 (5%)	2 (5%)
Amnestic MCI (%)		119 (71%)	73 (68%)	

MCI mild cognitive impairment, *rMDD* major depressive disorder with remission, *F* female, *M* male.

Table 2. Pairwise descriptive statistics of natural-log-transformed plasma ccf-mtDNA and lactate against demographic and clinical variables of interest.

Ccf-mtDNA (natural log scaled copies/µL, mean ± SD; [median:IQR])					
Variable	**Group (*n*)**			**Statistics**	**Adjusted *P* value**
Diagnostic group	MCI (168)	MCI + rMDD (108)	rMDD (56)	KW $\chi^2 = 3.50$	0.21
	9.34 ± 1.01 [9.41, 0.76]	9.51 ± 0.81 [9.55, 0.77]	9.38 ± 0.77 [9.47, 0.90]		
Amnesia	Amnestic MCI (192)	Non-amnestic MCI (84)		W = 7355	0.39
	9.35 ± 1.00 [9.42, 0.78]	9.52 ± 0.76 [9.55, 0.82]			
Smoking	Yes (11)	No (286)		W = 1645	0.78
	9.65 ± 0.92 [9.37, 0.78]	9.38 ± 0.92 [9.45, 0.83]			
Age				$\rho = -0.13$	0.048
Gender	F (199)	M (133)		W = 11174	0.049
	9.50 ± 0.75 [9.54, 0.75]	9.25 ± 1.10 [9.37, 0.80]			
Plasma lactate				$\rho = -0.21$	0.00008
Presence of moderate to severe psychiatric comorbidity	Yes (83)	No (240)		W = 11032	0.17
	9.52 ± 0.83 [9.57, 0.77]	9.35 ± 0.95 [9.41, 0.81]			
ApoE-ε4 allele carrier*	Carrier (82)	Non-carrier (199)		W = 9502	0.050
	9.54 ± 0.82 [9.59, 0.97]	9.29 ± 0.94 [9.40, 0.74]			
Total cholesterol/HDL ratio**				$\rho = -0.12$	0.11
Heart age**				$\rho = -0.015$	0.88
Type II diabetes	Yes (37)	No (295)		W = 5903	0.42
	9.42 ± 0.72 [9.34, 0.85]	9.39 ± 0.93 [9.45, 0.80]			
Plasma lactate (µM, mean ± SD)					
Diagnostic group	MCI (168)	MCI + MDD (108)	MDD (56)	KW $\chi^2 = 14.8$	0.0024
	571 ± 253 [544, 306]	683 ± 402 [549, 510]	762 ± 313 [715, 506]		
Amnesia	Amnestic MCI (192)	Non-amnestic MCI (84)		W = 7928	0.82
	614 ± 331 [546, 336]	615 ± 309 [542, 387]			
Smoking	Yes (11)	No (286)		W = 2105	0.082
	807 ± 288 [877, 472]	643 ± 334 [599, 268]			
Age				$\rho = -0.06$	0.30
Gender	F (199)	M (133)		W = 11544	0.082
	608 ± 307 [548, 363]	686 ± 350 [611, 437]			
Presence of moderate to severe psychiatric comorbidity	Yes (83)	No (240)		W = 12107	0.0097
	748 ± 382 [677, 557]	603 ± 299 [545, 335]			
ApoE-ε4 allele carrier*	Carrier (82)	Non-carrier (199)		W = 7450	0.29
	590 ± 285 [551, 339]	644 ± 319 [578, 374]			
Total cholesterol/HDL ratio**				$\rho = 0.14$	0.078
Heart age**				$\rho = 0.16$	0.054
Type II diabetes	Yes (37)	No (295)		W = 3443	0.0017
	827 ± 380 [771, 469]	615 ± 311 [547, 376]			

*Subset of genotyped participants ($n = 280$); **subset of participants with blood panels ($n = 221$).

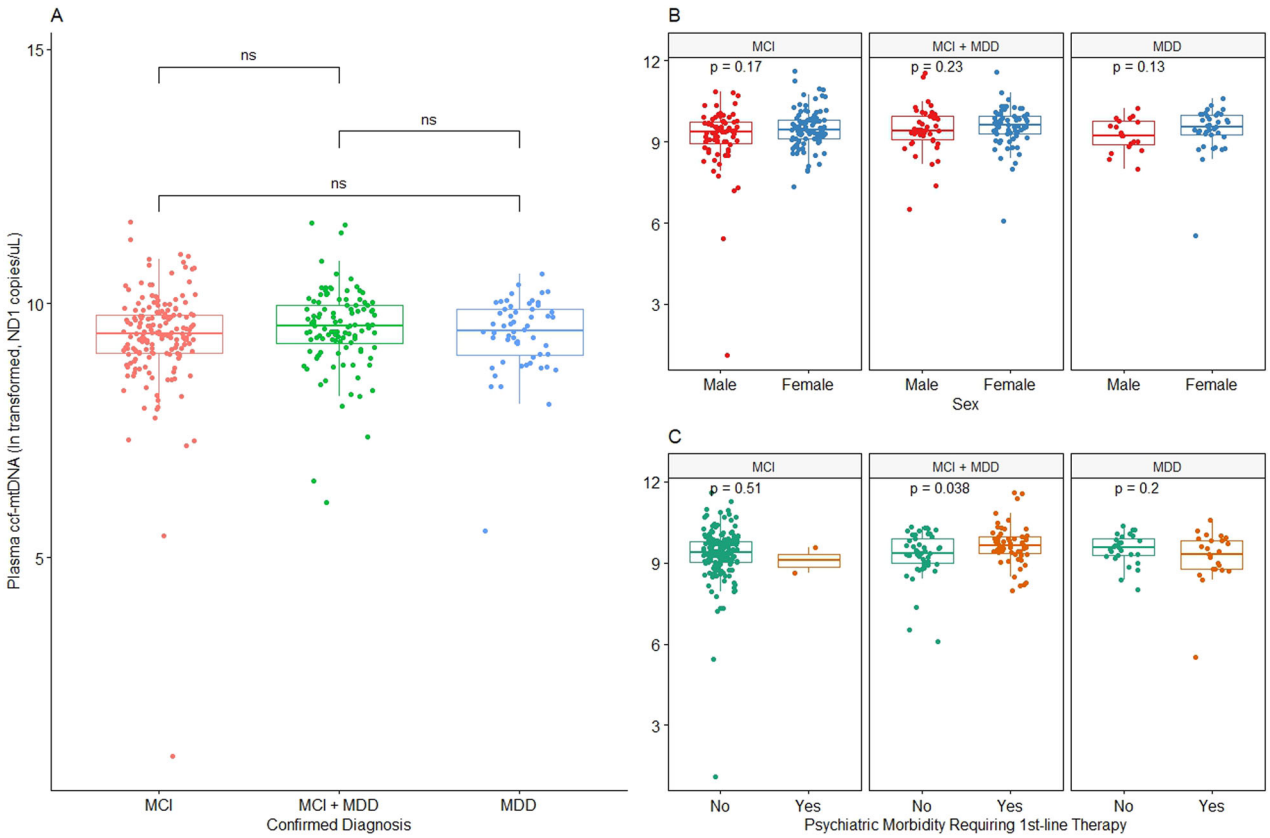

Fig. 1 Circulating cell free mitochondrial DNA in mild cognitive impairment and remitted major depressive disorder. A Boxplot of plasma ccf-mtDNA represented by natural log-transformed ND1 (copies/μL) across diagnostic groups ($\chi^2 = 3.50$, $P = 0.174$). **B** Boxplot of ln-transformed plasma ccf-DNA in each diagnostic group by gender; presented *P* values are unadjusted. **C** Boxplot of ln-transformed plasma ccf-DNA in each diagnostic group by presence of moderate to severe psychiatric comorbidities requiring first line therapy; presented *P* values are unadjusted. It is important to highlight that the plasma concentration of ccf-mtDNA measured by ND1 and ND4 demonstrated linear correlation of Pearson's r^2 of 0.93 (Supplemental Fig. 1). No noticeable nuclear DNA was quantified as measured by reference genes PPIA and B2M in all assayed samples. Demonstrating reliability of data.

ApoE-ε genotyping

The total count of successfully *ApoE*-genotyped individuals included in the analysis was 280 of 312, where rs4293558 genotyping was unsuccessful in 31 individuals, and inconsistency occurred in rs7412 quality control probes (3 total) which has been removed from the analysis. No statistical differences of ε4 or ε2 counts between diagnostic groups, age, race, and gender were found (Supplemental Table 2).

Single-nucleotide variation quality control and nuclear-encoded mitochondrial gene extraction

A total of 530,163 variants were genotyped, and upon initial quality control, 330,546 SNVs remained. The SNVs were pruned, and the remaining 123,328 SNVs were used for PCA. Upon extraction of NMt genes, a total of 4870 NMt SNVs were left for analysis.

Principal component analysis of the pruned genome wide and mitochondrial SNVs demonstrated similar structures, separating self-identified races (Supplemental Table 2). The first two PCs of the GW and NMt SNVs were linearly correlated (Pearson's $r^2 > 0.98$) and were used as covariates representing ancestry.

Nuclear-encoded mitochondrial single nucleotide variants

There were no NMt SNVs that were significantly associated with MCI or rMDD from logistic regression analysis. Of 4870 NMt SNVs, 135 variants were found in non-white participants. The average NMt alternative allele per individual was 2095 ± 137, and 0.43 ± 0.03 alleles per genotyped SNV. Burden and SKAT-O test for MCI as binary trait after adjusting for age, gender, rMDD, ccf-mtDNA, and lactate suggested difference by MCI ($n_{cases} = 262$, $n_{controls} = 50$) diagnosis status (Burden $P = 0.011$; SKAT-O $P = 0.018$), however, upon adjustment with the first 2 ancestry-related genetic PCs, no significance was found (Burden $P = 0.21$; SKAT-O $P = 0.18$). The burden and SKAT-O test for rMDD ($n_{cases} = 162$, $n_{controls} = 150$) as binary trait were similar ($Burden_{unadjusted}$ $P = 0.018$, $Burden_{adjusted}$ $P = 0.22$; $SKAT\text{-}O_{unadjusted} = 0.0013$, $SKAT\text{-}O_{adjusted} = 0.30$). The results of the same analyses for subgroup of self-identified white participants, confirmed by ancestry-related PCs, also identified no differences between MCI diagnosis status (Burden $P_{mci} = 0.80$, SKAT-O $P_{mci} = 0.37$), and rMDD (Burden $P_{mdd} = 0.37$, SKAT-O $P_{mdd} = 0.27$).

DISCUSSION

In this study, we examined lactate and ccf-mtDNA in plasma, nuclear-encoded mitochondrial SNVs, and ApoE in individuals with MCI and/or rMDD. In contrast to our hypothesis, we found decreased plasma lactate was associated with individuals with MCI or MCI+rMDD, compared to individuals with unimpaired cognition with rMDD. Furthermore, no additive effects of MCI and rMDD were found on mitochondrial markers, which is consistent with findings from biomarker studies of PACt-MD in cerebrospinal fluid (CSF) amyloid, tau markers, as well as neuroimaging data [42, 43]. Notably, the negative correlation of plasma lactate and ccf-mtDNA was only present in individuals with current MCI. While no differences in the level of ccf-mtDNA was found across diagnostic

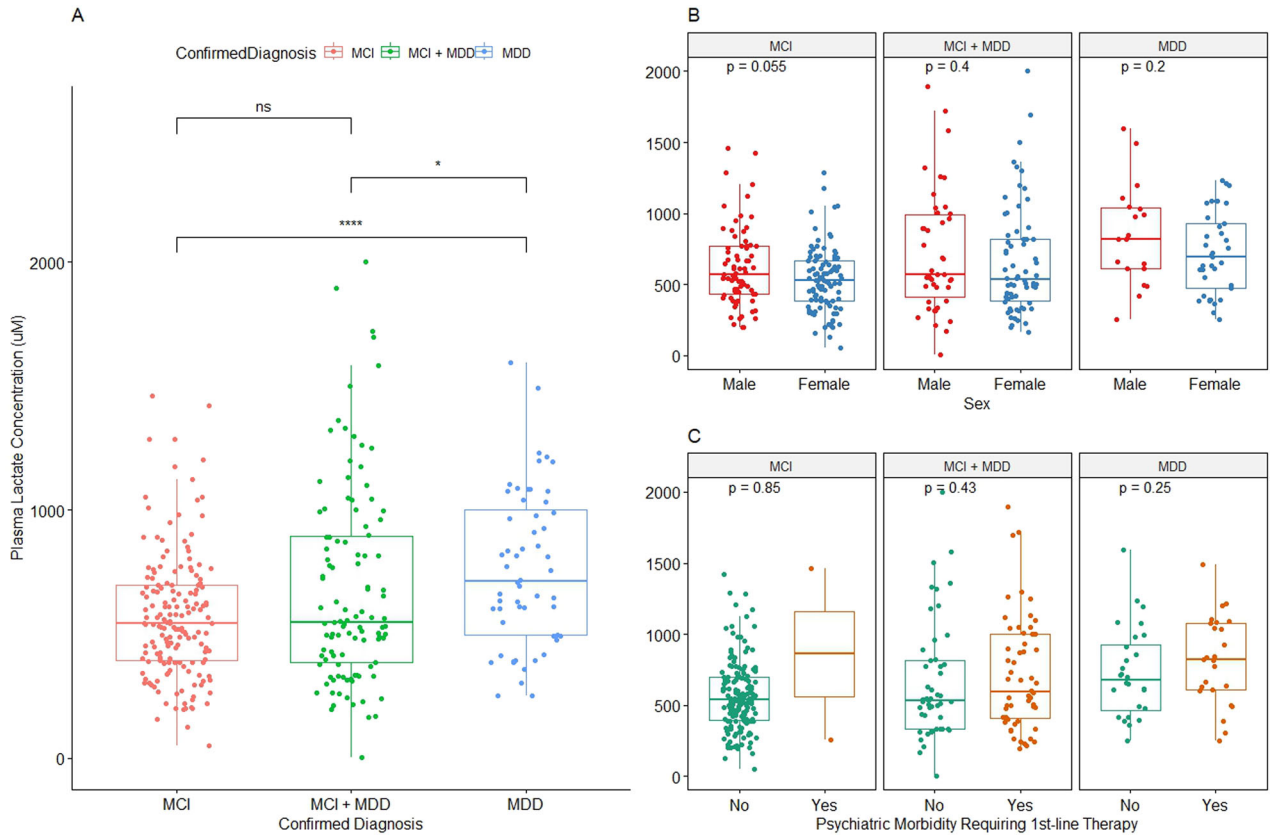

Fig. 2 Lactate levels in mild cognitive impairment and remitted major depressive disorder. A Boxplot of plasma lactate (μM) across diagnostic groups ($\chi^2 = 14.8$, $P = 0.00061$). Statistically significant lower lactate in MCI + rMDD ($^{*}Z = -2.35$, $P_{adjust} = 0.0141$) and MCI ($^{****}Z = -3.82$, $P_{adjust} = 0.0002$) observed in comparison to rMDD group. **B** Boxplot of ln-transformed plasma ccf-DNA in each diagnostic group by gender; presented *P* values are unadjusted. **C** Boxplot of ln-transformed plasma ccf-DNA in each diagnostic group by presence of moderate to severe psychiatric comorbidities requiring first line therapy; presented *P* values are unadjusted.

groups, we found that increased plasma ccf-mtDNA to be associated with ApoE-ε4 genotype in individuals with MCI.

Recent studies suggested ccf-mtDNA as a possible peripheral marker of aging-related inflammation in the elderly, while there is limited evidence of its association with cognitive impairment [33, 44, 45]. To our knowledge, this is the first study investigating peripheral ccf-mtDNA in MCI and MCI+rMDD, and possible association with *ApoE*-ε genotypes. Although there has been limited investigation of plasma ccf-mtDNA in elderly population, increased plasma lactate has been associated with late-life depression, frailty, physical inactivity, and sarcopenia [26, 32, 46]. Our results suggest that ccf-mtDNA may be affected by *ApoE*-ε4 genotype, as well as associated with altered metabolic markers (lactate and TC/HDL) in patients with MCI, but not in cognitively unimpaired individuals.

Lactate has been widely investigated in the brain and cerebrospinal fluid as a marker of metabolic and mitochondrial dysfunction across both MCI and MDD, where compensatory anaerobic glycolysis may contribute to elevated lactate [47–49]. However, a limited number of studies investigated peripheral lactate. To our knowledge, this is the first large study to investigate plasma lactate levels in a clinical cohort of MCI and rMDD in elderly participants, as well as co-current MCI+rMDD. Increased plasma lactate at rest has been previously associated with several metabolic disorders as T2D, obesity, and cardiovascular diseases [50, 51]. Recent evidence also suggested that elevated plasma lactate is associated with increased risk of MCI and increased C-reactive protein concentration in a Chinese community study, although included participants were aged 18–88 years [30]. Counterintuitively, Radford-Smith et al. recently reported that decreased plasma lactate is associated with increased susceptibility to MDD [52]. On the other hand, Singh et al. reported elevated plasma lactate levels in a study of 10 elderly patients with MCI with matched healthy controls [53]. In our study, we found significantly decreased lactate levels in individuals with MCI, regardless of rMDD. With caution, it suggests that presence of MCI may be associated with decreased plasma lactate. Similarly, patients with AD also have significantly decreased plasma lactate, which is also implicated with prolonged disease duration [54].

Furthermore, it is noteworthy that the negative correlation of lactate and ccf-mtDNA is found only in individuals with MCI and MCI+rMDD. The major sources of plasma lactate, at rest, are skeletal muscle via glycolytic pathway, which may represent bodily deficit of non-OXPHOS energy substrate at basal level [54]. Increase in ccf-mtDNA has been indicated in individuals with T2D-related cognitive impairment, where individuals with both altered metabolism and mitochondrial stress – increase in ccf-mtDNA and decreased cellular mtDNA was observed [55]. Our results suggest that decrease in plasma lactate may be associated with increased mitochondrial stress and damage, specifically in individuals with MCI, possibly representing altered metabolism along with mitochondrial stress, where deficiencies in glycolytic function together with mitochondrial damage may be present in the periphery. This trend was consistent on the association of *ApoE*-ε4 on ccf-mtDNA, where increase of ccf-mtDNA was shown only in groups with MCI. While plasma lactate was nominally correlated with TC/HDL and heart age, the trend was only apparent in MCI +rMDD, suggesting the differences in plasma lactate across diagnostic groups is not only reflective of cardiovascular

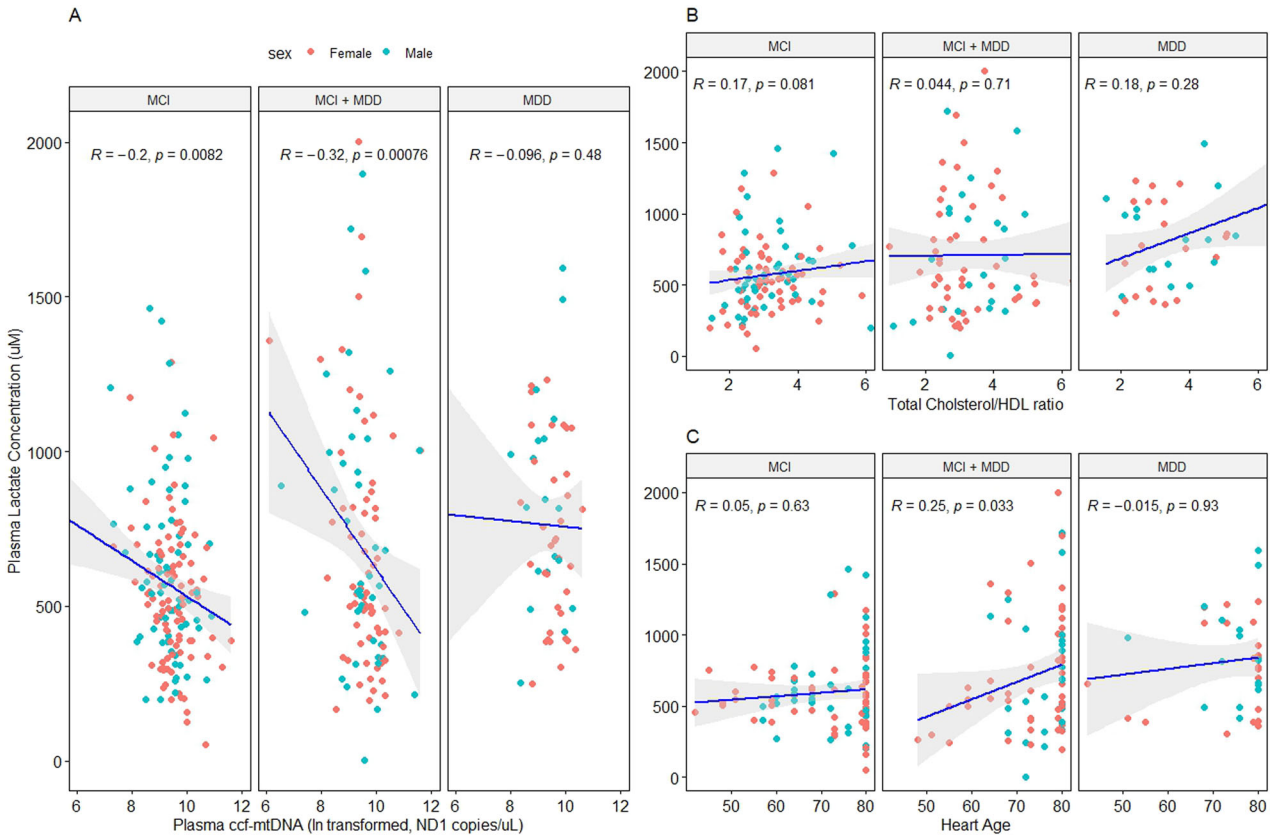

Fig. 3 Correlation analysis of blood-based mitochondrial biomarkers and cholesterol levels or heart age. A Correlation plot of plasma ccf-mtDNA represented by natural log-transformed ND1 (copies/μL) and lactate, gender labeled in color (female—red; male—blue). **B** Correlation plot of plasma lactate and total cholesterol to HDL ratio by diagnosis groups. **C** Correlation plot of plasma lactate and heart age by diagnosis groups.

metabolic differences, but more so MCI-indicated condition. Previously, Diniz et al. reported increased senescence associated secretory phenotype (SASP) in the MCI+rMDD cohort in PACt-MD [2]. Recent bodies of evidence suggest that mtDNA release from apoptotic stress is a major factor in both aging and cell senescence [56, 57]. Hence, it may be plausible that co-occurrence of both accelerated senescence with mitochondrial dysfunction, along with the metabolic effects of *APOE*-ε4, may be a systemic feature associated with MCI [58]. Future studies are needed to investigate the interactions of cell senescence, mitochondrial dysfunction, and systemic inflation to elucidate their role in MCI and progression to ADRD.

Although genetic variations of genes associated with mitochondrial function have been implicated across neurodegenerative disorders, gene-set aggregate analysis of our curated SNVs conservatively curated based on MitoCarta 3.0 did not identify significant difference between diagnostic status [59–63]. Despite our findings, it would be worthwhile to investigate the effects of genetic variations across significant at-risk conditions of late-life ADRD.

While our study has several strengths, limitations should be noted. We have performed post-hoc cross-sectional analysis on only the baseline measurements of a longitudinal clinical trial, and we have not performed further analysis on the association of mitochondrial markers with cognitive measures, imaging, or detailed medical measures. While the blood was taken in the morning, fasting was not a requirement. The plasma lactate level is transiently affected in postprandial states, which has not been fully accounted for in the analysis [64]. The level of chronic and acute exercise has not been accounted in the analysis, which may have affected the basal lactate level that has been measured [65]. Hence, although we found no outliers from the normal blood lactate range (e.g. >2 mM), unknown variability in the basal lactate level affected by physical activity may have inaccurately captured baseline metabolic state. In healthy older adults, chronic exercise has been suggested to significantly alter lactate threshold levels, but has nominal to no effects on baseline lactate levels [66]. Our analysis has been limited to blood-based markers, which may not reflect alterations in the brain, but rather represent peripheral metabolic stress. Although there has been evidence suggesting weak or no correlation between levels of plasma lactate with CSF or brain lactate, plasma-derived lactate is transported across the blood-brain barrier contributing up to ~10% of total brain metabolism at basal condition [67, 68]. Hence, it may be plausible that decreased plasma lactate can affect the brain lactate metabolism as a reservoir, especially under increased demand and stress. A future study to investigate the relationship between peripheral mitochondrial markers with CSF levels, cognitive measures, functional imaging, or longitudinal neurocognitive trajectories would be valuable. Our analysis did not include a control group without MCI and rMDD. We did not include clinical factors such as duration of rMDD, treatment type, duration of medication, and severity of major depression in our analysis, which may have influenced unaccounted heterogeneity in participants with rMDD included in our study. Hence, although the results may not be directly generalizable to cognitively unimpaired older adults without history of MDD, the results suggest association of mitochondrial metabolism, damage markers, and metabolic alterations with MCI.

In summary, this study investigated the potential links between MCI, rMDD, and their co-occurrence through the examination of mitochondrial markers. While no significant association was found

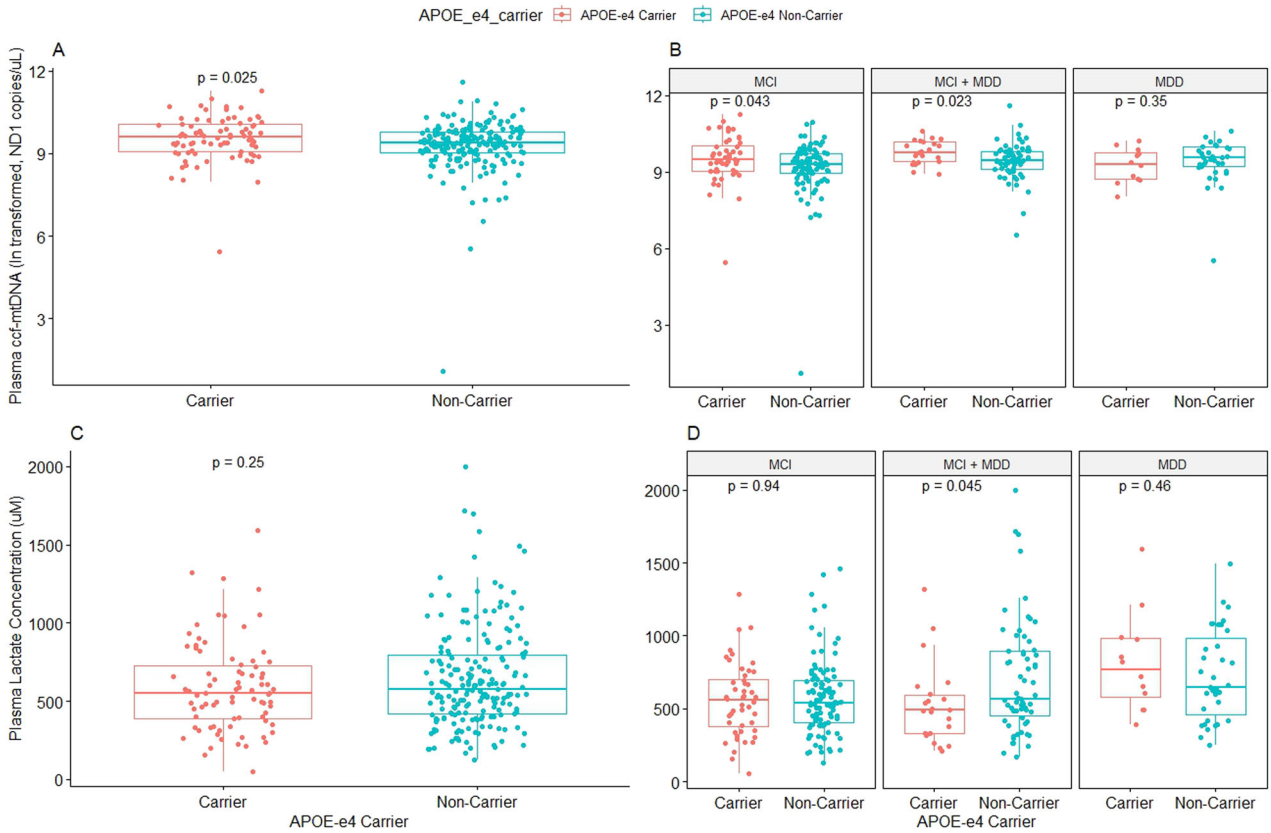

Fig. 4 Influence of APOE-ε4 allele carrier on ccf-mtDNA and lactate. A Boxplot of ccf-mtDNA (natural log-transformed ND1 copies/μL) by APOE-ε4 allele carrier group ($\chi^2 = 5.04$, $P = 0.025$). **B** Boxplot of ccf-mtDNA (natural log-transformed ND1 copies/μL) by APOE-ε4 allele carrier group, faceted by diagnosis groups. **C** Boxplot of plasma lactate concentration by APOE-ε4 allele carrier group, faceted by diagnosis groups. **D** Boxplot of lactate by APOE-ε4 allele carrier group, faceted by diagnosis groups.

with circulating ccf-mtDNA, patients with MCI+rMDD exhibited a nominal association. Plasma lactate levels varied among diagnostic groups, suggesting possible implications in disease progression, however towards lower levels mainly in MCI. ApoE-ε4 carriers displayed higher ccf-mtDNA levels. However, no significant associations were observed between nuclear-encoded mitochondrial genes and MCI or rMDD. While we found no statistically significant differences in the mitochondrial markers among MCI patients with or without rMDD, these findings contribute to a deeper understanding of the metabolic changes in MCI and rMDD, which may be potentially associated with peripheral metabolic shift in MCI and the underlying pathophysiology. Further research is needed to explore the precise roles of mitochondrial abnormalities in the development and progression of these neurodegenerative conditions.

DATA AVAILABILITY

The data generated and analyzed in the current study is available from the corresponding author and the PACt-MD Study Group upon request.

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ACKNOWLEDGEMENTS

This work was made possible by a grant from the Canada Brain Research Fund of Brain Canada, with the financial support of Health Canada, the Chagnon Family, and the CAMH Discovery Fund. The PACt-MD Study Group comprised: Benoit H. Mulsant, MD, MS (Principal Investigator); Tarek K. Rajji, MD (Co-PI; site PI, Centre for Addiction and Mental Health, Lead, neurostimulation and neurophysiology); Nathan Herrmann, MD (Co-PI; site PI, Sunnybrook Health Sciences Centre), Bruce G. Pollock, MD, PhD (Co-PI); Daniel Blumberger, MD, MSc (Co-Investigator); Christopher Bowie, PhD, C.Psych (Co-Investigator; lead, cognitive remediation and neuropsychology); Meryl Butters, PhD (Consultant, neuropsychology); Corinne Fischer, MD (Co-Investigator; site PI, St. Michael's Hospital); Alastair Flint, MD (Co-Investigator; site PI, University Health Network); Angela Golas, MD (lead, CSF); Ariel Graff, MD (Lead, neurochemistry); James L. Kennedy, MD (Lead, genetics); Sanjeev Kumar, MD (Co-Investigator); Krista Lanctot, PhD, (site PI, Sunnybrook Health Sciences Centre), Lillian Lourenco, MPH (study co-manager), Linda Mah, MD, MHS (Co-Investigator; site PI, Baycrest Health Sciences); Shima Ovaysikia, MA (study co-manager); Mark Rapoport, MD (Co-Investigator); Kevin Thorpe, MSc (Biostatistician); Nicolaas P.L.G. Verhoeff, MD, PhD (Co-Investigator); Aristotle Voineskos, MD, PhD (Lead, neuroimaging). This study was funded by Canadian Institute for Health Research to ACA, and Mitacs to JC. We also acknowledge the contribution of Kathleen Bingham, MD; Lina Chiuccariello, PhD; Tiffany Chow, MD; Pallavi Dham, MD; Breno Diniz, MD, PhD; Dielle Miranda, Carmela Tartaglia, MD; and the PACt-MD Research Staff.

AUTHOR CONTRIBUTIONS

JC contributed to conceptualization, design, data curation, investigation, analysis and interpretation of the data, and original draft of the study. TC and ELB contributed in data curation, investigation, validation, analysis of data, review and editing. HJB contributed to the data curation, investigation, analysis of data, review and editing. JLK, CEF, AJF, NH, KLL, LM, BHM, and BGP contributed to the study design, analysis and interpretation of data, manuscript review and editing. TKR and ACA supervised the study, and performed conceptualization, design, analysis and interpretation of the data, review and edit of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS DECLARATION

All participants provided written informed consent as approved by the University of local Research Ethics Boards (University of Toronto REB# 42061, Centre for Addiction and Mental Health protocol# 1333) and Clinical Trials Ontario. All research activities and methods were performed in accordance with the guidelines and regulations.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41398-024-03155-9.

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