BMC Neurology

RESEARCH

Open Access

Investigating the genetic association of mitochondrial DNA copy number with neurodegenerative diseases

Huan Xia[1,5†], Zi-Hao Wang[1†], Xiao-Bei Wang[1†], Mei-Rong Gao[2], Sen Jiang[3], Xin-Yu Du[4] and Xin-Ling Yang[5*]

Abstract

Objective This study aims to investigate the causal relationship between Mitochondrial DNA (mtDNA) copy number and several common neurodegenerative diseases (NDs).

Methods We conducted a bidirectional two-sample Mendelian randomization (MR) analysis using data from genome-wide association studies (GWAS) as instrumental variables (IVs). After screening for relevance and potential confounders, we estimated the association between mtDNA copy number and NDs, including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), and Multiple sclerosis (MS). Additionally, we validated our findings using GWAS data on mtDNA copy number from Longchamps et al., sourced from the Genetics Epidemiology Consortium and the UK Biobank (UKB) aging study cohort.

Results A GWAS analysis of 395,718 UKB participants found no significant association between mtDNA copy number and the risk of NDs, including AD (OR = 0.956, $P = 0.708$), PD (OR = 1.223, $P = 0.179$), ALS (OR = 0.972, $P = 0.374$), and MS (OR = 0.932, $P = 0.789$). Similarly, reverse MR analysis revealed no significant relationship between genetic predictions of NDs and mtDNA copy number: AD (OR = 0.987, $P = 0.062$), PD (OR = 0.997, $P = 0.514$), ALS (OR = 0.974, $P = 0.706$), and MS (OR = 1.003, $P = 0.181$).

Conclusion Although mitochondrial dysfunction is implicated in the pathogenesis of NDs, no clear evidence supports a causal role for mtDNA copy number. The relationship between mtDNA copy number and NDs is likely mediated by more complex molecular regulatory mechanisms. Further research is required to elucidate these intricate interactions.

Keywords Neurodegenerative diseases, Mitochondrial DNA copy number, Mendelian randomization analysis, Genome-wide association studies

†Huan Xia, Zihao Wang, and Xiaobei Wang have contributed equally to this work and share first authorship.

*Correspondence:
Xin-Ling Yang
yangxinling2014@163.com
[1]Department of Neurology, The Second Affiliated Hospital of Xinjiang Medical University, Xinjiang, China
[2]Department of Dermatology, The Second Hospital of Jilin University, Changchun, China
[3]Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[4]Department of Neurology, Jiangsu Provincial People's Hospital, Jiangsu, China
[5]Xinjiang Medical University, Xinjiang, China

Introduction

Neurodegenerative diseases (NDs) impact the central nervous system, characterized by progressive degradation of neuronal function and structure, typically resulting in cognitive and motor impairment [1]. Neuroinflammation, mediated by microglia and astrocytes, is a prominent feature common to NDs, including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), and Multiple sclerosis (MS) [2–4]. Although the primary therapeutic strategy for NDs targets harmful protein aggregates, the causal link between these aggregates and neuronal loss or synaptic damage remains under debate. With the increase in human life expectancy, the prevalence of NDs is on the rise [5, 6].

Mitochondria, essential subcellular organelles, produce cellular energy via oxidative phosphorylation in the respiratory chain [7]. This process occurs through the electron transport chain and oxidative phosphorylation [8]. Additionally, mitochondria produce molecules that protect against oxidative stress, aid in programmed cell death, and support cellular respiration [9]. Mitochondrial enzymes, rich in various oxidoreductases, and mitochondrial dysfunction are thought to be contributors to reactive oxygen species (ROS) production within the cellular environment [10]. Human mitochondrial DNA (mtDNA) is made up of 16,569 base pairs that encode 37 genes; this includes 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs), and 13 peptides involved in the electron transport chain [11]. The 13 peptides are essential for assembling respiratory chain complexes I to V. Markesbery's hypothesis centers on the 13 proteins needed for the electron transport chain, encoded by mtDNA [12]. The mtDNA is especially vulnerable to oxidative stress due to its close proximity to oxidative metabolism sites and lack of protective histones and repair mechanisms [13]. Damaged neurons induce mitochondrial dysfunction, leading to mtDNA damage and potentially triggering NDs onset and progression [14]. Recent studies in human fibroblasts indicate that point mutations in mtDNA's non-coding regulatory regions may significantly influence human aging [15].

Mounting evidence suggests an association between NDs and mitochondrial dysfunction caused by mtDNA mutations or proteomic abnormalities [16]. The mitochondrial cascade hypothesis proposes that, with age, modifications and mutations in mtDNA accumulate in somatic brain cells, influencing disease phenotypes. Certain mutations, such as those associated with Leber's hereditary optic neuropathy, can induce neuronal damage by promoting ROS production [17]. Many studies report lower mtDNA levels in the blood and CSF of PD patients, though results vary [18]. Studies have reported reduced CSF mtDNA concentrations in PD patients compared to healthy controls, whereas others have found no notable differences. For specific groups, such as *LRRK2* mutation carriers, mtDNA levels in the CSF are higher [19]. A prospective cohort study conducted using the UK Biobank (UKB) that involved whole-exome sequencing (WES) of approximately 50,000 individuals and genotyping of around 500,000 individuals found that mtDNA copy number is significantly associated with the prevalence and incidence of dementia and PD, and a higher mtDNA copy number is associated with a lower risk of developing NDs [20]. In AD research, a cohort study involving 1,201 individuals observed a reduction in mtDNA copy number in AD patients [21]. For PD, a two-year longitudinal study tracking 27 newly diagnosed, untreated PD patients and 22 healthy controls noted an increase in mtDNA copy number in PD patients [22]. Conversely, a South African case-control study found no significant link between mtDNA copy number and PD [23]. Additionally, an analysis of 363 peripheral blood samples, 151 substantia nigra pars compacta (SNpc) tissue samples, and 120 frontal cortex tissue samples from community-based PD cases (diagnosed according to UK PD Society Brain Bank criteria) and 262 peripheral blood samples, 33 SNpc tissue samples, and 37 frontal cortex tissue samples from controls without clinical evidence of PD found a reduction in mtDNA copy number in PD patients [24]. Research on MS also revealed a similar trend. A case-control study showed a decrease in mtDNA copy number in MS patients [25]. Furthermore, familial studies suggested that mtDNA copy numbers are elevated in patients with ALS [26]. Currently, the causal relationship between mtDNA copy number and NDs remains unconfirmed. Additionally, the question of whether NDs influence mtDNA copy number in reverse causality remains unresolved.

Mendelian randomization (MR) is a novel method in genetic epidemiology research design. It utilizes genetic variants closely linked to specific exposure factors, known as single nucleotide polymorphisms (SNPs), as instrumental variables (IVs). This approach minimizes confounding variables and bypasses reverse causation, enhancing the precision of causal inference between exposures and outcomes in the study [27]. Previous studies have examined the causal relationships between mtDNA copy number and diseases such as diabetes [28], stroke [29], and cardiac metabolic disorders [30] through MR designs. However, the causal links between mtDNA copy number and NDs (such as AD, PD, ALS, and MS) have not yet been investigated using MR. NDs may not only be influenced by mtDNA copy number, but may also affect it. To examine this bidirectional causal relationship, we used bidirectional MR analysis. This approach enables the simultaneous evaluation of the causal effects between mtDNA copy number and NDs.

Materials and methods

Study design

This study utilized the latest genome-wide association studies (GWAS) summary statistics for a bidirectional two-sample MR analysis to investigate the association between mtDNA copy number and NDs. Furthermore, the potential for NDs to induce changes in mtDNA copy number was examined. MR analysis employs SNPs as IVs, allowing us to test the causal relationship between risk factors and outcomes, overcoming reverse causation bias and confounding. To ensure the validity of the MR analysis, we followed three key assumptions: (1) the chosen SNPs have an association with mtDNA copy number; (2) the association between SNPs and NDs is not influenced by confounding factors; (3) SNPs only indirectly influence NDs outcomes by affecting mtDNA copy number.

The analysis encompassed four neurodegenerative disorders, specifically AD, PD, ALS, and MS. Figure 1

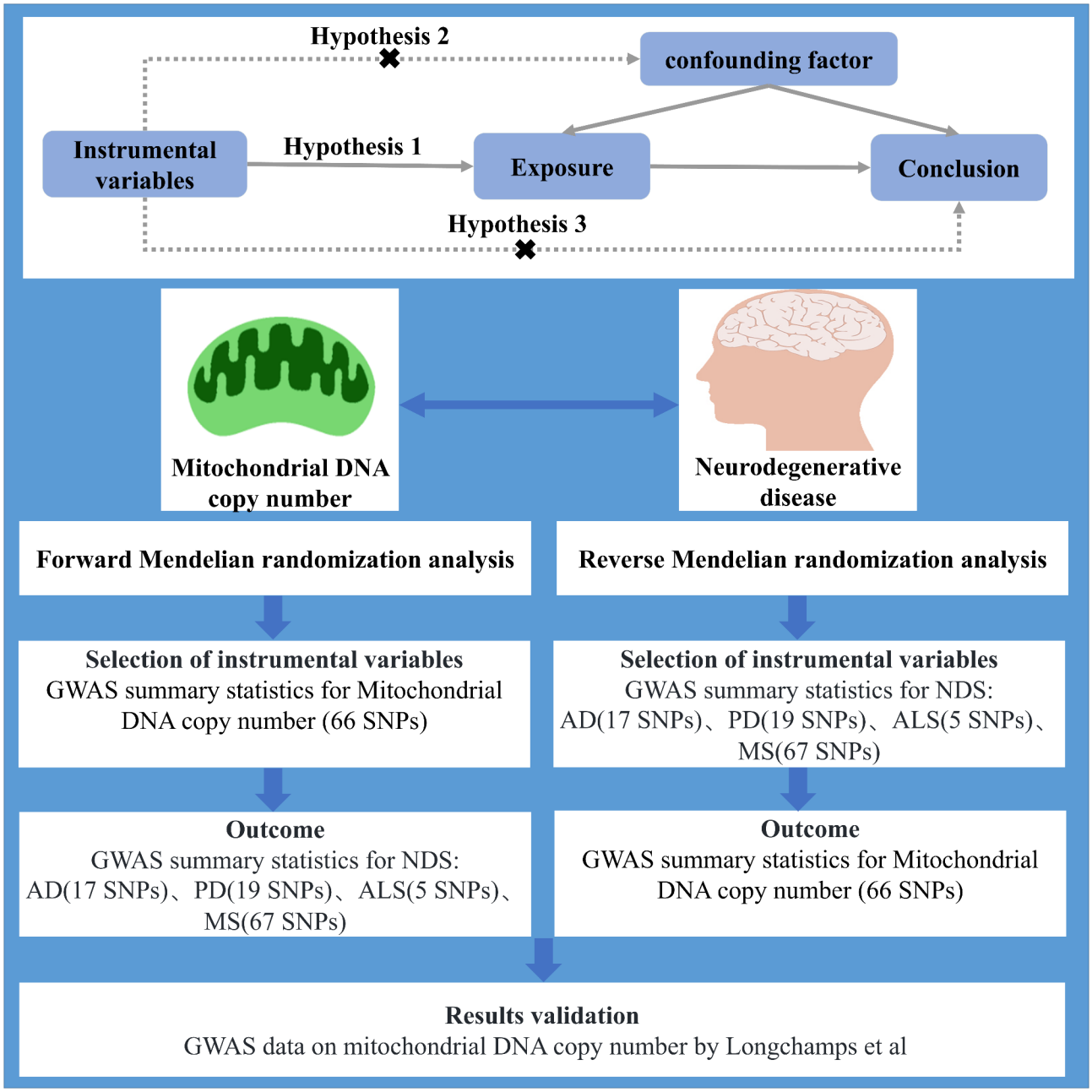

Fig. 1 This study depicts the bidirectional MR framework utilized to examine the causal link between mtDNA copy number and NDs. Eight MR analyses were conducted to explore the reciprocal relationships between mtDNA copy number and several NDs. All employed genetic instruments were SNPs. AD, Alzheimer's disease; PD, Parkinson's disease; ALS, Amyotrophic lateral sclerosis; and MS, Multiple sclerosis; GWAS, genome-wide association studies; SNPs, single nucleotide polymorphisms

depicts the bidirectional MR workflow. All the projects in the GWAS and related consortia upon which this study is based have been approved by the respective ethical committees and participants involved have provided signed informed consent.

Data source: MtDNA copy number

The GWAS summary statistics for mtDNA copy number included data from 395,718 UKB participants with varied ancestries [31]. Table 1 presents the relevant information from the GWAS summary statistics. This study employed "AutoMitoC," a novel method by Chong et al. for automated mitochondrial replication. This method provides an effective means for estimating mtDNA copy number through SNP array intensity. AutoMitoC encompasses preprocessing, background correction, probe hybridization detection, and mtDNA copy number estimation, facilitating swift and precise measurement of mtDNA levels in blood samples. The GWAS data were adjusted for age, sex, chip type, and blood cell counts.

Additionally, the study incorporated validation research by Longchamps et al. on data from 465,809 Caucasian subjects in a genomic epidemiology consortium and the UKB's aging cohort [32]. The UKB is a large longitudinal research project designed to explore the effects of genetics and environmental factors on human health. The project has recruited approximately 500,000 participants across the UK, collecting detailed information on their health, lifestyles, and genetics, providing researchers worldwide with a data resource for health studies.

Data source: NDs

We analyzed four large-scale GWAS datasets recently published to assess NDs. Genetic data for AD originated from the study conducted by Brian et al., providing a GWAS dataset of 63,926 Europeans, which includes 21,982 AD patients and 41,944 controls [33]. Data for PD were derived from a recently completed GWAS conducted by the International Parkinson Disease Genomics Consortium (IPDGC) [34]. This study integrated data from three earlier GWASs and 13 new datasets, including proxy cases from the UKB, totaling 33,674 cases and 449,056 controls. The analysis of ALS was based on GWAS data from Project MinE, covering 36,052 Europeans, with 12,577 patients and 23,475 controls [35]. The genetic data for MS were sourced from the International MS Genetics Consortium, featuring a dataset of 115,803 Europeans, including 47,429 patients and 68,374 controls [36]. Refer to Table 1 for detailed information.

Screening IVs

This study used the TwoSampleMR and MR-PRESSO packages to identify suitable IVs. We implemented rigorous criteria for screening. Specifically, we focused on genetic loci with *P*-values less than 5.0×10^{-8} to pinpoint SNPs with strong associations to disease risk. Furthermore, we required a linkage disequilibrium coefficient greater than 0.001 and a genetic distance below 10,000 kb for SNPs, to maintain independence among selected IVs and reduce biases from weak instruments. An F-statistic above 10 signifies strong instruments, whereas one below 10 suggests weak SNP-exposure associations. All analyses automatically excluded palindromic SNPs. The formula for the F-statistic is $F = R^2 / (1 - R^2) \times (N - K - 1) / K$, where N is the sample size of the exposure GWAS, K is the number of SNPs, and R^2 is the proportion of variance explained by SNPs in the exposure database [37].

Statistical analysis

This study primarily utilizes the inverse variance weighted (IVW) method, supplemented by the weighted median, simple mode, MR-Egger, and weighted mode approaches to bolster result credibility. The IVW method assesses the influence on outcomes by weighting SNPs according to their variance magnitudes. Sensitivity analyses included Cochran's Q test, leave-one-out analysis, MR-PRESSO, and the MR-Egger intercept to ensure robustness of the findings. Specifically, Cochran's Q evaluates IV heterogeneity; MR-PRESSO identifies and rectifies outlier-induced biases in IVs; the MR-Egger intercept examines pleiotropy, and the leave-one-out method concentrates on the stability and impact of each IV on the cumulative effect. We conducted a MR study to ascertain the mtDNA copy number's potential causal impact on NDs such as AD, PD, ALS, and MS. We employed genetic variants linked to mtDNA expression as IVs. GWASs of four NDs provided outcome data for MR analyses, with validation using mtDNA copy number GWAS data from 465,809 Caucasian UKB participants. Considering multiple association analyses ($n = 8$ tests) of mtDNA copy

Table 1 Information on the GWAS study data utilized in this study

Phenotype	SNPs	Cases	Controls	Sample size	Population	PMID
mtDNA copy number	237,961	NA	NA	395,718	UKB	35,023,831
mtDNA copy number	475,476	NA	NA	465,809	UKB	34,859,289
AD	10,528,610	21,982	41,944	63,926	European	30,820,047
PD	17,891,936	33,674	449,056	482,730	European	31,701,892
ALS	7,740,345	12,577	23,475	36,052	European	27,455,348
MS	6,304,359	47,429	68,374	115,803	European	31,604,244

number and NDs, we applied Bonferroni correction to adjust *P*-value thresholds, establishing a significance cutoff at 0.00625 (0.05/8). A *P*-value ≤ 0.05, falling short of the Bonferroni-adjusted significance level, indicated a potential causal link.

Results

The relationship between genetically predicted MtDNA copy number and NDs

The GWAS meta-analysis of mtDNA copy number identified 237,961 SNPs associated with mtDNA copy number, with 6,702 of them reaching genome-wide significance ($P < 5 \times 10^{-8}$). The dataset was refined by excluding 6,634 SNPs because of linkage disequilibrium or considerable imbalance ($r^2 < 0.001$), leaving 68 SNPs for in-depth analysis (Supplementary Table 1). The F-statistic for the genetic instrument associated with mtDNA copy number was 94.29 (ranging from 30.45 to 473.58). The IVW method showed no significant association between genetically predicted mtDNA copy number and AD (OR = 0.956; 95% CI 0.756–1.209; *P* = 0.708), PD (OR = 1.223; 95% CI 0.912–1.642; *P* = 0.179), ALS (OR = 0.972; 95% CI 0.912–1.035; *P* = 0.374), or MS (OR = 0.932; 95% CI 0.557–1.035; *P* = 0.789), as depicted in Fig. 2. The analysis suggested that mtDNA copy number does not statistically affect the risk of developing NDs. Supplementary Table 2 encompasses findings from four distinct analytical methods: weighted median, mode-based estimation, MR-Egger, and weighted mode. Aside from the causal relationship between mtDNA copy number and MS approaching statistical significance in heterogeneity analyses (*P* < 0.001) and MR-PRESSO tests

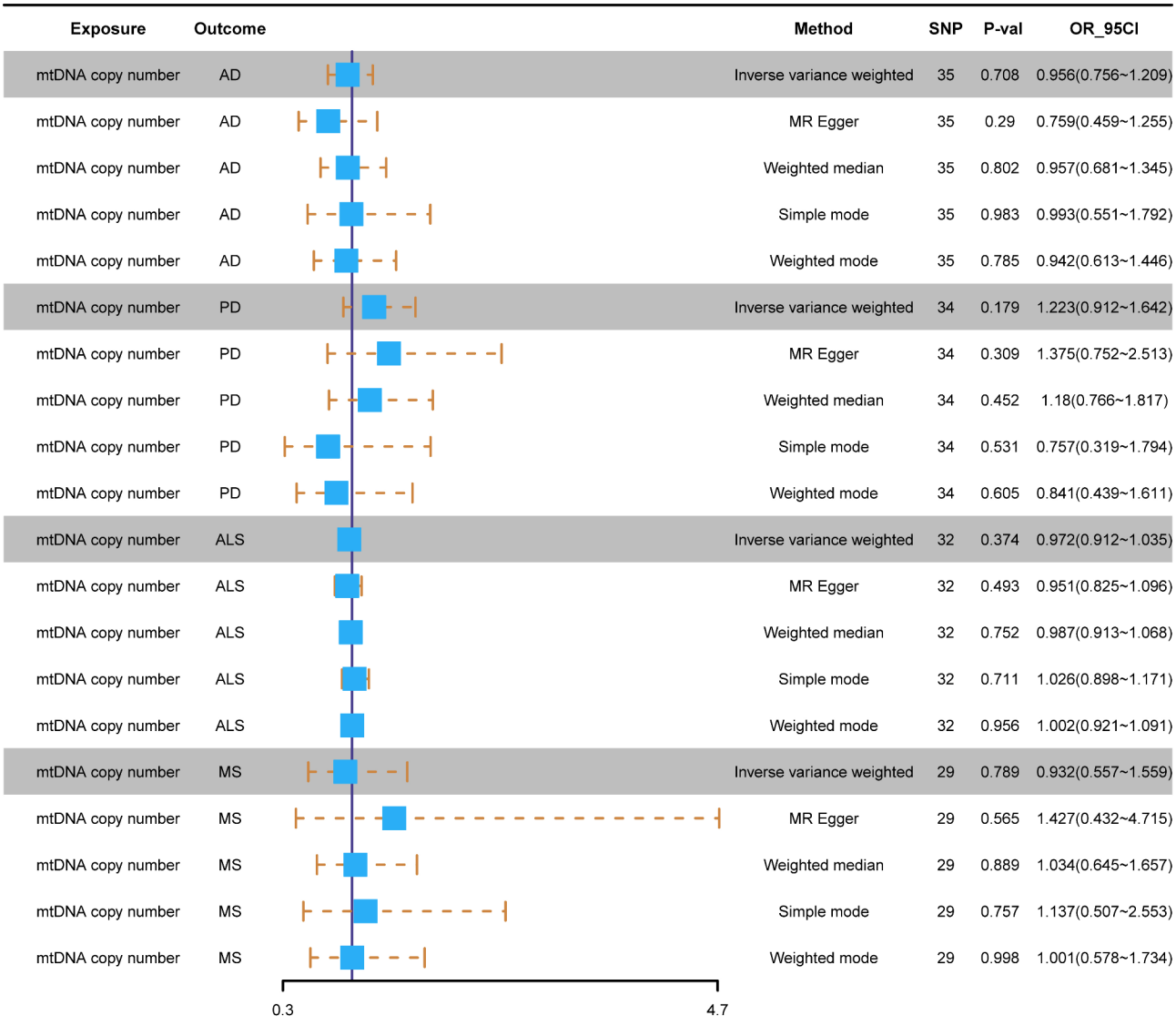

Fig. 2 MR analysis of the relationship between mtDNA copy number and the risk of AD, PD, ALS, and MS. CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism

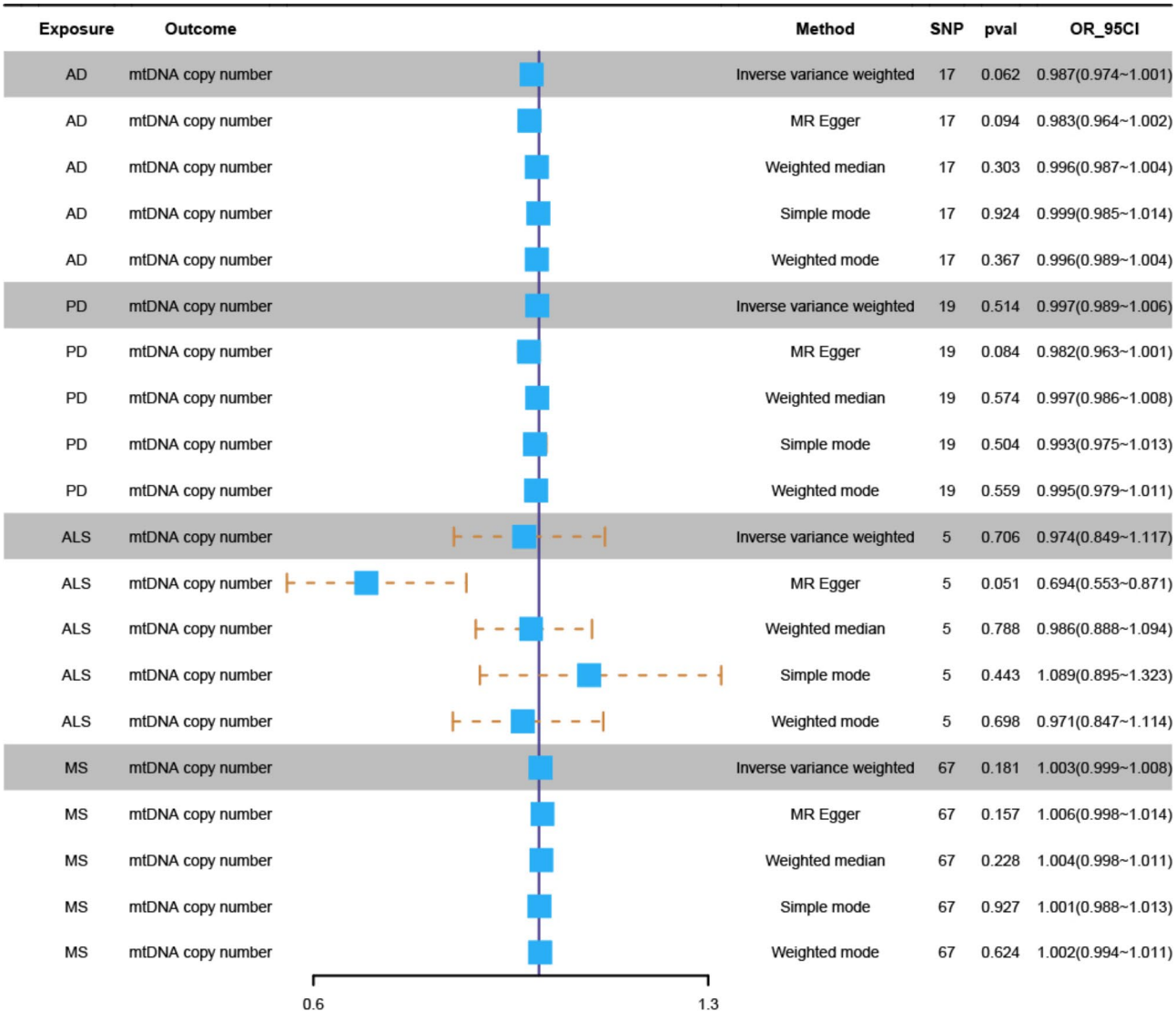

Fig. 3 Reverse MR analysis of the relationship between mtDNA copy number and the risks of AD, PD, ALS, and MS

($P < 0.001$), there was no evidence of heterogeneity or horizontal pleiotropy in other outcomes (Supplementary Table 3). Leave-one-out analyses for each SNP-NDs association are presented in Supplementary Fig. 1.

The relationship between gene-predicted NDs and MtDNA copy number

Our bidirectional MR analysis investigated the link between neurodegenerative disorders and mtDNA copy number, incorporating 17 SNPs associated with AD, 19 with PD, 5 with ALS, and 67 with MS. Genetic variants served as IVs in this analysis (Supplementary Table 4). The IVW method results showed genetic predictions for AD (OR = 0.987; 95% CI 0.974–1.001; $P = 0.062$), PD (OR = 0.997; 95% CI 0.989–1.006; $P = 0.514$), ALS (OR = 0.974; 95% CI 0.849–1.117; $P = 0.706$), and MS (OR = 1.003; 95% CI 0.999–1.008; $P = 0.181$) relative to mtDNA copy number, as seen in Fig. 3. The results of the four methods, including the weighted median, simple mode, MR-Egger, and weighted mode (Supplementary Table 5). We found no strong evidence of a significant correlation between mtDNA copy number and AD, PD, ALS, or MS. Statistical analyses identified significant pleiotropy and heterogeneity in the associations between mtDNA copy number and AD and MS ($P < 0.001$), but no such effects were observed for PD or ALS (Supplementary Table 6). Supplementary Fig. 2 illustrates the leave-one-out sensitivity analysis for the association between each SNP and mtDNA copy number.

Bidirectional MR of validation data

We incorporated 70 SNPs related to mtDNA copy number in our validation dataset (Supplementary Table 7). The forward MR IVW method indicated that genetically

predicted mtDNA copy number is not associated with AD (OR = 0.964; $P = 0.757$), PD (OR = 1.220; $P = 0.187$), ALS (OR = 0.957; $P = 0.187$), MS (OR = 0.837; $P = 0.488$) (Supplementary Table 8). The reverse MR IVW method results showed that the genetic prediction of AD (OR = 0.988; $P = 0.079$), PD (OR = 0.998; $P = 0.579$), ALS (OR = 0.973; $P = 0.701$), MS (OR = 1.003; $P = 0.182$) is not related to mtDNA copy number (Supplementary Table 9). IVW analysis of the validation dataset failed to provide robust evidence for a correlation between mtDNA copy number and NDs including AD, PD, ALS, and MS. The MR-Egger intercept analysis revealed no indication of horizontal pleiotropy influencing the outcomes. The relationship between MS and mtDNA copy number exhibits significant heterogeneity (Cochran's Q = 70.84, $P < 0.001$), as well as pleiotropy according to MR-PRESSO analysis ($P < 0.001$). There is heterogeneity (Cochran's Q = 42.14, $P = 0.07$) and pleiotropy (MR-PRESSO, $P = 0.12$) in the association between ALS and mtDNA copy number. The mtDNA copy number exhibits significant statistical heterogeneity (Cochran's Q, $P < 0.001$) and pleiotropy (MR-PRESSO, $P < 0.001$) when evaluated as a potential exposure factor linking AD and MS (Supplementary Table 10). The results of the leave-one-out analysis are presented in Supplementary Figs. 3 and 4.

Discussion

Mitochondrial diseases arise from mutations in nuclear genes, which reduce mtDNA expression, or from primary mtDNA mutations that impair the function or abundance of mtDNA copy number [38]. To accommodate metabolic demands, the regulation of mtDNA content within cells and tissues is necessary. Regulation of mtDNA levels is a complex process that involves a balance between replication and degradation [39]. Observational studies have investigated the relationship between mtDNA and diseases such as AD [40], PD [23], ALS [26], and MS [25]. Among these studies, few have focused on the relationship between mtDNA copy number and AD, PD, ALS, and MS, and there have been contentious findings regarding diseases [20]. Different observational studies have presented conflicting results regarding the association between mtDNA copy number and PD. Some studies have revealed a decreased correlation between mtDNA copy number and PD [24], yet others have identified an increased correlation [22]. In addition, some studies have not observed a significant correlation between PD and mtDNA copy number [23]. An analysis of the blood DNA from 114 individuals—including ALS patients, asymptomatic genetic carriers, and family members without the gene—revealed a significant increase in mtDNA copy number in the ALS cohort [26]. Another neuropathological autopsy excluded the possibility of reduced mitochondrial copy numbers in ALS, determining that the observed decrease in mitochondrial gene expression and related metabolic deficiencies were not caused by diminished mtDNA [41], aligning with the outcomes of this study. Although mitochondrial DNA copy number may be associated with the risk of NDs, insufficient direct evidence exists to support it as a mechanistic factor. The regulation of mtDNA copy number is likely to involve a range of complex molecular mechanisms, such as mitochondrial dysfunction, alterations in energy metabolism, modulation of oxidative stress responses, and activation of apoptotic pathways. These mechanisms, including mitochondrial dysfunction and oxidative stress modulation, may serve as targets for future therapeutic interventions.

This study is the first to use bidirectional two-sample MR to comprehensively explore the link between mtDNA copy number and NDs. Despite the large sample size, we found no causal link between genetically predicted mtDNA copy number and NDs. Furthermore, existing research indicates that oxidative stress and immune dysregulation associated with NDs could intensify fluctuations in mtDNA copy number [42–44]. Our study did not reveal a significant correlation between genetically predicted NDs and mtDNA copy number levels.We believe the underlying reasons for these findings are likely complex. To explain these findings, we propose the following hypotheses: The progression of NDs may be indirectly associated with mtDNA copy number, influenced by various biological processes and molecular regulatory mechanisms. This includes mitochondrial dysfunction, alterations in energy metabolism, regulation of oxidative stress responses, and activation of apoptotic pathways [45–47]. Additionally, the interplay between genetic predispositions [48] and environmental factors [49] may significantly influence mtDNA replication, repair, and maintenance, subsequently altering the risk associated with NDs.

Our study has several strengths. Initially, we utilized a two-sample MR analysis, leveraging SNPs as IVs to investigate the putative causal relationship between mtDNA copy number and NDs. Subsequently, we performed MR analysis with extensive GWAS datasets, further validated by independent datasets, thereby reinforcing the assessment validity and the causality's convincingness between mtDNA copy number and NDs. Moreover, to thoroughly explore the causal nexus between mtDNA copy number and various NDs, we conducted eight MR analyses to uncover bidirectional correlations. In addition to verifying the association between mtDNA copy number and NDs risk, this study represents the first systematic application of MR analysis to evaluate NDs' conceivable impact on mtDNA copy number.

However, this study is subject to certain limitations. Firstly, given the maternal inheritance of the

mitochondrial genome, as well as studies suggesting that sex, hormone levels, and other biological factors may contribute to differences in mitochondrial DNA copy number between females and males [50], sex differences are possible. Although the MR approach reduces confounding bias, the lack of individual-level data limits our ability to perform direct age- or sex-stratified analyses. Secondly, this study found that directional pleiotropy had a minor impact on the research outcomes through MR-Egger regression analysis. Nevertheless, we cannot overlook the potential bias introduced by pleiotropy or IVs events. Thirdly, the abundance of mtDNA copy number in this study was determined by the intensity of mtDNA genotyping probes, whereas assessments based on WES are generally considered more reliable estimates of mtDNA copy number. Since mtDNA copy number is measured in blood samples instead of specific neural tissues, direct inferences regarding mitochondrial changes in affected neurons across various NDs are not feasible. Currently, the relationship between blood mtDNA copy number and tissue-specific mitochondrial changes in these affected neurons remains unclear. Future research should adopt cell-type-specific strategies, such as single-cell sequencing and tissue-specific profiling, to further investigate the role of mitochondrial dysfunction in different neuronal populations within various NDs. Furthermore, large-scale analyses of diverse population samples, focusing on GWAS and comprehensive omics data, will be crucial for investigating the impact of mitochondrial genome variations on NDs.

Conclusion

Although mitochondrial dysfunction is implicated in the pathogenesis of NDs, no clear evidence supports a causal role for mtDNA copy number. The relationship between mtDNA copy number and NDs is likely mediated by more complex molecular regulatory mechanisms. Further research is required to elucidate these intricate interactions.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-025-04176-7.

Supplementary Material 1

Acknowledgements
The authors thank all individuals and researchers who contributed to and shared data with the GWAS study, IEU OpenGWAS project, NHGRI-EBI Catalog, and additional GWAS datasets.

Author contributions
HX: Writing - review & editing, Data curation, Formal analysis. ZW: Writing - original draft, Formal analysis, Writing - review & editing. XW: Funding acquisition, Formal analysis. MG: Data curation, Resources. SJ: Supervision. XD: Investigation, Writing - review & editing. XY: Resources, Software. LY: Conceptualization, Funding acquisition.

Funding
This research was supported by the Autonomous Region Key Research and Development Project (2023B03003), the Tian-Shan Talent Program (2022TSYCLJ0066), the National Natural Science Foundation of China (82371258), the Natural Science Foundation of Xinjiang Uygur Autonomous Region (2023D01C131), and the Central Guiding Local Science and Technology Development Special Fund Project (ZYYD2022C17).

Data availability
All data are available from public databases (https://gwas.mrcieu.ac.uk, https://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics, https://www.ukbiobank.ac.uk).

Declarations

Ethics approval
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 13 November 2024 / Accepted: 3 April 2025
Published online: 16 April 2025

References

1. Nimmo J, Byrne RAJ, Daskoulidou N, Watkins LM, Carpanini SM, Zelek WM, et al. The complement system in neurodegenerative diseases. Clin Sci (Lond). 2024;138:387–412.
2. Iemmolo M, Bivona G, Piccoli T, Nicosia A, Schiera G, Di Liegro CM, et al. Effects of Cerebrospinal Fluids from Alzheimer and Non-Alzheimer Patients on Neurons-Astrocytes-Microglia Co-Culture. Int J Mol Sci. 2024;25:2510.
3. Liu Y, Kwok W, Yoon H, Ryu JC, Stevens P, Hawkinson TR et al. Imbalance in glucose metabolism regulates the transition of microglia from homeostasis to Disease Associated Microglia stage 1. J Neurosci. 2024;:e1563232024.
4. Oshima T, Kater MSJ, Huffels CFM, Wesseling EM, Middeldorp J, Hol EM, et al. Early amyloid-induced changes in microglia gene expression in male APP/PS1 mice. J Neurosci Res. 2024;102:e25295.
5. Sun L-H, Lange CM, Hansen M, Kumsta C. Neuronal waste management: new roles for autophagy genes in the extrusion of protein aggregates and in longevity. Autophagy. 2024;:1–3.
6. Zhang Q, Lin Z, He Y, Jiang J, Hu D. Mendelian Randomization Analysis Reveals No Causal Relationship Between Plasma α-Synuclein and Parkinson's Disease. Mol Neurobiol. 2023;60:2268–76.
7. Casimir P, Iwata R, Vanderhaeghen P. Linking mitochondria metabolism, developmental timing, and human brain evolution. Curr Opin Genet Dev. 2024;86:102182.
8. Ukolova IV, Borovskii GB. OXPHOS Organization and Activity in Mitochondria of Plants with Different Life Strategies. Int J Mol Sci. 2023;24:15229.
9. Clemente-Suárez VJ, Redondo-Flórez L, Beltrán-Velasco AI, Ramos-Campo DJ, Belinchón-deMiguel P, Martinez-Guardado I, et al. Mitochondria and Brain Disease: A Comprehensive Review of Pathological Mechanisms and Therapeutic Opportunities. Biomedicines. 2023;11:2488.
10. Ba M, St J, Cd W. Mitochondria-Targeted Catalase Does Not Suppress Development of Cellular Senescence during Aging. Biomedicines. 2024;12.
11. Saravanan S, Lewis CJ, Dixit B, O'Connor MS, Stolzing A, Boominathan A. The Mitochondrial Genome in Aging and Disease and the Future of Mitochondrial Therapeutics. Biomedicines. 2022;10:490.
12. Bradley-Whitman MA, Timmons MD, Beckett TL, Murphy MP, Lynn BC, Lovell MA. Nucleic acid oxidation: an early feature of Alzheimer's disease. J Neurochem. 2014;128:294–304.
13. Hahn A, Zuryn S. Mitochondrial Genome (mtDNA) Mutations that Generate Reactive Oxygen Species. Antioxid (Basel). 2019;8:392.
14. Garone C, De Giorgio F, Carli S. Mitochondrial metabolism in neural stem cells and implications for neurodevelopmental and neurodegenerative diseases. J Transl Med. 2024;22:238.

15. Jauhari A, Baranov SV, Suofu Y, Kim J, Singh T, Yablonska S, et al. Melatonin inhibits cytosolic mitochondrial DNA-induced neuroinflammatory signaling in accelerated aging and neurodegeneration. J Clin Invest. 2021;131:e150328.
16. Kulkarni PG, Mohire VM, Waghmare PP, Banerjee T. Interplay of mitochondria associated membrane proteins and autophagy: Implications in neurodegeneration. Mitochondrion. 2024;:101874.
17. Park J-S, Davis RL, Sue CM. Mitochondrial Dysfunction in Parkinson's Disease: New Mechanistic Insights and Therapeutic Perspectives. Curr Neurol Neurosci Rep. 2018;18:21.
18. Dabravolski SA, Nikiforov NG, Zhuravlev AD, Orekhov NA, Grechko AV, Orekhov AN. Role of the mtDNA Mutations and Mitophagy in Inflammaging. Int J Mol Sci. 2022;23:1323.
19. Shang D, Huang M, Wang B, Yan X, Wu Z, Zhang X. mtDNA Maintenance and Alterations in the Pathogenesis of Neurodegenerative Diseases. Curr Neuropharmacol. 2023;21:578–98.
20. Yang SY, Castellani CA, Longchamps RJ, Pillalamarri VK, O'Rourke B, Guallar E, et al. Blood-derived mitochondrial DNA copy number is associated with gene expression across multiple tissues and is predictive for incident neurodegenerative disease. Genome Res. 2021;31:349–58.
21. Liou C-W, Chen S-H, Lin T-K, Tsai M-H, Chang C-C. Oxidative Stress Biomarkers and Mitochondrial DNA Copy Number Associated with APOE4 Allele and Cholinesterase Inhibitor Therapy in Patients with Alzheimer's Disease. Antioxid (Basel). 2021;10:1971.
22. Ortega-Vázquez A, Sánchez-Badajos S, Ramírez-García MÁ, Alvarez-Luquín D, López-López M, Adalid-Peralta LV, et al. Longitudinal Changes in Mitochondrial DNA Copy Number and Telomere Length in Patients with Parkinson's Disease. Genes (Basel). 2023;14:1913.
23. Müller-Nedebock AC, Meldau S, Lombard C, Abrahams S, van der Westhuizen FH, Bardien S. Increased blood-derived mitochondrial DNA copy number in African ancestry individuals with Parkinson's disease. Parkinsonism Relat Disord. 2022;101:1–5.
24. Pyle A, Anugrha H, Kurzawa-Akanbi M, Yarnall A, Burn D, Hudson G. Reduced mitochondrial DNA copy number is a biomarker of Parkinson's disease. Neurobiol Aging. 2016;38:216.e7-216.e10.
25. Al-Kafaji G, Bakheit HF, Alharbi MA, Farahat AA, Jailani M, Ebrahin BH, et al. Mitochondrial DNA Copy Number in Peripheral Blood as a Potential Non-invasive Biomarker for Multiple Sclerosis. Neuromolecular Med. 2020;22:304–13.
26. Stoccoro A, Mosca L, Carnicelli V, Cavallari U, Lunetta C, Marocchi A, et al. Mitochondrial DNA copy number and D-loop region methylation in carriers of amyotrophic lateral sclerosis gene mutations. Epigenomics. 2018;10:1431–43.
27. Wang Z-Y, Ma D-R, Li M-J, Liang Y-Y, Hu Z-W, Li S-J, et al. Association between irritable bowel syndrome and Parkinson's disease by Cohort study and Mendelian randomization analysis. NPJ Parkinsons Dis. 2024;10:70.
28. Wang W, Luo J, van Willems K, Hägg S, Grassmann F, Hart T. Assessment of the bi-directional relationship between blood mitochondrial DNA copy number and type 2 diabetes mellitus: a multivariable-adjusted regression and Mendelian randomisation study. Diabetologia. 2022;65:1676–86.
29. Martens LG, Luo J, Wermer MJH, van Willems K, Hägg S, Grassmann F, et al. The association between mitochondrial DNA abundance and stroke: A combination of multivariable-adjusted survival and Mendelian randomization analyses. Atherosclerosis. 2022;354:1–7.
30. Qin P, Qin T, Liang L, Li X, Jiang B, Wang X, et al. The role of mitochondrial DNA copy number in cardiometabolic disease: a bidirectional two-sample mendelian randomization study. Cardiovasc Diabetol. 2024;23:45.
31. Chong M, Mohammadi-Shemirani P, Perrot N, Nelson W, Morton R, Narula S, et al. GWAS and ExWAS of blood mitochondrial DNA copy number identifies 71 loci and highlights a potential causal role in dementia. Elife. 2022;11:e70382.
32. Longchamps RJ, Yang SY, Castellani CA, Shi W, Lane J, Grove ML, et al. Genome-wide analysis of mitochondrial DNA copy number reveals loci implicated in nucleotide metabolism, platelet activation, and megakaryocyte proliferation. Hum Genet. 2022;141:127–46.
33. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. Nat Genet. 2019;51:414–30.
34. Pihlstrøm L, Wiethoff S, Houlden H. Genetics of neurodegenerative diseases: an overview. Handb Clin Neurol. 2017;145:309–23.
35. van Rheenen W, Shatunov A, Dekker AM, McLaughlin RL, Diekstra FP, Pulit SL, et al. Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. Nat Genet. 2016;48:1043–8.
36. International Multiple Sclerosis Genetics Consortium. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. Science. 2019;365:eaav7188.
37. Au Yeung SL, Luo S, Schooling CM. The Impact of Glycated Hemoglobin (HbA1c) on Cardiovascular Disease Risk: A Mendelian Randomization Study Using UK Biobank. Diabetes Care. 2018;41:1991–7.
38. Song M, Ye L, Yan Y, Li X, Han X, Hu S, et al. Mitochondrial diseases and mtDNA editing. Genes Dis. 2024;11:101057.
39. Vincent AE, Chen C, Gomes TB, Di Leo V, Laalo T, Pabis K et al. A stagewise response to mitochondrial dysfunction in mitochondrial DNA maintenance disorders. Biochim Biophys Acta Mol Basis Dis. 2024;:167131.
40. Kim KM, Meng Q, Perez de Acha O, Mustapic M, Cheng A, Eren E, et al. Mitochondrial RNA in Alzheimer's Disease Circulating Extracellular Vesicles. Front Cell Dev Biol. 2020;8:581882.
41. Mehta AR, Gregory JM, Dando O, Carter RN, Burr K, Nanda J, et al. Mitochondrial bioenergetic deficits in C9orf72 amyotrophic lateral sclerosis motor neurons cause dysfunctional axonal homeostasis. Acta Neuropathol. 2021;141:257–79.
42. Bogatyreva AI, Gerasimova EV, Kirichenko TV, Markina YV, Tolstik TV, Kiseleva DG, et al. Mitochondrial DNA copy number in patients with systemic sclerosis. Front Mol Biosci. 2023;10:1313426.
43. Winstanley YE, Liu J, Adhikari D, Gonzalez MB, Russell DL, Carroll J, et al. Dynamics of Mitochondrial DNA Copy Number and Membrane Potential in Mouse Pre-Implantation Embryos: Responses to Diverse Types of Oxidative Stress. Genes (Basel). 2024;15:367.
44. Zhang J, Shang J, Wang F, Huo X, Sun R, Ren Z, et al. Decreased mitochondrial D-loop region methylation mediates an increase in mitochondrial DNA copy number in CADASIL. Clin Epigenetics. 2022;14:2.
45. Currim F, Shukla S, Singh J, Gohel D, Mane M, Shinde A, et al. Neuronal exosomal miRNAs modulate mitochondrial functions and cell death in bystander neuronal cells under Parkinson's disease stress conditions. Neurotoxicology. 2024;101:102–16.
46. Liang H, Ma Z, Zhong W, Liu J, Sugimoto K, Chen H. Regulation of mitophagy and mitochondrial function: Natural compounds as potential therapeutic strategies for Parkinson's disease. Phytother Res. 2024. https://doi.org/10.1002/ptr.8156.
47. Pszczołowska M, Walczak K, Miśków W, Mroziak M, Chojdak-Łukasiewicz J, Leszek J. Mitochondrial disorders leading to Alzheimer's disease-perspectives of diagnosis and treatment. Geroscience. 2024. https://doi.org/10.1007/s11357-024-01118-y.
48. Özsoy M, Stummer N, Zimmermann FA, Feichtinger RG, Sperl W, Weghuber D, et al. Role of Energy Metabolism and Mitochondrial Function in Inflammatory Bowel Disease. Inflamm Bowel Dis. 2022;28:1443–50.
49. Murphy MP, O'Neill LAJ. A break in mitochondrial endosymbiosis as a basis for inflammatory diseases. Nature. 2024;626:271–9.
50. Gupta R, Kanai M, Durham TJ, Tsuo K, McCoy JG, Kotrys AV, et al. Nuclear genetic control of mtDNA copy number and heteroplasmy in humans. Nature. 2023;620:839–48.

Publisher's note