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RESEARCH

Mitochondrial DNA Copy Number as a Hidden Player in the Progression of Multiple Sclerosis: A Bidirectional Two-Sample Mendelian Randomization Study

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Abstract
The relationship between mitochondrial DNA copy number (mtDNA-CN) and multiple sclerosis (MS) progression remains unclear, as previous observational studies have reported conflicting results. This study aimed to clarify the association between mtDNA-CN and MS progression using a bidirectional two-sample Mendelian randomization (MR) approach. MR analyses were conducted using the latest summary statistics from genome-wide association studies (GWAS) on mtDNA-CN and MS progression. Single-nucleotide polymorphisms (SNPs) associated with mtDNA-CN were extracted from 383,476 participants of European ancestry in the UK Biobank, while SNPs associated with MS severity were obtained from the International Multiple Sclerosis Genetics Consortium (IMSGC), comprising 12,584 cases of European ancestry. The inverse variance weighted (IVW) method was used as the primary analysis. Potential heterogeneity and pleiotropy were evaluated, and sensitivity analyses were performed to ensure the robustness of the results. The forward MR analysis using the IVW method revealed no significant association between mtDNA-CN and MS progression ($P = 0.487$). However, reverse MR analysis identified a causal association between MS progression and mtDNA-CN ($\beta = -0.010$, 95% CI = -0.019 to -0.001, $P = 0.036$). No evidence of heterogeneity or horizontal pleiotropy was found in the analyses. Sensitivity analyses yielded consistent results. Our findings suggest that MS progression may causally influence mtDNA-CN, highlighting the crucial role of mitochondria in the pathophysiology of MS. However, further research is needed to confirm mtDNA-CN as a reliable biomarker and a deeper understanding of the molecular mechanisms is necessary to develop targeted therapeutic interventions.

Keywords Bidirectional · Genome-wide association studies · Mendelian randomization · Mitochondrial DNA copy number · Multiple sclerosis

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system (CNS). It is characterized by progressive neuronal loss, primarily impacting young adults [1]. The pathogenesis of MS involves the dysregulation of both adaptive and innate immune cells targeting CNS autoantigens, leading to chronic neuroinflammation and subsequent neuronal damage [2]. Most patients initially present with a relapsing–remitting form of MS (RRMS), characterized by alternating episodes of acute demyelination and recovery. Over one to two decades, the majority of RRMS patients transition to a more severe condition marked by continuous neuronal degeneration without acute relapses, known as secondary progressive MS (SPMS). A smaller subset of patients, approximately 10 to 15%, exhibit primary progressive MS (PPMS), which is characterized by a steady progression of symptoms from the onset [3].

Aging is associated with a chronic inflammatory state, a notable risk factor for the onset and progression of chronic diseases such as MS [4]. This prolonged inflammatory state during aging can induce mitochondrial dysfunction [5]. Mitochondria contain their own genetic material, known as mitochondrial DNA (mtDNA), with multiple copies in

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each cell. mtDNA plays crucial roles in cellular proliferation, maintaining mitochondrial membrane potential, regulating apoptosis, managing oxidative stress, storing energy, and producing adenosine triphosphate (ATP) via oxidative phosphorylation (OXPHOS) [6, 7]. Alterations in mitochondrial function and content have been identified as potential contributors to neurodegeneration. Specifically, fluctuations in mitochondrial DNA copy number (mtDNA-CN) within biological samples have been observed during both the early and advanced stages of neurodegenerative diseases such as MS [8]. These fluctuations in mtDNA levels may underlie progressive bioenergetic deficits and impairments in the OXPHOS pathway, ultimately leading to changes in the respiratory capacity of neurons and the development of neurodegeneration [8, 9].

Previous observational investigations have reported an association between mtDNA-CN and MS, but their findings have been inconsistent [10–14]. These studies primarily utilized cross-sectional or case–control designs, which are vulnerable to confounding variables and reverse causation [15]. Furthermore, the directionality of the relationship between mtDNA-CN and MS progression remains unclear, as it is uncertain whether mitochondrial dysfunction contributes to MS severity or if disease progression itself affects mtDNA-CN levels. To address these gaps, our study utilizes a bidirectional two-sample Mendelian randomization (MR) framework, leveraging large-scale genome-wide association studies (GWAS) data, to evaluate the causal relationship between these variables while minimizing the impact of confounding factors and reverse causation biases.

MR uses single-nucleotide polymorphisms (SNPs) identified through GWAS as instrumental variables. The SNP alleles are randomly allocated to individuals during gamete development prior to exposure to environmental confounders. Consequently, MR can effectively mitigate the impacts of confounding variables, enabling the investigation of causal relationships and their directionality [16].

In the present study, we aimed to investigate the bidirectional causal relationship between mtDNA-CN and MS progression using the MR method.

Methods

Study Design

We conducted bidirectional two-sample MR analyses to investigate mtDNA-CN and MS progression associations. For this purpose, we used the latest GWAS summary statistics to assess the effect of exposure on the resulting outcome. The overall study design is outlined in the flowchart shown in Fig. 1.

The MR analysis was performed based on three assumptions. First, the SNPs are associated with the exposure. Second, the SNPs are independent of confounders in the exposure-outcome relationship. Third, the outcome is affected by the SNPs only through the exposure [16] (Fig. 2).

Data Sources

Genetic associations with mtDNA-CN were analyzed using data from 383,476 participants of European ancestry from the UK Biobank [17]. In this dataset, a total of 11,453,766 SNPs were analyzed. mtDNA-CN estimates were derived using the AutoMitoC pipeline as described by Chong et al. [17], which provides the most comprehensive genetic evaluation available to date. Summary statistics for MS severity were obtained from the International Multiple Sclerosis Genetics Consortium (IMSGC). The IMSGC conducted GWAS of age-based MS severity scores in 12,584 European ancestry cases to identify genetic variants potentially affecting MS severity [18]. This dataset included approximately 7,776,916 SNPs. Neurological disability was assessed using the Expanded Disability Status Scale (EDSS), an ordinal numerical scale that increases with neurodegenerative progression [19]. Aging effects were controlled by converting individual EDSS scores to the age-related MS severity (ARMSS) score, with disability ranked within age-specific strata [20]. Table 1 presents the detailed information on the utilized GWAS.

Genetic Instrument Selection

Instrumental variables (IVs) were defined as SNPs with genome-wide significant associations with the exposure. For mtDNA-CN, a threshold of $P < 5 \times 10^{-8}$ was selected to extract substantial SNPs. For genetic variation related to MS progression, the threshold was set at $P < 5 \times 10^{-5}$ to ensure a sufficient number of IVs. Adopting a $P < 5 \times 10^{-5}$ threshold can increase the number of IVs and potentially the power of MR analysis, but it is crucial to implement strategies to address the accompanying risks of weak instrument bias and pleiotropy [21, 22]. To assess instrument strength and validity, we calculated the F-statistic ($F = \beta^2/\mathrm{se}^2$) to minimize weak instrument bias. IVs with an F-statistic less than 10 were considered weak and excluded from further analysis. Additionally, we employed different robust MR methods and performed sensitivity analyses to evaluate the robustness and consistency of our findings, as detailed in the following section.

Linkage disequilibrium (LD) was minimized by applying an $r^2 < 0.001$ threshold within a clumping distance of 10,000 kb window. We used ANNOVAR [23] to annotate the missing effect allele frequencies compared to the 1000 Genomes Project data [24]. IVs were extracted based on the

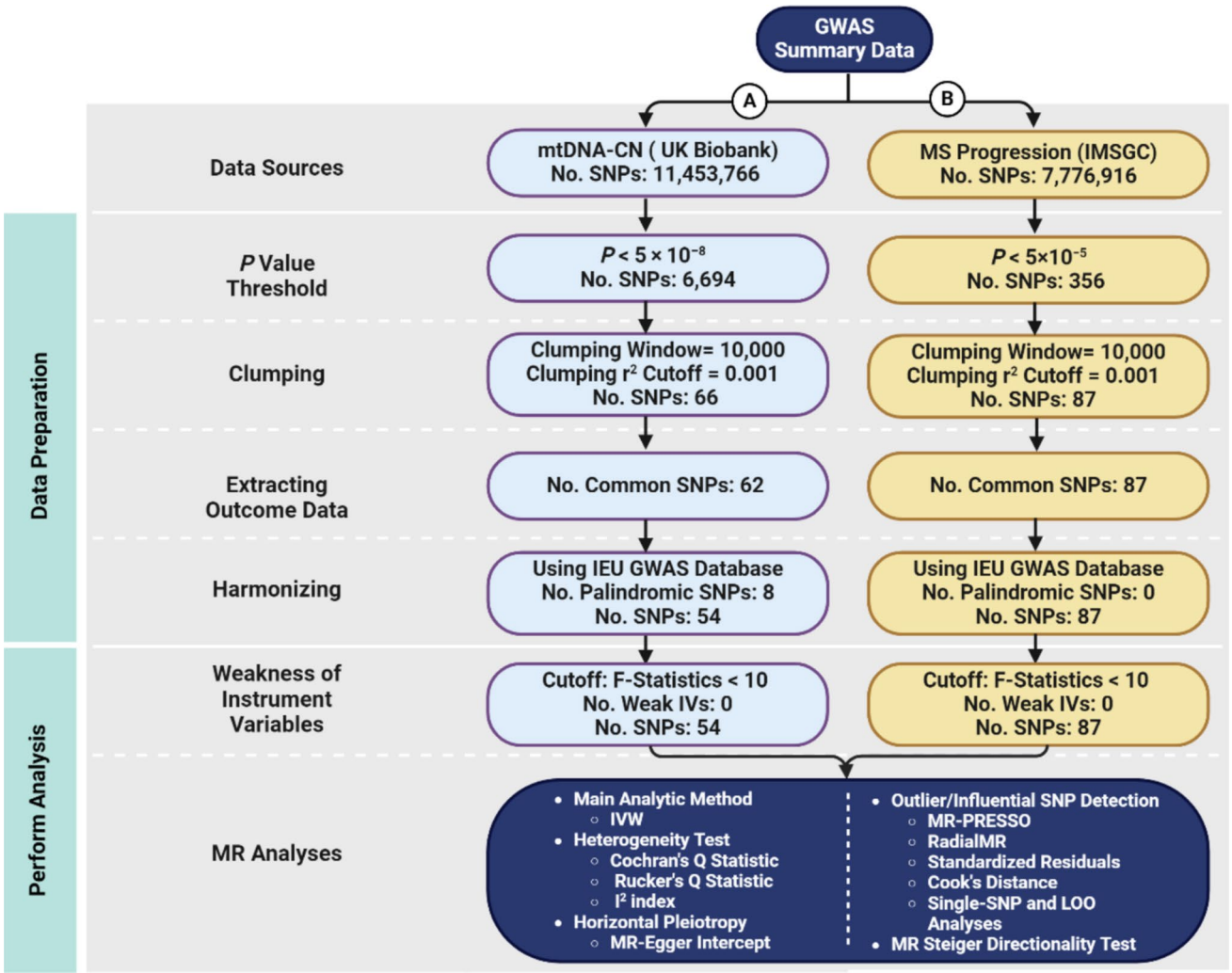

Fig. 1 The forward (workflow A) and reverse (workflow B) Mendelian randomization designs assess the causal relationship between exposure and outcome. Abbreviations: GWAS, genome-wide association studies; IVW, inverse variance weighted; LOO, leave-one-out; MR, Mendelian randomization; MS, multiple sclerosis; mtDNA-CN, mitochondrial DNA copy number; SNP, single-nucleotide polymorphism

outcome of interest, followed by clumping. The effect sizes for the instruments on the outcomes and exposures were harmonized to the same reference allele. Palindromic SNPs were excluded if present.

Mendelian Randomization Analysis

The inverse variance weighted (IVW) method was employed as the primary technique to estimate the causal relationship between exposure and outcome. To ensure the reliability and robustness of the results, several additional approaches were implemented, including Penalized IVW, Robust IVW, Penalized Robust IVW, MR-Egger, Penalized MR-Egger, Robust MR-Egger, Penalized Robust MR-Egger, Simple Median, Weighted Median, Penalized Weighted Median, Simple Mode, Weighted Mode, Robust Adjusted Profile Score (RAPS), MR-Constrained Maximum Likelihood (MR-cML), Debiased IVW (dIVW), Mode-Based Estimation (MBE), and MR-Lasso [25].

Heterogeneity in the analysis was assessed using multiple approaches, including Cochran's Q statistic, Rucker's Q statistic for MR-Egger, and the I^2 index [26, 27]. Horizontal pleiotropy was evaluated through intercept tests using the MR-Egger technique [28]. To detect and correct potential outliers, the Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test was applied by removing identified outliers [29, 30]. In addition, RadialMR was used to identify outliers with potential pleiotropy [31]. Cook's distance and standardized residuals were also applied to determine influential or outlier SNPs [30]. Leave-one-out (LOO) analysis was performed, and the corresponding plot was depicted to evaluate the effect of potentially pleiotropic SNPs on causal

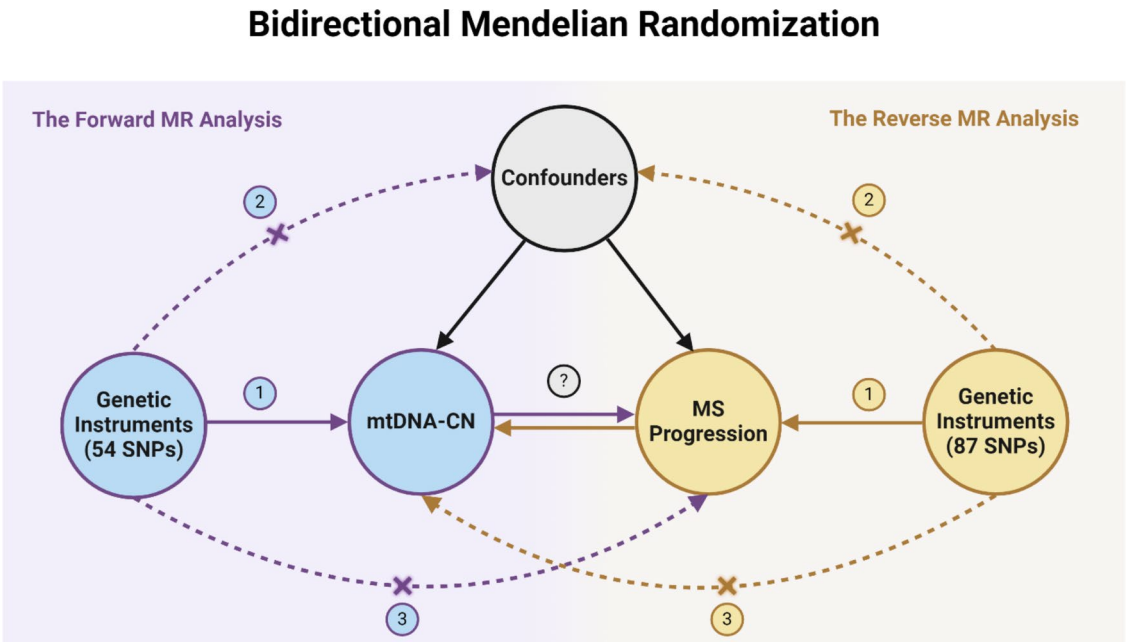

Fig. 2 The three assumptions of Mendelian randomization analysis are (1) the relevance assumption, (2) the independence assumption, and (3) the exclusion-restriction assumption. Abbreviations: MR, Mendelian randomization; MS, multiple sclerosis; mtDNA-CN, mitochondrial DNA copy number; SNP, single-nucleotide polymorphism

Table 1 Features of genome-wide association studies

Trait	Resource	Sample size (all/European)	Covariates	PubMed ID
mtDNA-CN	UK biobank	395,718/383,476	Blood cell counts, 20 genetic principal components, age, sex, and chip type	35023831
MSP	IMSGC	12,584/12,584	Sex, age at onset, date of birth, center, genotyping platform, and the first ten principal components	37380766

IMSGC, International Multiple Sclerosis Genetics Consortium; *mtDNA-CN*, mitochondrial DNA copy number; *MSP*, multiple sclerosis progression

estimates by sequentially excluding each SNP [26]. Funnel, forest, and scatter plots were created to identify directional pleiotropy, visualize genetic association, and inspect causal estimates for outliers [32]. Furthermore, the MR Steiger directionality test examined the causal direction [33].

All statistical analyses were conducted using R software (V.4.3.2), with the packages "TwoSampleMR," "MRPRESSO," "MendelianRandomization," "MRPracticals," and "mr.raps." The results were reported as β coefficients with corresponding 95% confidence intervals (CIs), and associations with $P < 0.05$ were considered statistically significant.

Results

Causal Association between Mitochondrial DNA Copy Number and Multiple Sclerosis Progression

A total of 6694 SNPs associated with mtDNA-CN were identified at genome-wide significance ($P < 5 \times 10^{-8}$). After filtering out 6628 SNPs due to high LD ($r^2 > 0.001$) or based on the LD reference panel, 66 SNPs remained for the primary analysis. From the outcome of GWAS, 62

SNPs were extracted. During harmonization, eight palindromic SNPs (rs10835540, rs17850455, rs12052715, rs2038480, rs342293, rs289713, rs8176645, rs72660908) were excluded, leaving 54 SNPs for the initial analysis (see Supplementary File: Table S1). Moreover, the poor instrumental bias can be ignored for the *F*-statistic range of 29.54 to 441.00 (Supplementary File: Table S1). First, no satisfactory estimation was achieved; however, this was improved by identifying potential outliers using methods such as MR-PRESSO, RadialMR, standardized residuals, and Cook's distance (see Supplementary File: Fig. S1).

Ultimately, with 43 SNPs serving as IVs, no significant evidence was found for an association between mtDNA-CN and MS progression ($P > 0.05$; see Fig. 3).

Rucker's Q test ($Q = 15.141$, $P = 0.999$), Cochran's Q test ($Q = 15.166$, $P = 0.999$), and the I^2 statistic ($I^2 = 0.0\%$) indicated no heterogeneity in the relationship between mtDNA-CN and MS progression. Additionally, the MR-Egger intercept test did not detect any evidence of horizontal pleiotropy (MR-Egger intercept $= 0.001$, $P = 0.877$). A visual examination of the scatter plot showing the association between exposure and outcome (Supplementary File: Fig. S2) along with a LOO plot (Supplementary File: Fig. S3) was conducted to assess the impact of potential outliers. Funnel and forest plots were depicted for the causal association between mtDNA-CN and MS progression (Supplementary File: Figs. S4 and S5).

MR Steiger analysis confirmed the correct causal direction ($P < 0.05$). Furthermore, a scatter plot summarizing the results of multiple MR methods was used as a visual tool to compare and contrast the findings obtained from different MR analytical approaches (Fig. 4).

Causal Association between Multiple Sclerosis Progression and Mitochondrial DNA Copy Number

In the reverse MR analyses, we identified a total of 356 SNPs associated with MS progression at genome-wide significance ($P < 5 \times 10^{-5}$). After excluding 269 SNPs due to high LD ($r^2 > 0.001$) or the LD reference panel, 87 SNPs were maintained for the main analysis. These 87 SNPs were extracted from the outcome GWAS. During harmonization, no SNPs were removed due to palindromic issues, resulting in the retention of all 87 SNPs for the initial analysis (see Supplementary File: Table S2). The instrumental variable bias was statistically negligible, as indicated by the *F*-statistic ranging from 16.50 to 32.90 (see Supplementary File: Table S2). First, no estimation accuracy was achieved. However, this was improved by identifying potential outliers through the MR-PRESSO method, RadialMR, standardized residuals, and Cook's distance (see Supplementary File: Fig. S6).

Our analysis identified a causal association between MS progression ($N = 76$ SNPs) and mtDNA-CN using IVW ($\beta =$

Methods	nSNPs	P-value	Beta (95% CI)
Inverse Variance Weighted (IVW)	43	0.487	-0.066 (-0.254 to 0.121)
Penalized IVW	43	0.487	-0.066 (-0.254 to 0.121)
Robust IVW	43	0.560	-0.066 (-0.289 to 0.156)
Penalized Robust IVW	43	0.560	-0.066 (-0.289 to 0.156)
MR-Egger	43	0.670	-0.100 (-0.558 to 0.359)
Penalized MR-Egger	43	0.670	-0.100 (-0.558 to 0.359)
Robust MR-Egger	43	0.618	-0.110 (-0.540 to 0.321)
Penalized Robust MR-Egger	43	0.618	-0.110 (-0.540 to 0.321)
Simple Median	43	0.780	-0.038 (-0.309 to 0.232)
Weighted Median	43	0.637	-0.063 (-0.327 to 0.200)
Penalized Weighted Median	43	0.637	-0.063 (-0.327 to 0.200)
Simple Mode	43	0.995	-0.001 (-0.483 to 0.481)
Weighted Mode	43	0.627	-0.095 (-0.468 to 0.277)
Robust Adjusted Profile Score (RAPS)	43	0.493	-0.067 (-0.275 to 0.124)
MR-Constrained Maximum Likelihood (MR-cML)	43	0.484	-0.067 (-0.255 to 0.121)
Debiased IVW (dIVW)	43	0.487	-0.067 (-0.257 to 0.122)
Mode-Based Estimation (MBE)	43	0.620	-0.095 (-0.471 to 0.281)
MR-Lasso	43	0.487	-0.066 (-0.254 to 0.121)

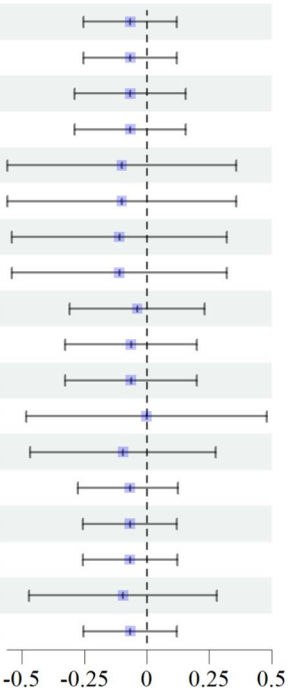

Fig. 3 A forest plot displaying the results from various forward Mendelian randomization analyses. The outcomes are presented as β coefficients, with 95% confidence intervals (CIs)

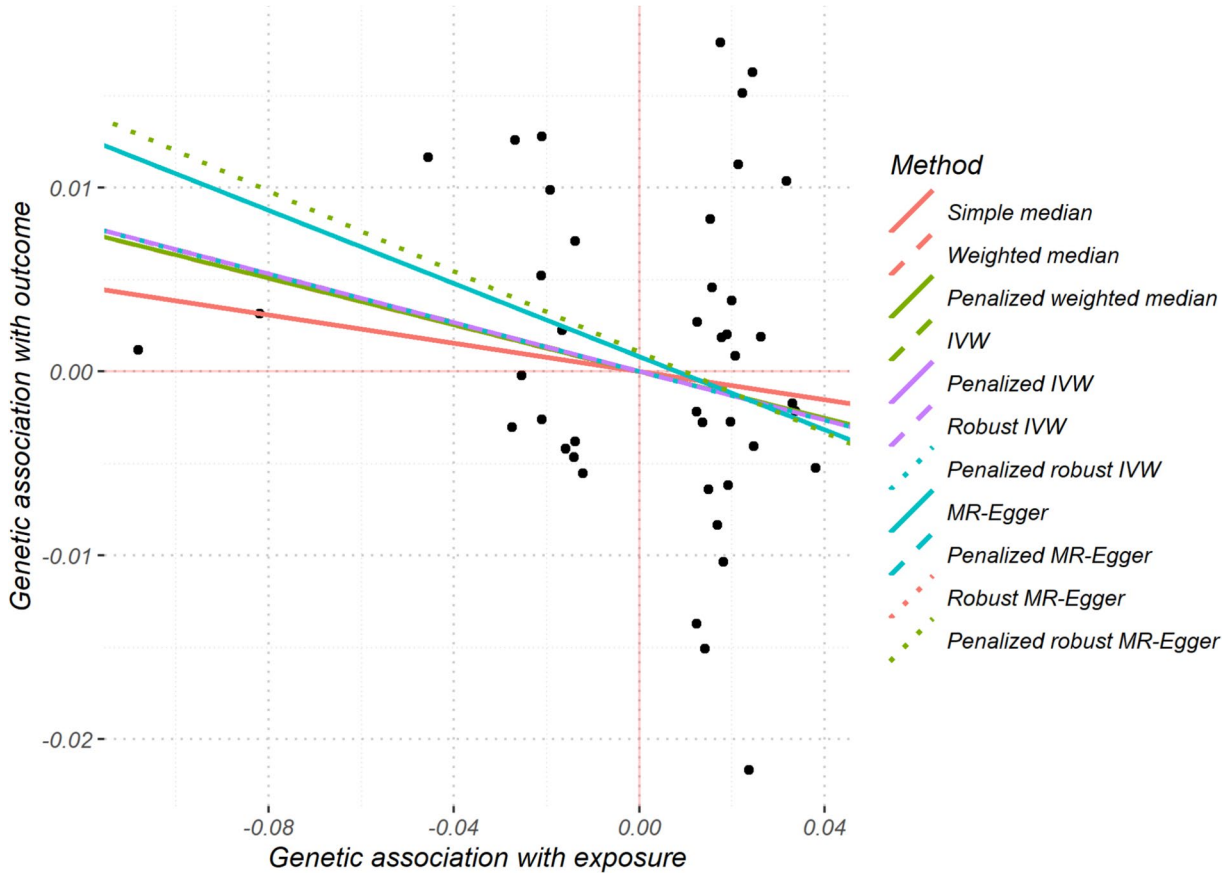

Fig. 4 A scatter plot visualizing and comparing findings obtained from Mendelian randomization methods

−0.010, 95% CI = − 0.019 to −0.001, P = 0.036), providing evidence that MS progression leads to a reduction in mtDNA-CN. This result was consistent across various MR approaches, including Penalized IVW (β = −0.010, 95% CI = − 0.019 to −0.001, P = 0.036), Robust IVW (β = −0.009, 95% CI = − 0.018 to −0.001, P = 0.029), Penalized Robust IVW (β = −0.009, 95% CI = − 0.018 to −0.001, P = 0.029), RAPS (β = −0.010, 95% CI = − 0.020 to 0.000, P = 0.043), MR-cML (β = −0.010, 95% CI = − 0.019 to −0.001, P = 0.035), dIVW (β = −0.010, 95% CI = − 0.020 to −0.001, P = 0.036), and MR-Lasso (β = −0.010, 95% CI = − 0.019 to −0.001, P = 0.036). However, the results were not statistically significant for other methods (P > 0.05) (see Fig. 5).

Rucker's Q (Q = 41.683, P = 0.999), Cochran's Q value (Q = 41.712, P = 0.999), and an I^2 statistic of 0.0% all indicated no heterogeneity in the analysis. An MR-Egger intercept analysis (MR-Egger intercept = 0.0002, P = 0.864) showed no evidence of horizontal pleiotropy. Moreover, visual examinations of the scatter plot (Supplementary File: Fig. S7) and LOO plot (Supplementary File: Fig. S8) were conducted to assess the impact of outliers. Funnel and forest plots related to the causal association of MS progression with mtDNA-CN were also created (Supplementary File: Figs. S9 and S10). MR Steiger analysis confirmed the correct causal direction (P < 0.05). A scatter plot using various MR methods was used to visualize and compare the findings from different MR approaches (Fig. 6). The MR analysis process and the findings are available publicly on GitHub: https://hani-sabaie.github.io/mtDNACN-MSP-MR/MR-Report.html.

Discussion

In the present study, we investigated the causal role of mtDNA-CN in MS progression, as well as the reverse relationship using genetic correlation, two-sample, and bidirectional MR methods. We found that mtDNA-CN had no causal association with MS progression. However, reverse MR analysis revealed that MS progression is associated with a decrease in mtDNA-CN levels.

mtDNA is more vulnerable to oxidative stress and inflammatory damage than nuclear DNA, leading to a higher incidence of mutations and replication errors over time. This vulnerability is due to the absence of histones and inefficient DNA repair mechanisms in mtDNA [34]. The D-Loop, a

Methods	nSNPs	P-value	Beta (95% CI)
Inverse Variance Weighted (IVW)	76	0.036	-0.010 (-0.019 to -0.001)
Penalized IVW	76	0.036	-0.010 (-0.019 to -0.001)
Robust IVW	76	0.029	-0.009 (-0.018 to -0.001)
Penalized Robust IVW	76	0.029	-0.009 (-0.018 to -0.001)
MR-Egger	76	0.272	-0.011 (-0.032 to 0.009)
Penalized MR-Egger	76	0.272	-0.011 (-0.032 to 0.009)
Robust MR-Egger	76	0.171	-0.011 (-0.028 to 0.005)
Penalized Robust MR-Egger	76	0.171	-0.011 (-0.028 to 0.005)
Simple Median	76	0.077	-0.011 (-0.024 to 0.001)
Weighted Median	76	0.079	-0.011 (-0.024 to 0.001)
Penalized Weighted Median	76	0.079	-0.011 (-0.024 to 0.001)
Simple Mode	76	0.165	-0.023 (-0.057 to 0.010)
Weighted Mode	76	0.178	-0.022 (-0.054 to 0.009)
Robust Adjusted Profile Score (RAPS)	76	0.043	-0.010 (-0.020 to 0.000)
MR-Constrained Maximum Likelihood (MR-cML)	76	0.035	-0.010 (-0.019 to -0.001)
Debiased IVW (dIVW)	76	0.036	-0.010 (-0.020 to -0.001)
Mode-Based Estimation (MBE)	76	0.166	-0.022 (-0.054 to 0.009)
MR-Lasso	76	0.036	-0.010 (-0.019 to -0.001)

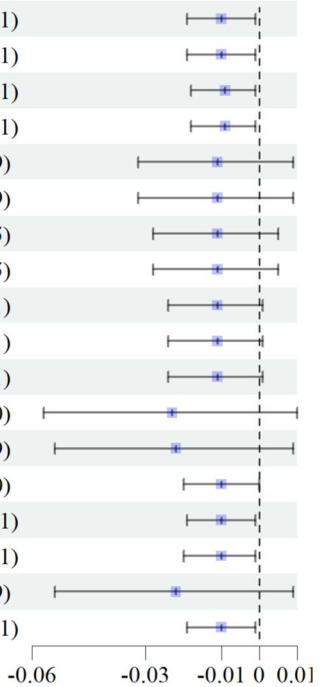

Fig. 5 A forest plot of various reverse Mendelian randomization methods. The results are presented as β coefficients with an equivalent confidence interval (CI) of 95%

non-coding region of the mitochondrial genome responsible for its replication, is highly prone to mutations. Alterations in this region can lead to decreased mtDNA-CN [35]. A recent study using next-generation sequencing of the mitochondrial genome has observed a more significant number of mutations in D-Loop in MS patients compared to healthy individuals [36]. Damage to mtDNA may trigger mechanisms that increase mtDNA-CN to protect the mitochondrial genome and preserve normal function [37, 38]. However, excessive mtDNA damage can decrease mtDNA-CN, resulting in mitochondrial dysfunction [37, 39]. Evidence from cell models suggests that reduced mtDNA-CN is associated with decreased mitochondrial protein expression, diminished respiratory enzyme activity, and changes in cellular morphology, highlighting the link between mtDNA-CN and mitochondrial dysfunction [40]. Our findings suggest that decreased mtDNA-CN is likely a consequence of impaired mitochondrial function in patients with MS. Although direct experimental evidence specifically elucidating the causal pathways between MS progression and reduced mtDNA-CN is currently limited, several plausible biological mechanisms based on established pathophysiological processes in MS may account for this observation. One possible explanation is that chronic inflammation and progressive demyelination in MS trigger a cascade of cellular and metabolic changes, leading to the redistribution of ion channels, an increase in Na^+/K^+-ATPase activity and higher ATP consumption. In response, mitochondria may undergo adaptive changes to balance the energy supply and demand [41]. Concurrently, the prolonged inflammatory state of the disease generates an environment of oxidative stress resulting from the release of ROS by activated macrophages and microglia [42], as well as increased glutamate release due to neuronal injury [41]. Furthermore, tumor necrosis factor-alpha (TNF-α) impairs the OXPHOS process through calcium-regulated mechanisms [41]. Oxidative stress and excitotoxicity resulting from neuronal damage lead to progressive mitochondrial impairment, including mtDNA alterations, increased heteroplasmy, dysfunctions in OXPHOSP subunits, and altered mitochondrial transport [43, 44]. These mitochondrial modifications result in decreased ATP production, impairing the Na^+/K^+-ATPase. This triggers Na^+ accumulation within the neuronal cytoplasm, which activates the Na^+/Ca^{2+} channel. Increased intracellular Ca^{2+} initiates an apoptotic cascade, ultimately leading to neuronal death, Wallerian degeneration, and irreversible neurological impairment [43]. While prior studies have not specifically quantified the direct impact of MS progression on mtDNA-CN, our interpretation aligns with the broader understanding of mitochondrial involvement in MS pathogenesis. Further mechanistic investigations are needed to more precisely characterize the causal relationships.

Mitochondrial dynamics, particularly mtDNA-CN, display substantial tissue-specific variations that can influence disease mechanisms and progression. A recent study has

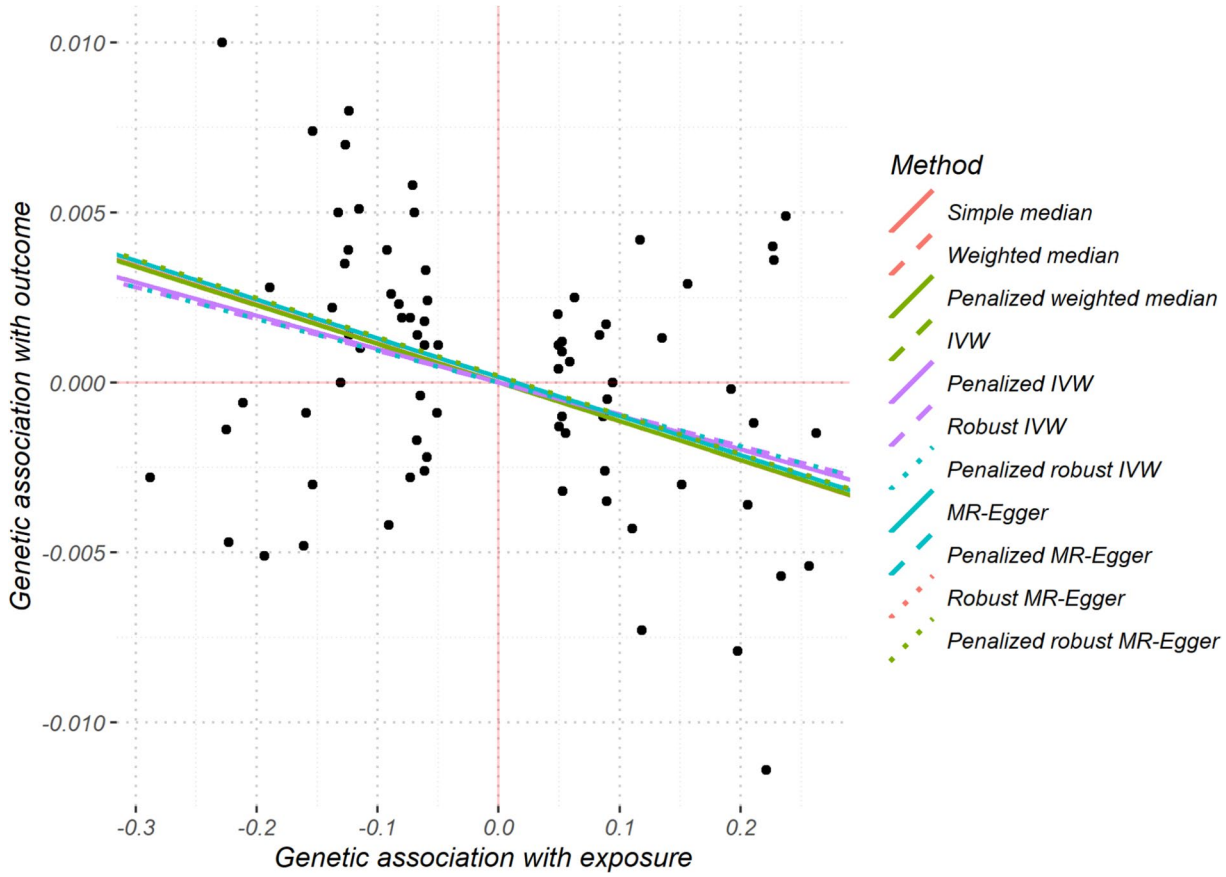

Fig. 6 Comparison of different Mendelian randomization approaches. All methods indicate a negative relationship between multiple sclerosis progression and mitochondrial DNA copy number

demonstrated considerable variations in mtDNA-CN across different tissues in both humans and mice, with up to 50-fold differences in humans and 200-fold in mice [45]. The study quantified mtDNA-CN in thousands of samples, revealing significant tissue-specific and inter-individual differences [45]. Given the multisystem involvement of MS, including both the immune and central nervous systems, the tissue-specific contributions of mtDNA alterations warrant careful consideration. Measurements of mtDNA-CN in peripheral blood reflect systemic mitochondrial function and oxidative stress, which may be influenced by immune cell activation and inflammation [10, 43]. Conversely, mtDNA-CN in cerebrospinal fluid (CSF) and brain tissue may provide more direct insights into mitochondrial dysfunction within the CNS [14].

Observational studies are often subject to confounding biases, making it challenging to establish causality. To our knowledge, this is the first study that used the MR approach to investigate the causal effect of MS progression on mtDNA-CN and vice versa. Previous observational studies have revealed controversial findings regarding mtDNA-CN and MS severity. For instance, Leurs et al. [11] found a non-significant correlation between EDSS and mtDNA-CN. Sedky et al. [13] reported a negative relationship between EDSS and mtDNA-CN, which was not statistically significant.

Conversely, three observational studies reported a significant correlation between decreased mtDNA-CN and MS progression, aligning with our results. Varhaug et al. [14] observed a significant inverse correlation between disease duration and mtDNA-CN. Al-Kafaji et al. [10] found that patients with disease durations exceeding 10 years had lower mtDNA-CN than those with shorter disease durations. López-Armas et al. [12] showed a linear correlation between mild to moderate disability in MS patients and mtDNA-CN. These findings suggest that mtDNA-CN could be a valuable biomarker for assessing MS progression. While mtDNA-CN provides insight into mitochondrial dysfunction, other biomarkers, such as neurofilament light chain (NfL) [46] and glial fibrillary acidic protein (GFAP) [47], have been widely used to assess different pathological aspects of MS. NfL, which indicates neuroaxonal damage, and GFAP, a marker of astroglial activation, have both shown potential in tracking disease progression [46–48]. Integrating mtDNA-CN with

established biomarkers like NfL and GFAP may enhance our ability to track MS progression, as mtDNA-CN reflects mitochondrial health, NfL indicates neuroaxonal integrity, and GFAP highlights astroglial activation. Future studies should explore the combined utility of these biomarkers to provide a more comprehensive picture of MS pathophysiology and disease monitoring. Moreover, decreased mtDNA-CN has also been found in other neurodegenerative conditions, including Alzheimer's disease, Huntington's disease, and Parkinson's disease [8], indicating that reduced mtDNA-CN may be a common feature across neurodegenerative disorders, including MS.

Recent advancements in understanding mitochondrial dysfunction in neurodegenerative diseases, particularly MS, suggest novel avenues for preventing neuronal loss. Targeting mitochondrial pathways could provide new therapeutic strategies [43]. Interventions aimed at addressing mitochondrial dysfunction could be integrated into current MS treatment regimens, particularly in progressive forms of the disease. Therapeutic strategies aimed at boosting mitochondrial function or reducing oxidative stress might complement existing immunomodulatory treatments [43]. mtDNA-CN holds promise within a personalized medicine framework, offering potential for stratifying MS patients based on their mitochondrial dysfunction and tailoring treatments accordingly. This approach could enable early intervention for patients at higher risk of rapid progression and facilitate targeted therapies that mitigate mitochondrial damage, ultimately leading to more personalized and effective approaches for MS patients, thereby improving clinical outcomes.

While our study provides valuable insights, it is important to acknowledge its limitations. First, it is essential to note that the GWAS data used were primarily derived from populations of European ancestry which limits the generalizability of our findings to non-European populations. Further research is needed to validate these findings in diverse populations to ensure that clinical applications, such as diagnosis and treatment strategies, are practical across all ethnic groups. Second, the use of GWAS data may be influenced by population stratification and relatedness issues. Third, while reverse causality in MR studies can be difficult to interpret and may not establish a clear causal pathway, additional experimental studies are needed to elucidate the underlying mechanisms through which MS progression might influence changes in mtDNA-CN. These additional studies could help determine whether the observed association reflects a direct causal relationship or arises from complex, multifactorial processes. Fourth, the use of a statistical threshold of $P < 5 \times 10^{-5}$ when selecting genetic instruments associated with MS progression may heighten the risk of pleiotropy and weak instrument bias. To mitigate these potential concerns, we employed several approaches, including evaluating the *F*-statistic, utilizing robust MR techniques, and conducting sensitivity analyses. Finally, future studies with larger sample sizes from GWAS of MS progression could provide more valuable insights into the causal relationship.

Conclusion

This study highlights the potential link between mitochondrial dysfunction and the progression of MS, particularly through mtDNA-CN. Integrating tissue-specific analyses can enhance our understanding of mitochondrial contributions to MS progression and may provide more precise biomarkers for monitoring disease severity and therapeutic response. Further research is essential to validate the role of mtDNA-CN as a reliable biomarker. Gaining deeper insights into underlying molecular mechanisms will be critical for developing targeted therapeutic interventions that could complement existing immunomodulatory therapies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12035-025-04980-9.

Author Contribution HS, MA, and MSD conceived and designed the study. HS and ATR analyzed the data, and DH provided critical guidance and support in data analysis. HS, ATR, MS, TS, SS, ASZ, and MZ drafted the initial manuscript. HS, ATR, and MS generated Tables and Figures. AHS, HV, LF, HH, FA, and MH provided critical revision. MA and MSD supervised, edited, and finalized the manuscript. All authors reviewed and approved the final manuscript.

Data Availability The original data used are publicly available and the details of the MR analyses and their findings are available through the provided HTML link: https://hani-sabaie.github.io/mtDNACN-MSP-MR/MR-Report.html.

Declarations

Ethics Approval and Consent to Participate The local ethics committee approved this study at the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences (Research Approval Code: 43014163).

Consent for Publication Not applicable.

Competing interests The authors declare no competing interests.

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