The association between cytomegalovirus infection and neurodegenerative diseases: a prospective cohort using UK Biobank data

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Summary

Background Certain viral infections have been linked to the development of neurodegenerative diseases. This study aimed to investigate the association between cytomegalovirus (CMV) infection and five neurodegenerative diseases, spinal muscular atrophy (SMA) and related syndromes, Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS), and disorders of the autonomic nervous system (DANS).

Methods This prospective cohort included white British individuals who underwent CMV testing in the UK Biobank from January 1, 2006 to December 31, 2021. A Cox proportional hazard model was utilized to estimate the future risk of developing five neurodegenerative diseases in individuals with or without CMV infection, adjusted for batch effect, age, sex, and Townsend deprivation index in Model 1, and additionally for type 2 diabetes, cancer, osteoporosis, vitamin D, monocyte count and leukocyte count in Model 2. Bidirectional Mendelian randomization was employed to validate the potential causal relationship between CMV infection and PD.

Findings A total of 8346 individuals, consisting of 4620 females (55.4%) and 3726 males (44.6%) who were white British at an average age of 56.74 (8.11), were included in this study. The results showed that CMV infection did not affect the risk of developing AD (model 1: HR [95% CI] = 1.01 [0.57, 1.81], $P = 0.965$; model 2: HR = 1.00 [0.56, 1.79], $P = 0.999$), SMA and related syndromes (model 1: HR = 3.57 [0.64, 19.80], $P = 0.146$; model 2: HR = 3.52 [0.63, 19.61], $P = 0.152$), MS (model 1: HR = 1.16 [0.45, 2.97], $P = 0.756$; model 2: HR = 1.16 [0.45, 2.97], $P = 0.761$) and DANS (model 1: HR = 0.65 [0.16, 2.66], $P = 0.552$; model 2: HR = 0.65 [0.16, 2.64], $P = 0.543$). Interestingly, it was found that participants who were CMV seronegative had a higher risk of developing PD compared to those who were seropositive (model 1: HR = 2.37 [1.25, 4.51], $P = 0.009$; model 2: HR = 2.39 [1.25, 4.54], $P = 0.008$) after excluding deceased individuals. This association was notably stronger in males (model 1: HR = 3.16 [1.42, 7.07], $P = 0.005$; model 2: HR = 3.41 [1.50, 7.71], $P = 0.003$), but no significant difference was observed in the female subgroup (model 1: HR = 1.28 [0.40, 4.07], $P = 0.679$; model 2: HR = 1.27 [0.40, 4.06], $P = 0.684$). However, a bidirectional Mendelian randomization analysis did not find a genetic association between CMV infection and PD.

Interpretation The study found that males who did not have a CMV infection were at a higher risk of developing PD. The findings provided a new viewpoint on the risk factors for PD and may potentially influence public health approaches for the disease.

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Keywords: Cytomegalovirus; Parkinson's disease; GWAS; Cox proportional hazards model; Bidirectional Mendelian randomization analysis

Research in context

Evidence before this study

A systematic search was performed in PubMed from database inception up to October 1, 2023, with primary keywords "virus", "herpesviruses", "cytomegalovirus", "neurodegenerative disease", "Parkinson's disease", "Alzheimer's disease", "amyotrophic lateral sclerosis" and "multiple sclerosis (MS)". Previous research has suggested that viral infection, especially herpesviruses including herpes simplex virus type 1 (HSV1), HSV2, Varicella zoster virus (VZV), and cytomegalovirus (CMV), may be responsible for neuroinflammation and weakened immune system, potentially leading to the progression of neurodegenerative diseases such as Alzheimer's disease (AD). However, there is no evidence to quantify the risk of neurodegenerative diseases in individuals with CMV infection, nor is there any information on the genes and pathways that may be involved in CMV affecting Parkinson's disease (PD).

Added value of this study

In this study, we identified a higher risk of developing PD in male individuals with CMV seronegative than those with CMV seropositivity in a prospective cohort from UK Biobank. However, the risk of developing PD did not change in the female subgroup. We also found that lower levels of CMV antigens, including pp28, pp52, and pp150, were associated with a higher risk of developing PD in males, suggesting a negative association between the titer levels and the risk. Furthermore, a bidirectional Mendelian randomization analysis showed no genetic association between CMV infection and PD. This report is the first to measure the relationship between CMV infection and the risk of developing PD.

Implications of all the available evidence

Further research, including clinical and animal experiments, is needed to elucidate the precise mechanism by which CMV infection may reduce the risk of developing PD, and it may lead to a shift in public policy for PD.

Introduction

Neurodegenerative diseases (NDDs) are a heterogeneous group of neurological disorders adversely affecting the lives of millions of people worldwide and entailing the progressive loss of neurons and collapse of the structure and function of neural networks, culminating in impaired memory, cognition, behavior, sensory and motoric function. There is increasing evidence to suggest that viral infections could be related to the development of neurodegenerative diseases. Several studies have shown that viral hepatitis, skin infections, mucous membrane infections and influenza increase the risk of developing some neurodegenerative diseases, such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), general dementia, vascular dementia, Parkinson's disease (PD), and multiple sclerosis (MS).[1–4] Evidence from epidemiologic, postmortem, animal, and cellular studies suggests that herpesviruses may be responsible for the seeding and deposition of amyloid-beta (Aβ), a classic symptom of Alzheimer's disease.[5,6] In addition, fragmented DNA released because of viral infections can spark neuroinflammation and lead to the apoptosis of dopamine neurons in the nigrostriatal of the brain, potentially resulting in Parkinson's disease. Recent evidence also found that the COVID-19 pandemic can result in short- and long-term cognitive impairments,[5] further establishing a potential link between viral exposure and neurodegeneration.

Cytomegalovirus (CMV) is highly prevalent in humans and may cause exacerbation of neuroinflammation.[5] Studies in neurodegenerative diseases have suggested that neuroinflammation is not only a result of neurodegeneration but also a crucial player in this process.[6] This study aimed to explore the link between CMV infection and five neurodegenerative diseases, including spinal muscular atrophy (SMA) and related syndromes, PD, AD, MS, and disorders of the autonomic nervous system (DANS), using data from the UK Biobank.

Epidemiological studies are often complicated by various confounding factors that are difficult to control for. Observational studies may not be able to fully address these issues. However, Mendelian randomization (MR) can help minimize confounders and reduce the impact of reverse causality, as genetic variations are randomly assigned at conception and remain constant throughout an individual's life.[7] By using disease-associated SNPs identified through Genome-wide

association study (GWAS) analysis as instrumental variables, we can conduct MR analyses to investigate causal relationships between CMV and PD. These genetic analysis methods, including GWAS and MR, have also been employed in other studies to investigate the associations between infectious factors and diseases. For example, to examine the link between severe COVID-19 and respiratory failure,[8] as well as the relationship between COVID-19 and systemic lupus erythematosus, these methods were used.

Methods

Study population

CMV data were collected from the UK Biobank, a large-scale, long-term prospective cohort of over 500,000 participants aged 40–69 in the UK, since 2006. The full UK Biobank study protocol is available online (https://www.ukbiobank.ac.uk/media/gnkeyh2q/study-rationale.pdf). Strict quality control was conducted to exclude ineligible samples. We excluded (1) genotyped ineligible samples (Field ID: 22010); (2) samples not undergone calculating by principal component analysis (PCA), which is necessary for subsequent GWAS analysis; (3) highly related individuals in genetic kinship to other participants (Field ID: 22021); (4) individuals with sex chromosome aneuploidy (Field ID: 22019). For quality control of genotype data (SNPs from the Haplotype Reference Consortium, UK10K database and 1000 Genomes project), we performed information (INFO) scores, minor allele frequency (MAF) scores and evaluated the *P*-value of Hardy–Weinberg equilibrium (HWE). We limited the race of participants to white British. All patients with a baseline diagnosis of one of the five NDDs were excluded from the prospective study.

The exposures and outcomes

Data from individuals who underwent CMV antigens testing were obtained from the UK Biobank, including CMV pp28 antigen (Field ID: 23009), CMV pp52 antigen (Field ID: 23008), CMV pp150 antigen (Field ID: 23007) and CMV seropositive (Field ID: 23054). It was defined as CMV seropositive if two or more of following antigens are positive: antigen pp150 > 100, antigen pp52 > 150, antigen pp28 > 200. Brenner et al. have confirmed the robustness and effectiveness of CMV infection detection using CMV Monoplex Serology based on three proteins: pp52, pp28, and pp150. The sensitivity of this method ranges from 92.3% to 100.0% (median 97.4%), with a specificity ranging from 91.8% to 98.7% (median 96.6%).[9] Age and sex were collected as demographic data. The sex of participants in the study was determined by a combination of the NHS record and the self-reported questionnaire. Other data were collected as a baseline, including medical history (cancer history and type 2 diabetes [T2D]) and biological samplings (such as level of vitamin D, white blood cell (WBC) leukocyte count and monocyte count). We then limited the analysis to white British samples. The final 8346 samples with genotypes and valid CMV measurements were ultimately included in the analysis.

The diagnostic data for participants in the UK Biobank were collected from hospital inpatient data, primary care data, death registries and self-reported medical condition. Five neurodegenerative diseases were identified, based on the International Classification of Diseases Tenth Revision (ICD-10-CM): SMA and related syndromes (G12), Parkinson's Disease (G20), Alzheimer's Disease (G30), multiple sclerosis (G35) and disorders of autonomic nervous system' (G90). Participants were tested for CMV antigens at baseline and were then followed up until the date of diagnosis of five NDDs, death, loss to follow-up, or December 31, 2021.

Statistics

Kolmogorov–Smirnov test was used to assess the normality of quantitative data. Levene's test was used to assess homogeneity of variances. The mean ± standard deviation was reported if the data followed a normal distribution. The independent samples t test was used for between-group comparisons of normally distributed data with homogeneous variances, such as age and Townsend deprivation index (TDI) at baseline. For nonnormally distributed quantitative data, such as vitamin D, monocyte count, leukocyte WBC cell count and CMV titers at baseline, the median and interquartile range (IQR) were presented and between-group comparisons were conducted using the Mann–Whitney U test. Categorical data, including sex, T2D, cancer, and osteoporosis (OP), were reported as counts and percentages, and between-group comparisons were conducted using the chi-square test. When the prerequisites for the chi-square test were not met, Fisher's exact test was used. Additionally, CMV titers were natural log transformation.

Multivariable Cox regression model was used to evaluate the CMV infection (CMV titers of pp28, pp52, pp150, and CMV seronegative/seropositivity) and the risk of developing SMA and related syndromes (G12), PD (G20), AD (G30), MS(G35), and DANS (G90). The proportional hazard assumption was examined using Schoenfeld residuals. We did not note any violations of the proportional hazard assumption in models involving incidents of these five situations. Previous studies have identified age, sex, T2D, cancer, OP, vitamin D, immune status and socioeconomic status as risk factors for PD.[10–13] Therefore, we included the following potential confounders in our model, such as batch effect, Townsend deprivation index (TDI, calculated with 12 crucial deprivation indicators that reflect regional socioeconomic status), age, sex, T2D, cancer, OP, vitamin D, monocyte count, and leukocyte WBC cell count in our models. Model 1 includes four confounding factors:

batch effect, age, sex, and TDI. Model 2 was further adjusted for T2D, cancer, OP, vitamin D, monocyte count, and leukocyte count. Hazard ratios (HRs) with 95% confidence intervals (Cis) were estimated to determine the risk of the occurrence of the 5 diseases. To further investigate the possible connection between CMV infection and the risk of developing PD, a restricted cubic spline was used to evaluate the nonlinear relation between exposure (CMV titers of pp28, pp52, and pp150) and incident PD. The cumulative incidence rates of PD were estimated based on different titers of CMV antigens which were divided into Q1-Q4 quartiles. Several sensitivity analyses were conducted to examine the robustness of our results. We treated death as a competing risk and conducted an additional sensitivity analysis by excluding dead individuals. We also conducted an analysis treating death and PD as a composite endpoint. To mitigate multiple testing issues for various CMV infection, a False discovery rate (FDR) correction was used to correct for multiple comparisons. Finally, a stratified analysis based on sex was employed to compare the HRs of PD in male and female subgroups after excluding the death individuals, and a further comparison among different quartiles was done using Q4 as the reference.

All statistical analyses were performed using R3.5.3 software (R Foundation for Statistical Computing, Vienna, Austria). Two-sided $P < 0.05$ was considered statistically significant and the P value was adjusted using FDR method for multiple comparisons.

Genome-wide association study (GWAS) and multimarker analysis of genomic annotation (MAGMA) analyses for instrumental variables

The genome-wide data for PD was derived from a study by the International Parkinson's Disease Genomics Consortium and included 33,674 cases and 449,056 controls, with a total of 17,891,936 SNPs analyzed by GWAS.[14] Quality control was applied to genotype data, including SNPs from the Haplotype Reference Consortium, UK10K database and 1000 Genomes project, by imposing an INFO of greater than 0.8, an MAF of greater than 0.001 and an HWE of greater than 1e-10.[15] After quality control, approximately 13.7 million SNPs were available for subsequent GWAS analysis. The GWAS analysis was performed using PLINK 2.0 software, adjusting for batches (Field ID: 22000), age (Field ID: 21022), sex (Field ID: 31), and genetic principal components of the top 10 (Field ID: 22009). Independent loci associated with CMV were identified using the clump program in PLINK. Functional mapping and annotation (FUMA) of GWAS were used to elucidate the biological function of pleiotropic loci. Multimarker analysis of genomic annotation (MAGMA) gene set analysis was used to investigate the biological function of the lead SNPs. The identified motifs were then mapped to nearby genes.

Two-sample bidirectional Mendelian randomization (BMR) analysis

The clump program in PLINK software was used to identify loci that were independently associated with CMV phenotypes and could be used as instrumental variables. The P value thresholds for these variables ranged from 1×10^{-5}, 5×10^{-6} to 5×10^{-7}, with a r^2 threshold of 0.001 within a window of 500 kb. To assess the causal relation between CMV and PD, we employed inverse variance weighting (IVW) as the main method of Mendelian randomization. To ensure the reliability of the results, various sensitivity analyses were performed. First, Q tests with IVW and MR−Egger were used to evaluate violations of the hypothesis. Second, MR−Egger was applied to estimate horizontal pleiotropy, ensuring that genetic variance is independently associated with exposure and outcome. To further increase the stability of the results, additional MR analyses with different modeling assumptions and strengths (weighted median and weighted mode) were applied. Third, MR-PRESSO was used to identify outliers and correct for horizontal pleiotropy. Fourth, leave-one-out analysis was conducted to assess the potential influence of individual SNPs. All MR analyses were performed using the Mendelian randomization and MRPRESSO packages.

Ethics

UKB received approval from the National Information Governance Board for Health and Social Care and the National Health Service North West Centre for Research Ethics Committee (Ref: 11/NW/0382). All participants provided informed consent through electronic signature at baseline assessment.

Role of funding source

The funders played no part in the design of the study, collection, analysis, interpretation of data and the writing of the report.

Results

Clinical characteristics of the participants

The clinical characteristics of the participants were analyzed. Baseline data are presented in Table 1. The mean age of the participants was 56.74 ± 8.11 years, and most of them were female (55.4%). Of the 8346 participants, 4696 were CMV seropositive and 3650 were CMV seronegative. The mean age of those who tested negative for CMV was 55.29 ± 8.30 years, with 1972 (54.0%) female and 1678 (46.0%) male, while the mean age of those who tested positive for CMV was 57.87 ± 7.78 years, with 2648 (56.4%) female and 2048 (43.6%) male. The mean TDI for all participants was −1.54 ± 2.94, with a TDI of −1.67 ± 2.83 for CMV seronegative and −1.44 ± 3.02 for CMV positive. Approximately 4.9% of participants, 4.3% of

	Overall	CMV seropositivity	
		Negative	Positive
N	8346	3650	4696
Age (mean (SD))	56.74 (8.11)	55.29 (8.30)	57.87 (7.78)
Sex			
Female	4620 (55.4%)	1972 (54.0%)	2648 (56.4%)
Male	3726 (44.6%)	1678 (46.0%)	2048 (43.6%)
TDI (mean (SD))	−1.54 (2.94)	−1.67 (2.83)	−1.44 (3.02)
T2D			
No	7935 (95.1%)	3493 (95.7%)	4442 (94.6%)
Yes	411 (4.9%)	157 (4.3%)	254 (5.4%)
Cancer			
No	6877 (82.4%)	3063 (83.9%)	3814 (81.25)
Yes	1469 (17.6%)	587 (16.1%)	882 (18.85)
OP			
No	8097 (97.0%)	3554 (97.4%)	4543 (96.7%)
Yes	249 (3.0%)	96 (2.6%)	153 (3.3%)
Vitamin D (median [IQR])	45.20 [31.60, 60.00]	44.85 [31.40, 59.40]	45.70 [31.80, 60.50]
Monocyte count (median [IQR])	0.45 [0.36, 0.57]	0.45 [0.36, 0.56]	0.45 [0.36, 0.57]
White blood cell leukocyte count (median [IQR])	6.62 [5.65, 7.82]	6.59 [5.60, 7.79]	6.70 [5.70, 7.89]
CMV_pp28_qc (median [IQR])	375.00 [85.00, 2192.75]	77.00 [45.00, 126.00]	1938.00 [894.75, 3201.25]
CMV_pp52_qc (median [IQR])	1272.50 [67.25, 5792.00]	57.00 [29.00, 115.00]	5365.50 [3356.00, 7368.25]
CMV_pp150_qc (median [IQR])	382.50 [40.00, 2617.00]	35.00 [22.00, 58.00]	2328.50 [1118.75, 3819.25]

CMV, cytomegalovirus; pp28, CMV antigen pp28; pp52, CMV antigen pp52; pp150, CMV antigen pp150; IQR, interquartile range; OP, osteoporosis; SD, standard deviation; T2D, type 2 diabetes; TDI, Townsend deprivation index.

Table 1: **Baseline characteristics of the study participants stratified by CMV seropositivity.**

seronegative individuals and 5.4% of seropositive individuals had T2D. Additionally, 17.6% of the participants, 16.1% seronegative and 18.8% seropositive individuals had cancer. Of the participants, 3.0% had OP, compared to 2.6% and 3.3% in seronegative individuals and seropositive individuals, respectively. The mean level of vitamin D across all individuals was 45.20 [31.60, 60.00] nmol/L, while it was 44.85 [31.40, 59.40] and 45.70 [31.80, 60.50] in seronegative individuals and seropositive individuals, respectively. The mean monocyte and overall WBC count in seronegative individuals were 0.45 [0.36, 0.56] $x10^9$ cells/L and 6.59 [5.60, 7.79] $x10^9$ cells/L, respectively, while in seropositive individuals, they were 0.45 [0.36, 0.57] $x10^9$ cells/L and 6.70 [5.70, 7.89] $x10^9$ cells/L, respectively. On average, the monocyte and WBC counts were 0.45 [0.36, 0.57] 109 cells/L and 6.62 [5.65, 7.82] cells/L, respectively. The mean serum titers of CMV pp28, pp52, and pp150 were 375.00 [85.00, 2192.75], 1272.50 [67.25, 5792.00] and 382.50 [40.00, 2617.00], respectively. In seronegative individuals, these values were 77.00 [45.00, 126.00], 57.00 [29.00, 115.00], and 35.00 [22.00, 58.00], respectively, and in seropositive individuals, they were 1938.00 [894.75, 3201.25], 5365.50 [3356.00, 7368.25], and 2328.50 [1118.75, 3819.25]. The model I includes four confounding factors: batch effect, age, sex and TDI. And model II was further adjusted for T2D, cancer, OP, vitamin D, monocyte count, and leukocyte count (Table 1).

Relationship between the CMV infection and the risk of developing PD

The relationship between CMV infection and the Hazard ratio (HR) of developing five neurodegenerative diseases was explored through Cox regression. Cox regression analysis revealed that no significant associations were found between CMV infection and the risk of SMA and related syndromes (model 1: HR [95% CI] = 3.57 [0.64, 19.80], P = 0.146; model 2: HR [95% CI] = 3.52 [0.63, 19.61], P = 0.152), AD (model 1: HR [95% CI] = 1.01 [0.57, 1.81], P = 0.965; model 2: HR [95% CI] = 1.00 [0.56, 1.79], P = 0.999), MS (model 1: HR [95% CI] = 1.16 [0.45, 2.97], P = 0.756; model 2: HR [95% CI] = 1.16 [0.45, 2.97], P = 0.761), and DANS (model 1: HR [95% CI] = 0.65 [0.16, 2.66], P = 0.552; model 2: HR [95% CI] = 0.65 [0.16, 2.64], P = 0.543) (Fig. 1, Supplementary Table S1). However, we found that individuals with lower CMV pp52 or who were CMV seronegative had a higher risk of PD than those who were CMV seropositive (pp52 vs seropositive: HR [95% CI] = 1.12 [1.00, 1.25] in Model 1, adjusted for batch effect, age, sex, and TDI, and Model 2, further adjusted for T2D, cancer, OP, vitamin D, monocyte count, and leukocyte count; for seronegative vs

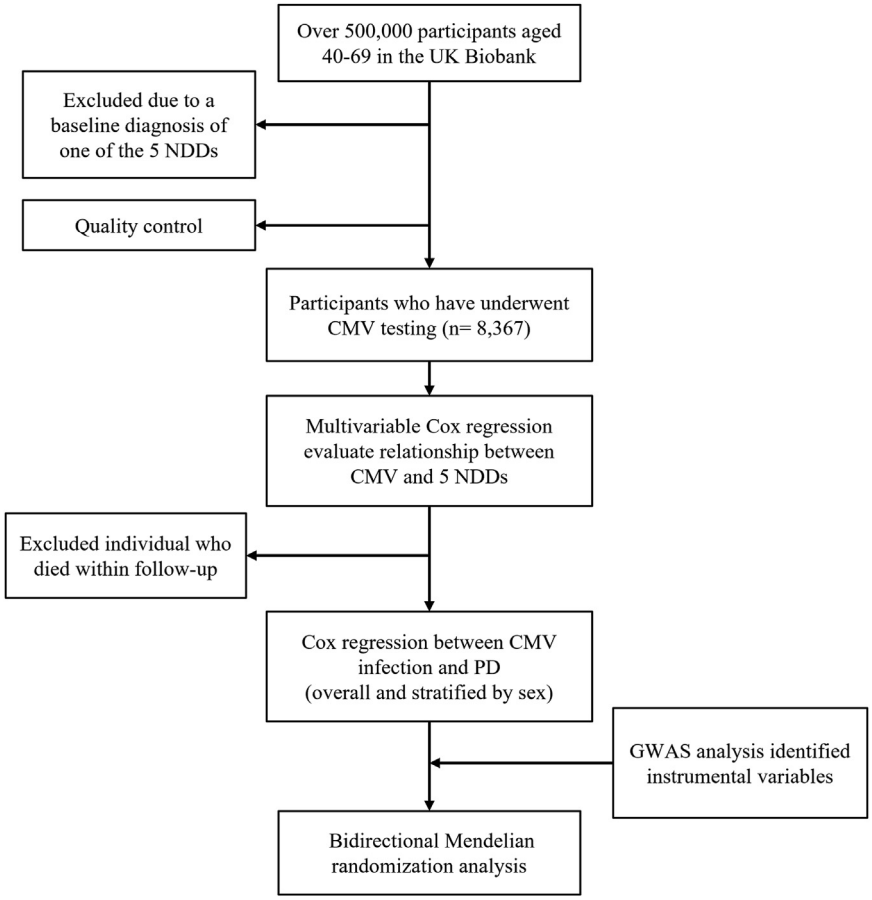

Fig. 1: **Flowchart of the study.**

seropositive: HR [95% CI] = 1.77 [1.03, 3.02] in Model 1 and Model 2). There were no significant correlations found between either CMV pp28 or pp150 and PD risk.

RCS analysis was conducted to evaluate the relationship between CMV infections and the risk of developing PD. The results showed a gradual decrease in HR as CMV titer increased (Fig. 2A–C). Nonlinear assessment results were not statistically significant ($P = 0.465$ for CMV pp28, $P = 0.474$ for CMV pp52, and $P = 0.244$ for CMV pp150), indicating no evidence of nonlinearity between CMV antigens and PD risk. During the follow-up period, participants with high titers (Q4) for CMV pp28, pp52 and pp150 had a lower incidence rate of PD compared to those with low titers (Q1) (Fig. 2D–F).

Through the cause-specific hazard method, a Competing Risk Model was employed to evaluate the impact of death on the PD incidence. The findings indicated a competitive risk relationship between death and PD (PD: Z = 3.705, $P = 0.054$; death: Z = 4.055, $P = 0.044$), suggesting that death may actually prevent the occurrence of PD. Furthermore, when using all-cause death and PD as the composite endpoint, the results did not show any significant difference between CMV seropositivity and seronegative individuals ($P = 0.067$, Supplementary Table S2). Only using PD as an endpoint, a sensitivity analysis was performed by excluding individuals who had died, demonstrating that CMV infection decreases the risk of developing PD even further ($P = 0.008$ in model 2, Table 2), compared to not excluding deceased individuals ($P = 0.038$ in model 2, Supplementary Table S3). This suggests an increased protective effect of CMV on PD after accounting for the competing risk event of death. Additionally, further analyses were conducted by stratifying the data by sex. Males with seronegative had significantly higher HRs of PD than those with seropositivity in the two models (model 1: HR 3.16 [1.42, 7.07], $P = 0.005$; model 2: HR 3.41 [1.50, 7.71], $P = 0.003$). However, no significant difference was observed in the female subgroup ($P = 0.684$ in model 2, Table 2). Furthermore, the lower levels of CMV antigens, including pp28 (model 1: HR = 1.30 [1.05, 1.60], $P = 0.016$; model 2: HR = 1.33 [1.07, 1.65], $P = 0.009$), pp52 (model 1: HR = 1.30 [1.09, 1.54], $P = 0.003$; model 2: HR = 1.31 [1.10, 1.55], $P = 0.002$) and pp150 (model 1: HR = 1.28 [1.07, 1.54],

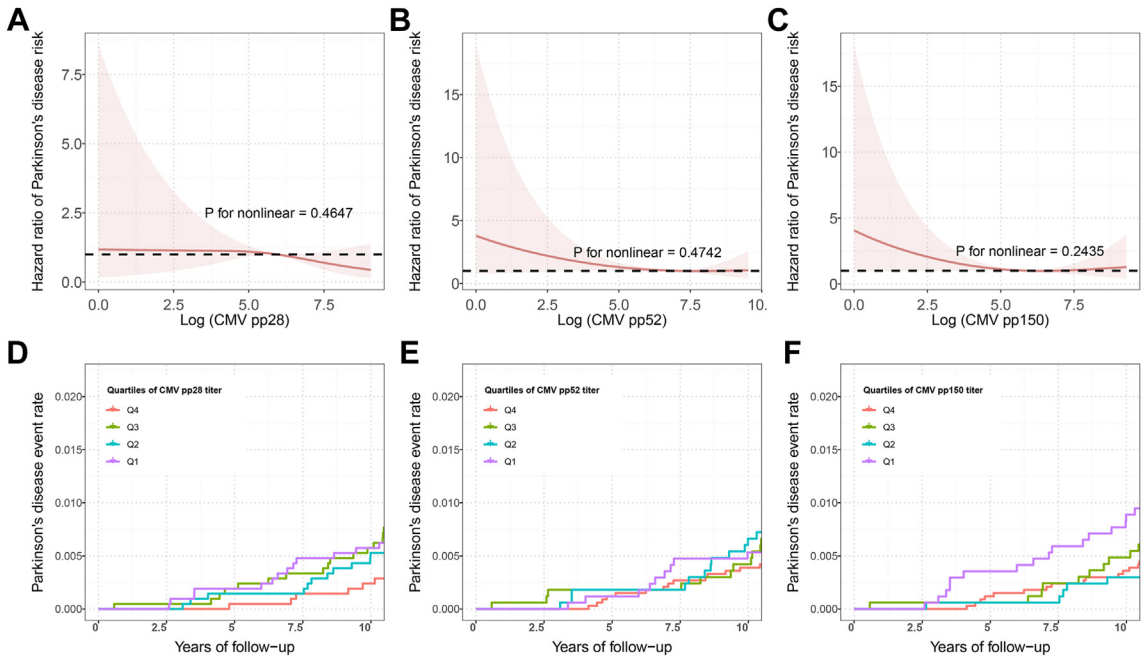

Fig. 2: **Restricted cubic splines (RCS) assessing the nonlinear association between CMV infection and Parkinson's disease (PD).** (A) No nonlinear association between CMV pp28 and PD was shown (*P* for nonlinear = 0.465). (B) No nonlinear association between CMV pp52 and PD was shown (*P* for nonlinear = 0.474). (C) No nonlinear association between CMV pp150 and PD was shown (*P* for nonlinear = 0.243). Shaded areas represent 95% CIs. (D) The event rate of PD patients with different levels of CMV pp28 titers was shown. (E) The event rate of PD patients with different levels of CMV pp52 titers was shown. (F) The event rate of PD patients with different levels of CMV pp150 titers was shown. Titer levels were divided into quartiles of Q1-Q4. Abbreviation: RCS, Restricted cubic splines; CMV, cytomegalovirus; PD, Parkinson's disease; CI, confidence interval.

P = 0.007; model 2: HR = 1.31 [1.09, 1.57], *P* = 0.004), were associations with the higher risk of developing PD in males (Table 2).

Moreover, upon conducting analysis based on CMV titer quartiles, it was revealed that male patients with low CMV antigen titers (Q1) of pp28, pp52 and pp150 had higher HRs of PD in both models when compared to those with high CMV titers (Q4). Nevertheless, this discrepancy was not statistically significant among female patients (Fig. 3).

GWAS analysis identified a significant SNP for human CMV

A GWAS of human CMV was conducted using data in the UK Biobank to identify significant SNPs and the genome-wide MAGMA analysis was used to enrich their pathways and explore the tissue-specific distribution.

A genome-wide significant SNP (rs2051738, $P = 1.38 \times 10^{-7}$) was identified through GWAS analysis. Fig. 4 and Supplementary Fig. S1 illustrate the –log10 (*P* value) of the GWAS of human blood CMV and the Q–Q plot, respectively, with no genome inflation found. rs2051738 is mapped to the gene *SHISA9*, and the protein is predicted to be located in the synapse and as a part of the AMPA glutamate receptor complex in the synapse. The genome-wide MAGMA analysis revealed that the 10 most significantly enriched pathways included the pid myc repression pathway and the glutamate gated calcium ion channel activity pathway ($P < 0.001$) (Supplementary Fig. S2).

The genome-wide MAGMA analysis revealed the tissue specificity of CMV associations, providing insights into the tissues where genes are most relevant to the disease. We found that the brain and cervical spinal cord were associated with the onset of PD and ranked 12th (Fig. 5).

Mendelian randomization (MR) analysis

We investigated the causality between CMV and PD through MR analysis. By using the instrumental variable rs2051738, our findings showed that CMV did not decrease the risk of PD (odds ratio [OR] = 0.49, 95% CI: 0.15 to 1.67, *P* = 0.26) with the threshold 5×10^{-7} by the IVW method (Fig. 6), suggesting no genetic causality between CMV infection and PD. This result was consistent with different thresholds (5×10^{-6} to 1×10^{-5}) and even under a loose threshold ($P < 1 \times 10^{-5}$, OR = 0.99, 95% CI: 0.69 to 1.42, *P* = 0.945) (Fig. 6).

Sensitivity analysis using the weighted mode (OR = 0.86, 95% CI: 0.42 to 1.78, *P* = 0.677) or weighted

Model	Sex	Virus	HR (95%CI)	P	FDR
Model 1	All	Lower CMV pp28	1.208 (1.017, 1.435)	0.032	0.053
		Lower CMV pp52	1.2 (1.05, 1.371)	0.007	0.024
		Lower CMV pp150	1.198 (1.036, 1.386)	0.015	0.024
		CMV seronegative vs seropositivity	2.369 (1.246, 4.506)	0.009	
Model 2	All	Lower CMV pp28	1.218 (1.024, 1.448)	0.026	0.026
		Lower CMV pp52	1.199 (1.049, 1.37)	0.008	0.020
		Lower CMV pp150	1.202 (1.039, 1.391)	0.013	0.020
		CMV seronegative vs seropositivity	2.385 (1.253, 4.541)	0.008	
Model 1	Female	Lower CMV pp28	1.05 (0.774, 1.423)	0.754	0.754
		Lower CMV pp52	1.041 (0.825, 1.314)	0.733	0.754
		Lower CMV pp150	1.048 (0.813, 1.351)	0.717	0.754
		CMV seronegative vs seropositivity	1.277 (0.401, 4.073)	0.679	
	Male	Lower CMV pp28	1.297 (1.049, 1.603)	0.016	0.016
		Lower CMV pp52	1.297 (1.094, 1.536)	0.003	0.008
		Lower CMV pp150	1.282 (1.07, 1.537)	0.007	0.011
		CMV seronegative vs seropositivity	3.163 (1.415, 7.072)	0.005	
Model 2	Female	Lower CMV pp28	1.064 (0.783, 1.446)	0.692	0.732
		Lower CMV pp52	1.041 (0.825, 1.314)	0.732	0.732
		Lower CMV pp150	1.051 (0.816, 1.355)	0.698	0.732
		CMV seronegative vs seropositivity	1.272 (0.398, 4.062)	0.684	
	Male	Lower CMV pp28	1.33 (1.073, 1.648)	0.009	0.004
		Lower CMV pp52	1.308 (1.104, 1.549)	0.002	0.004
		Lower CMV pp150	1.307 (1.087, 1.572)	0.004	0.004
		CMV seronegative vs seropositivity	3.405 (1.504, 7.711)	0.003	

Model 1: adjusting for batch, age, sex, TDI; Model 2: adjusting for batch, age, sex, TDI, T2D, Cancer, OP, Vitamin D, monocyte count, white blood cell leukocyte count. HR, hazard ratio; CI, confidence interval; FDR, False Discovery Rate.

Table 2: **Sensitivity analyses for CMV infection and the risk of developing Parkinson's disease in the overall population, females, and males, excluding death individuals.**

median (OR = 0.96, 95% CI: 0.59 to 1.57, $P = 0.884$) also yielded the same result. The MR−Egger regression intercept was –0.017 (95% CI: –0.072 to 0.038, $P = 0.539$), indicating no multiple effects. MR-PRESSO analysis showed no outliers, with a global test estimate of 8.51 ($P = 0.882$). The estimated value of MR-PRESSO was 0.98 (95% CI: 0.70 to 1.36, $P = 0.890$, Fig. 6A and B). Scatter plots and funnel plots suggested no horizontal pleiotropy between CMV and PD. Finally, reverse MR was applied to investigate the potential genetic causal effect between CMV and PD, with no evidence been found (Fig. 6C and D).

Discussion

This study used a large-scale prospective cohort data to investigate the associations of CMV infection with the risk of developing PD, AD, SMA and related syndromes, MS, and DANS. Our findings suggest that individuals with a history of CMV infection had a reduced risk of developing PD. In addition, this association was more significant in the male subgroup. However, CMV infection did not affect the incidence of AD, spinal muscular atrophy and related syndromes, MS, or DANS. Moreover, we identified an independent, significant SNP (rs2051738) in the SHISA9 gene, whose protein is a component of the AMPA glutamate receptor complex in the synapse. The causal relationship between CMV and PD was not validated.

In recent years, there has been increasing evidence suggesting that viral infections may be associated with neurodegenerative diseases, especially certain opportunistic pathogen such as herpes simplex virus type 1 (HSV1), HSV2, Varicella zoster virus (VZV), and cytomegalovirus (CMV).[16,17] An example is a high prevalence of HSV-1 and the subsequent occurrence of AD.[16] HSV-1 is an opportunistic infection of the nervous system. After infection, it replicates and remains latent in sensory neurons of the peripheral ganglia after infection.[1] A compromised immune environment can lead to reactivation of CMV, resulting in viral replication at the primary infected site of infection and spread to the brain and other areas. This infection is usually clinically asymptomatic. Recurrent HSV-1 reactivation has been implicated in the pathogenesis of amnestic mild cognitive impairment and AD.[16] In addition, during the COVID-19 pandemic, patients with PD were observed to have worsening motor disorders and an increase in their levodopa equivalent daily dose, which is part of the post-COVID-19 syndrome.[17] However, epidemiological studies of the association between CMV and PD are limited.

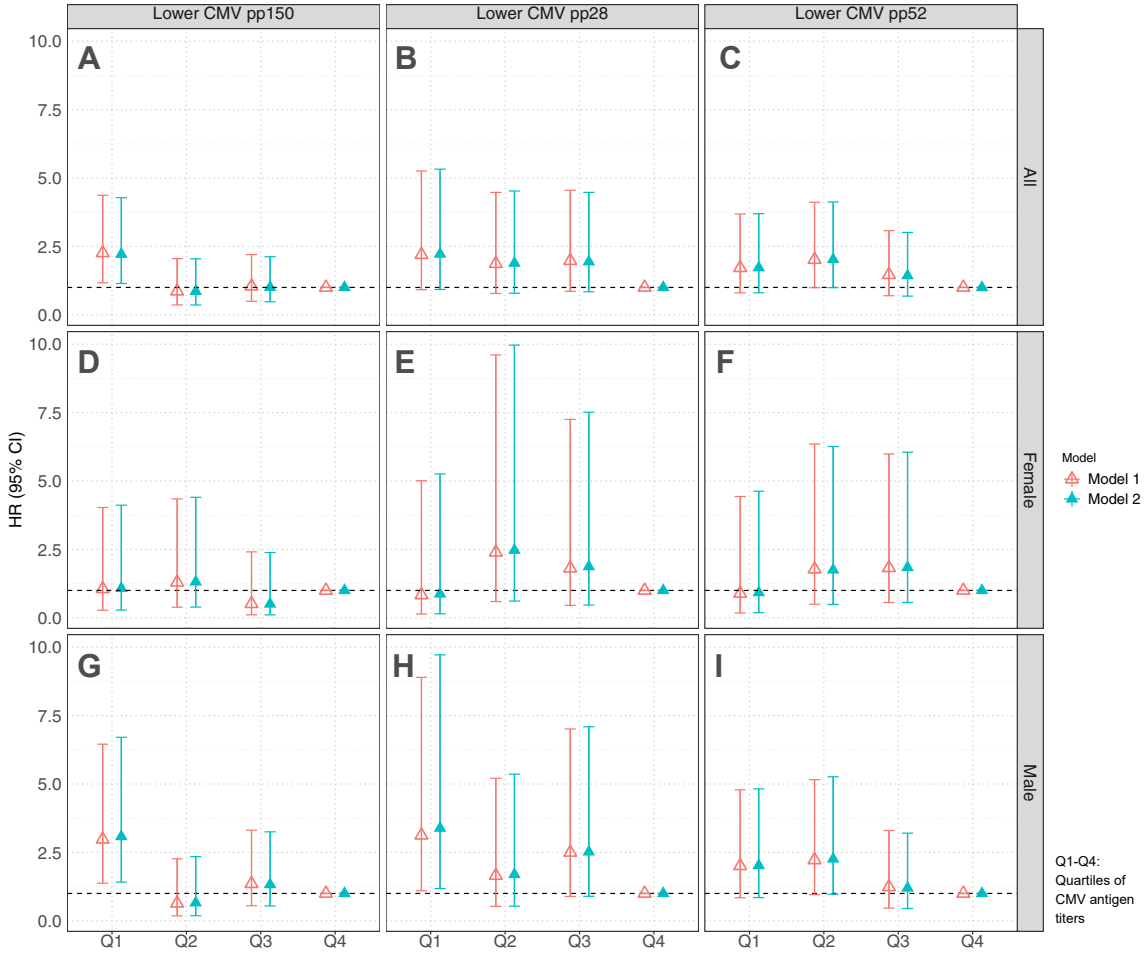

***Fig. 3*: The HR of PD is presented based on different CMV titers (Q1–Q4) stratified by sex.** Model 1 (red) was adjusted for batch, age, sex and TDI. Model 2 (blue) was adjusted for batch, age, sex, TDI, T2D, cancer, OP, vitamin D, monocyte count and white blood cell leukocyte count. (A–C) The hazard ratio (HR) of developing PD varies with different levels of pp150, pp28 and pp52 in overall population. (D–F) The HR of developing PD varies with different levels of pp150, pp28 and pp52 in females. (G–I) The HR of developing PD varies with different levels of pp150, pp28 and pp52 in males. Abbreviation: HR, hazard ratio; TDI, Townsend deprivation Index; T2D, type 2 diabetes; OP, osteoporosis.

Previous studies have suggested that CMV infection might elevate the risk of AD and expedite cognitive decline in older adults.[18] However, our study did not find the same association. It is conceivable that AD often occurs in individuals aged 65 and above, whereas the average age of participants in our study was around 56.74 years old. Thus, a prolonged follow-up duration may be needed to fully understand the impact of CMV on AD. Although it has also been reported that CMV infection was linked to the development of MS.[19] The results of cox regression in our study showed that CMV infection was not significantly associated with the risk of MS. This discrepancy may be attributed to the fact that the average age of MS onset is around 30 years old,[20] which is much younger than the age range of participants in our study (40–60). There is currently no research on the potential relationships between CMV and PD, SMA, and DANS.

The study unexpectedly discovered that CMV infection was associated with a decreased risk of developing PD. When looking at sex differences, it was observed that men with seropositivity or lower levels of CMV antigens were more likely to develop PD, while this relationship was not seen in women. Previous research has shown that men are twice as likely to develop PD compared to women. It is possible that the mechanisms involved in PD development differ between male and female patients.[21] For example, CMV infection may attribute to coronary artery disease in men by triggering an inflammation, while in women, other mechanisms such as humoral or immune responses may be more important.[22] In this study, no significant associations were found between the CMV infection and the risk of developing SMA and related syndromes and DANS. Despite the lack of CMV antigen testing in most

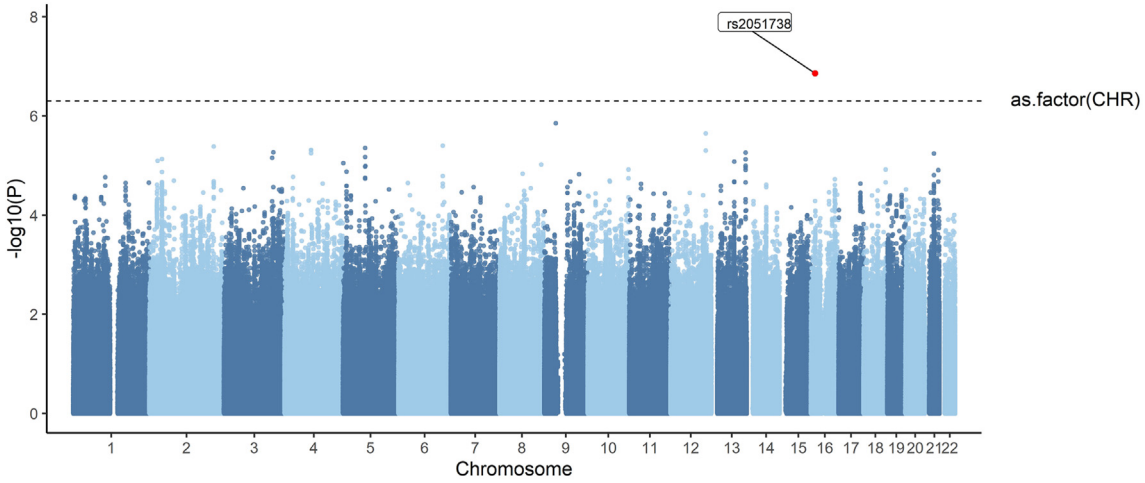

Fig. 4: **Genome-wide association study.** In Manhattan plots, each point represents the location of a SNP on the chromosome (x-axis) and –log10 (*P*-value) for the strength of its association (y-axis). The red line indicating the threshold for –log10 ($5E^{-7}$). The SNP above the red line is with strongly associated with the CMV. Abbreviation: SNP, single nucleotide polymorphism.

individuals in the UK Biobank, the large sample size and consideration of cofounding factors helped to minimize bias in the research.

Specific viral infections may reduce risk of developing PD through immune system response. As mentioned above, viral infection can increase the risk of occurrence or worsening of neurodegenerative disease. Viruses can release DNA fragments that cause neuroinflammation, leading to apoptosis of nigrostriatal dopamine neurons and the onset of PD. However, viruses can also activate the immune system and induce specific adaptive immunity and trained immunity. Virus-specific adaptive immunities, including cellular and humoral immune responses, are generally directed against the specific virus. While trained immunity is also useful in fighting against heterologous infections.[23–25]

Innate immunity has traditionally been viewed as a no-adaptive, first-line defense against invading microorganisms without immune memory.[26] However,

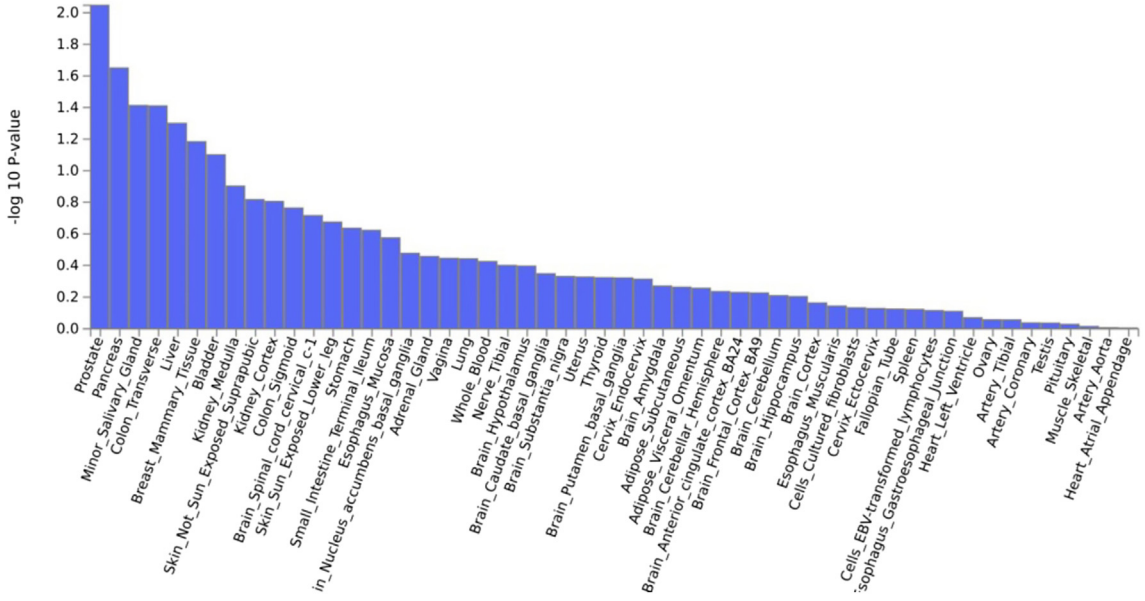

Fig. 5: **The genome-wide MAGMA analysis for SNPs.** The genome-wide MAGMA analysis revealed the tissue-specifical distribution of SNPs, providing a ranking of tissues that are significantly associated with CMV. Abbreviation: MAGMA, multimarker analysis of genomic annotation.

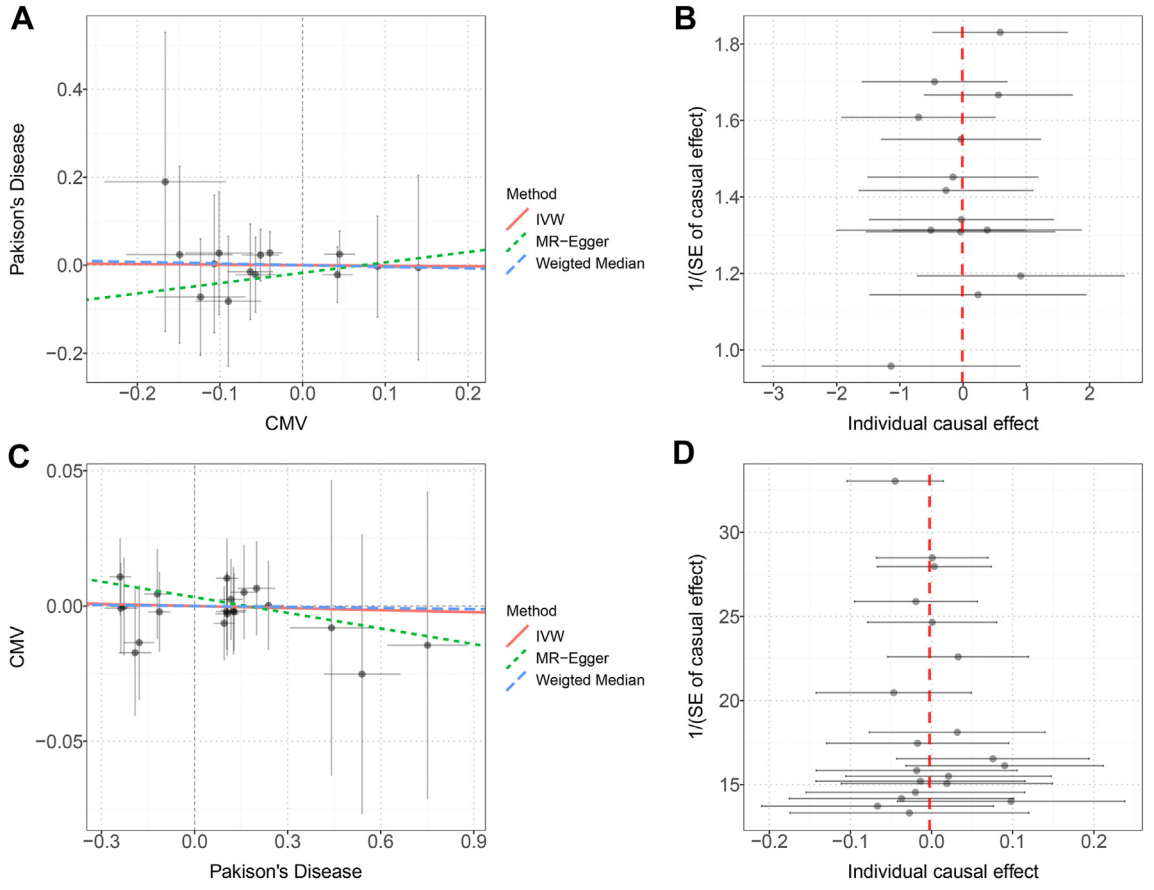

***Fig. 6:* No causality between the CMV infection and PD was established in BMR analysis.** (A) Scatter plot shows no causal effect of CMV on Parkinson's disease. Each point represents a SNP, with the line at each point showing its 95% confidence interval. The horizontal axis represents the effect of the SNP on the exposure factor (CMV), while the vertical axis represents the effect on the outcome factor (PD). The colored lines indicate the effect of exposure on outcome, with the red representing the IVW algorithm, the green representing the MR-Egger algorithm and the blue representing weighted median algorithm. (B) The funnel plot shows the heterogeneity of the SNPs, with the red representing the IVW algorithm, the green representing the MR-Egger algorithm and the blue representing weighted median algorithm. The points on both sides of the line are fairly symmetrical, with no significant outliers, suggesting low heterogeneity of the SNPs. (C) Scatter plot of the causal effect of Parkinson's disease on CMV. (D) Funnel plot of the causal effect of Parkinson's disease on CMV. Abbreviation: BMR, bidirectional Mendelian randomization.

researchers have discovered that innate immunity can also display a new form of "immune memory", similar to the adaptive immunity induced by certain infections or vaccines. This is known as trained immunity,[27] which is triggered by specific pathogens and provides protection against other pathogens and is attributed to epigenetic reprogramming of innate immune cells, such as neutrophils, monocytes, macrophages, natural killer cells, and γδ T cells.[28] Through trained immunity, the host has a high responsiveness and clearance capacity to the reinfected same or different pathogens and removes them by innate immune cells such as NK cells, DCs, and monocytes/macrophages. This hypothesis is also supported by the effect of attenuated live or viral vector vaccines.[27] For example, the Bacille Calmette-Guérin vaccine against tuberculosis has been shown to provide heterologous protection against non-tuberculous infections.[29] In particular, it has recently been proposed as a potential preventive strategy against COVID-19.[30] In addition, a replication-competent adenovirus type 4 encoding influenza virus H5 HA (Ad4-H5-Vtn) has been shown to induce durable systemic and mucosal immunity.[31] Similarly, attenuated CMV vaccine and vectored CMV vaccine have been found to increase CD4+ and CD8+ T cell levels,[32] suggesting that trained immunity may be effective against other pathogens.

Age is a major risk factor for many neurodegenerative diseases, as the aging process can lead to a decline in the efficiency of the innate immune and adaptive immune systems. In aging progression, the change of innate immune system is characterized by the dysfunction of the effector cells, such as microglia,

monocytes, dendritic cells and natural killer cells, and the adaptive immune systems show a decrease in the number, the diversity and sensitivity of T, B cells and their receptors, accompanying an accumulation of memory cells.[33] The combination of aging, genetic and environmental factors can create a perfect storm for the development and progression of PD.[34] However, the role of trained immunity in the development and progression of PD has not yet been thoroughly investigated. As inflammation is an important factor in the development of immunity, further research is needed to determine whether the protective effects of CMV infection are due to changes in immunity. Previous studies have found that CMV infection could accelerate CD8+ T cell senescence, whereas T cell senescence appears to be attenuated in PD patients.[35,36] This also supports that CMV may impact PD by altering the immune environment of affected individuals.

Currently, researchers are focusing on developing CMV vaccines that target specific antigens, including glycoprotein B, glycoprotein H, the pentameric complex and tegument phosphorylated proteins (TPP). These TPPs play crucial roles in the CMV life cycle, with pp65 involved in immune evasion, pp71 in gene expression, pp150 and pp28 in virus assembly and egress, and pp52 in inducing a specific and high immune response.[37] Since that CMV pp28, pp52 and pp150 have been linked to a decreased risk of developing PD, a potential CMV vaccine utilizing these antigens could potentially provide benefits for individuals at risk. With no current curative treatments for PD, exploring the use of CMV vaccines for preventing against the disorders shows promise. More research on the clinical application of CMV vaccines for this purpose is necessary.

In this study, we further identified an independent significant SNP (rs2051738) in the *SHISA9* gene that was associated with the CMV GWAS analysis. *SHISA9* encodes a subunit of the AMPA glutamate receptor complex in the synapse, which regulates AMPAR activity and short-term neuronal synaptic plasticity. In addition, AMPA receptor antagonists have been shown to reduce motor symptoms in animal models of PD.[38,39] This evidence supports a role for AMPAR in the pathophysiology of PD. However, the bidirectional Mendelian randomization analysis did not establish genetic causality between the change in SHISA9 caused by CMV infection and PD. Although a correlation was established between CMV infection and the onset of PD through Cox regression, this does not definitely indicate a genetic association. Our hypothesis suggests that CMV infection may decrease the risk of developing PD, but it is important to note that neither CMV infection nor its absence directly causes PD.

There are still some limitations to this study. First, our study was limited to white British individuals, so the results may not be generalizable to other populations such as Asian people. Second, the average age of PD onset is typically around 60 years old, whereas the individuals who underwent CMV testing had an average age of 56.74. This age discrepancy could potentially impact the results, as some individuals may not have developed PD yet and may need longer-term monitoring. Furthermore, the onset age of AD is much later, suggesting that the effects of CMV infection on AD may not have become apparent yet. Finally, the sex of the participants in the study was determined by a combination of the NHS record and the self-reported questionnaire, resulting in limited information about their biological identities. Overall, these limitations highlight the need for further research to fully understand the relationship between CMV infection, genetic factors, and the risk of developing neurodegenerative diseases such as PD.

In summary, the results of this study indicate that CMV infection may reduce the risk of developing PD in the future for males. Further studies, including clinical and animal experiments, are needed to explore the underlying pathogenesis. This finding may highlight the likely benefit of a CMV vaccine for males, as there are currently no curative therapies for PD.

Contributors

X.G. conceived and designed the study, H.T., K.W. and C.F. performed the analysis, X.M., Z.L., X.G. and C.F. finished the manuscript, X.Z., J.H. P.J., and S.P. collected the data, X.G., P.X. and L.H. verified the underlying data, W.L., H.L. and X.M. assisted the data analysis and revised the article. All authors have read and approved the final version of the manuscript.

Data sharing statement

Data relevant to the study were acquired from the UK Biobank Resource. The UK Biobank application number is 69550. Further details can be found on the official UK Biobank website (https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us/ethics). The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102757.

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