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A Hidden Menace? Cytomegalovirus Infection is Associated with Reduced Cortical Gray Matter Volume in Major Depressive Disorder

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Abstract

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CONFLICT OF INTEREST

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Human cytomegalovirus (HCMV) infection is associated with neuropathology in patients with impaired immunity and/or inflammatory diseases. However, the association between gray matter volume (GMV) and HCMV has never been examined in major depressive disorder (MDD) despite the presence of inflammation and impaired viral immunity in a subset of patients.

We tested this relationship in two independent samples consisting of 179 individuals with MDD and 41 healthy controls (HC) (sample 1) and 124 MDD participants and 148 HCs (sample 2). HCMV positive (HCMV+) and HCMV negative (HCMV−) groups within each sample were balanced on up to eleven different clinical/demographic variables using inverse probability of treatment weighting. GMV of 87 regions was measured with FreeSurfer. There was a main effect of HCMV serostatus but not diagnosis that replicated across samples. Relative to HCMV− subjects, HCMV+ subjects in sample 1 showed a significant reduction of volume in six regions ($p_{\text{uncorrected}}<0.05$). The reductions in GMV of the right supramarginal gyrus (standardized beta coefficient (SBC)=−0.26) and left fusiform gyrus (SBC=−0.25) in sample 1 were replicated in sample 2: right supramarginal gyrus ($p_{\text{uncorrected}}<0.05$, SBC=−0.32), left fusiform gyrus ($P_{\text{FDR}}<0.01$, SBC=−0.51). Posthoc tests revealed that the effect of HCMV was driven by differences between the HCMV+ and HCMV− MDD subgroups. HCMV IgG level, a surrogate marker of viral activity, was correlated with GMV in the left fusiform gyrus (r=−0.19, $P_{\text{uncorrected}}=0.049$) and right supramarginal gyrus (r=−0.19, $p_{\text{uncorrected}}=0.043$) in the HCMV+ group of sample 1. Conceivably, HCMV infection may be a treatable source of neuropathology in vulnerable MDD patients.

Keywords

Cytomegalovirus; Depression; Inflammation; Magnetic Resonance Imaging; Gray Matter Volume

INTRODUCTION

Approximately 50% of the US population is infected with human cytomegalovirus (HCMV). HCMV is not cleared after initial infection but establishes life-long latent infections, persisting in myeloid lineage cells, vascular pericytes, endothelial cells of the blood-brain barrier as well as glia, neurons, and neural precursor cells[1–3]. The capacity of HCMV to infect the brain may explain why it is a well-established cause of CNS damage[4]. This neuropathology is traditionally thought to occur only within limited clinical contexts such as in immunologically-naïve or immune-compromised patients (congenital infection, AIDS patients, transplant recipients) in whom the once dormant virus becomes reactivated. However, even in individuals who are not immunosuppressed, reactivation of HCMV occurs periodically when anti-viral immunity is weakened, i.e. during times of stress, including depression, as well as in the context of inflammation and cellular damage[5–8]. This raises the possibility that HCMV may also be a source of neuropathology in these populations.

HCMV IgG titer is a surrogate marker of viral activity with higher levels indicative of an active infection[9]. Neuro-immune stress pathways promote reactivation of HCMV via adrenergic signaling[10]. Consistent with this mechanism, HCMV IgG titer was found to be higher during exams in medical students[6, 11]; caregivers to disabled children had elevated IgG titers[5], and viral DNA shedding was higher in astronauts directly before and after

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space travel[12, 13]. Similarly, associations between psychological stressors and herpesviruses (including HCMV) levels are commonly reported[13–17]. Reactivation of HCMV is also induced by inflammatory mediators such as TNF[7] and inflammatory diseases such as sepsis are known drivers of HCMV reactivation, a risk factor for adverse outcomes in these patients[8].

Among patients with inflammatory disorders, including autoimmune disorders[18] and HIV[19], there is evidence that HCMV infection is a major driver of pathology. For example, HCMV positive (HCMV+) multiple sclerosis patients showed greater brain atrophy over time than HCMV negative (HCMV−) patients[20] and higher antibody levels during a first demyelinating event predicted greater loss of gray matter volume (GMV) over time[20]. Brain tissue donated by clergy showed that higher lifetime anti-HCMV IgG titers were associated with the presence of neurofibrillary tangles[21]. Further, the CD4+ response to the HCMV pp65 antigen was associated with a pathological diagnosis of Alzheimer's disease (AD)[21]. In depression, inflammation is present[22–24] but it is not known whether HCMV is a source of neuropathology in these patients.

Although the relationship between structural brain abnormalities and HCMV has not to our knowledge been studied in major depressive disorder (MDD), HCMV has been linked with depression in at least 13 studies[25–38]. This may be because depression is associated with impaired anti-viral immunity, rendering individuals vulnerable to both initial infection and subsequent reactivation of HCMV. Evidence for impaired adaptive immunity in depression takes the form of a decreased proliferative response of lymphocytes to mitogens, decreased natural killer cell function and lymphopenia *in vitro*[39], upregulated expression of inflammation-related genes together with down-regulated expression of antiviral genes[40, 41], poorer control of chronic viral infections[42], impairment of vaccine-induced immunity to the varicella-zoster virus[43], reduced vaccine-induced antibody response to hepatitis B[44], and a loss of childhood vaccine-induced immunity to measles[45]. Of note, loss of immunity to measles has been shown to occur in contexts where HCMV is known to cause disease, i.e. in patients undergoing chemotherapy[46], organ transplantion[47] or with HIV[48]. Further, experimental studies show that subjects exposed to rhinovirus or influenza are more likely to become infected and show clinical symptoms if they endorse recent stress[49, 50].

Taken together, there is mounting evidence that: (1) HCMV infection can damage the brain in immunosuppressed populations; (2) HCMV is a source of pathology in non-immune-suppressed populations with inflammatory diseases; (3) HCMV is reactivated by stress and inflammation; (4) a biotype of MDD is characterized by inflammation and impaired viral immunity; and (5) these depressed individuals may be vulnerable to reactivation of HCMV. We therefore hypothesized that HCMV may contribute to structural brain abnormalities in MDD. This is not just a question of theoretical interest but has treatment implications given the existence of well-tolerated anti-HCMV medications and the ongoing development of HCMV vaccines[51, 52].

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METHODS

Participants

Approval for the study was obtained from the Western Institutional Review Board and written informed consent was obtained from all participants. Two independent groups of participants were included in the study, 303 participants in sample 1, and 462 in sample 2.

Sample 1.—Participants were aged 18–55 years and either had no personal history of psychiatric illness (healthy controls, HC) or received a DSM-V diagnosis of MDD (with or without comorbid anxiety) based on the Mini International Neuropsychiatric Inventory (MINI). Data were collected between January 2015 and February 2017. Subjects completed PROMIS scales for depression and anxiety and the childhood trauma questionnaire (CTQ) for early-life stress measurement. Lifetime alcohol use was measured by the Customary Drinking and Drug use Record (CDDR) interview. Exclusion criteria included: comorbid psychiatric disorders (except anxiety disorders), neurological disorders, unstable medical conditions, a history of moderate-to-severe traumatic brain injury, a positive urine drug screen, and general MRI exclusion criteria (details in[53]).

Sample 2.—Participants (aged 18–55 years) met DSM-IV-TR criteria for MDD based on the Structured Clinical Interview for the DSM-IV-TR and unstructured psychiatric interviews. Data was collected between October 2010 and November 2016. Depressive symptoms were measured with the Montgomery-Asberg Depression Rating Scale (MADRS). Exclusion criteria included medical conditions or medications likely to influence CNS or immunological function, a history of drug or alcohol abuse within 6 months or a history of drug or alcohol dependence within 1 year (DSM-IV-TR criteria), and general MRI exclusion criteria. The same exclusion criteria applied to HCs (details in[54, 55]).

Anti-CMV IgG Antibodies and C-Reactive Protein

Morning blood samples were collected and frozen at −80 °C. Blood samples were processed using standard laboratory procedures. Thawed plasma samples were tested blind to diagnosis for IgG antibodies using a solid-phase ELISA (IBL America, catalog #EI2570–9601G). A sample was considered seropositive if it had an optical density value of >0.5, which is equivalent to approximately ten international units of antibody. Intra-and inter-assay coefficients of variation were 5% and 10%, respectively. Due to the non-normal distribution, the density value was converted to a z score.

For the sample 1, serum concentrations of c-reactive protein (CRP) were analyzed with the V-PLEX Neuroinflammation Panel-1 Human Kit (Meso Scale Diagnostics) with a lowest level of quantification (LLOQ) of 0.027 mg/L and intra- and inter-assay coefficients of variation of 2% and 10%, respectively. For sample 2, hs-CRP was measured immunoturbidimetrically with the Kamiya Biomedical K-Assay in a hospital laboratory with an LLOQ of 0.1 mg/L.

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Image acquisition

For both samples T1-weighted anatomical images were acquired on two identical 3T scanners (GE Discovery MR750) using an MPRAGE sequence with following parameters: FOV=240mm, 186 slices, slice thickness=0.9mm, voxel dimensions=0.938×0.938×0.9mm^3, image matrix=256×256, TR/TE=5/2.012 ms, acceleration factor R=2 in the phase encoding direction, flip angle=8 degrees.

Image preprocessing

Cortical reconstruction and volumetric segmentation were performed using FreeSurfer version 6.0.0[56]. Whole-brain GMVs were estimated from individual anatomical images including 68 cortical regions (34 regions per hemisphere) using the Desikan-Killiany atlas and 19 subcortical regions using FreeSurfer standard subcortical segmentation[57] (ventricles, cerebrospinal fluid, and white matter regions were excluded). Visual inspection of all cortical segmentation was performed before analysis for quality assurance purpose. FreeSurfer has been validated against histological measurements[58] and demonstrates good test-retest reliability[59].

Statistical analysis

Statistical analyses were performed using RStudio V1.1.463 and R version 3.5.3.

Confounding is a major concern in observational studies. Although multivariate regression is commonly used to control for potential confounds, it can be highly sensitive to the form of the model and interaction terms. Additionally, if the groups differ greatly in the distribution of covariates, regression can lead to biased estimation. In contrast, the propensity score approach, i.e. the inverse probability of treatment weighting (IPTW)[60, 61], offers a transparent and effective analytical tool for adjusting confounding factors. The method creates a weighted population that has similar baseline characteristics between groups which then only differ on the independent variable of interest, in this case, HCMV status.

Following standard guidelines[62], several steps were carried out to implement the IPTW: First, a propensity score was used to combine the information from the covariates into a single variable. The propensity score was defined as the likelihood of being HCMV+ and was determined from a multivariate logistic regression model containing the following independent variables for sample 1: age, sex, BMI, education, income, PROMIS depression score, PROMIS anxiety score, medication status (yes/no), early-life stress (CTQ score), number of depressive episodes (MINI interview), and lifetime alcohol use (CDDR interview). The propensity score for sample 2 was obtained from the same variables except that depression was measured with the MADRS and anxiety scores, income, number of depressive episodes, and lifetime alcohol use were not available. Standardized mean differences were calculated to examine covariate balance before and after IPTW. Second, the stabilized weights were calculated from the propensity score using the ‘ipw’ package. By down-weighting the characteristics of subjects who were over-represented and up-weighting subjects who were under-represented in the samples, this procedure is thought to allow the weighted samples to estimate the characteristics of samples derived from randomized

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experiments[63]. Finally, to estimate the main effect of HCMV on GMV, the main effect of diagnosis, and their interaction while accounting for the weights and estimating robust standard error, weighted generalised linear regression models from the 'Survey' package were used at each of the regions. The total intracranial volume (TIV) was added into the model as a covariate to adjust for individual differences in overall brain size. Posthoc tests were performed to estimate the effect of HCMV in the MDD and HC groups, separately.

We used a discovery/replication design[64] and set an uncorrected $p<0.05$ (two-tailed) as the threshold. The regions showing significant differences in sample 1 were selected as ROIs to be tested in sample 2. The ROIs that were significantly associated with HCMV status in both sample 1 and sample 2, and were in the same direction in both samples were considered statistically significant. The false discovery rate (FDR) was used to control for multiple testing. To assess the sensitivity of results to the selection of balancing covariates, a general linear model without-adjustment for any covariates except TIV was also performed. To evaluate the robustness of the results to potential unmeasured confounders, we calculated the E-value using the 'EValue' package[65]. The E-value estimates the minimum effect an unmeasured confounder would need to have in order to be able to explain away an observed association with the outcome of interest. To test for associations between HCMV IgG level, CRP concentrations, and GMVs, CRP was log-transformed and then correlation analyses were performed within the combined MDD/HC HCMV+ groups.

Data availability

The full analysis code, datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Study population and covariate balance

Out of a total of 765 subjects, we excluded 83 subjects from sample 1 and 190 subjects from sample 2, leaving a total of 492 subjects (Figure 1). Demographic characteristics before applying IPTW are summarized in Supplementary Table 1. After applying IPTW, the differences between subgroups diminished substantially. The plot of weights and propensity score distributions in Supplementary Figure 1 demonstrated that no extreme weights were present and the propensity weighting achieved balance between the HCMV+ and HCMV− groups. In the sample 1 HC subgroup, standardized mean differences (SMD range from 0.22–0.99) remained between the HCMV+ and HCMV− samples that could not be corrected for by IPTW due to the small sample size. In the sample 1 MDD subgroup and the sample 2 MDD and HC groups, standardized mean differences for all of the covariates were less than 0.1, indicative of well-balanced samples. Other than education in the sample 1 HC subgroup, there were no statistically significant group differences in any of the measured covariates between HCMV+ and HCMV− subgroups in both sets of MDD and HC samples (Table 1).

Main effect of HCMV

Relative to HCMV− subjects, HCMV+ subjects in sample 1 showed a reduction of GMV in 67 out of 87 cortical and subcortical ROIs although only six of these regions were

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statistically significant ($p_{uncorrected}$<0.05), i.e. the left pars orbitalis gyrus (standardized beta coefficient (SBC) =−0.38, [95%CI, −0.68 to −0.07]), left fusiform gyrus (SBC=−0.25, [95%CI, −0.49 to −0.01]), left inferior parietal lobule (SBC=−0.25, [95%CI, −0.49 to −0.01]), right lateral orbitofrontal gyrus (SBC=−0.23, [95%CI, −0.46 to −0.01]), right parahippocampal gyrus (SBC)=−0.32, [95%CI, −0.62 to −0.03]), and right supramarginal gyrus (SBC=−0.26, [95%CI, −0.51 to −0.01]). Note that an SBC of −1 indicates that seropositive HCMV individuals show a one standard deviation smaller volume than seronegative HCMV individuals. Relative to HCMV− subjects, HCMV+ subjects in sample 2 had reduced GMV in 73 out of 87 regions with seven regions significant (p<0.05, uncorrected). No regions were significantly larger in the HCMV+ versus the HCMV− groups in either of the samples (Supplementary Table 2).

Two out of the six regions that were significantly reduced in sample 1 also showed a significant reduction of GMV in sample 2, indicating a replication of the HCMV main effect for the left fusiform gyrus (SBC=−0.51, [95%CI, −0.80 to −0.23]) and the right supramarginal gyrus (SBC=−0.32, [95%CI, −0.64 to −0.01]) (Table 2, Figure 2). Posthoc analyses revealed a similar effect of HCMV in the MDD subgroups, but not in the HC subgroups, in both samples, suggesting that the effects of HCMV were mainly driven by the MDD participants (Table 2, Figure 2.C).

The unadjusted model showed similar results to the IPTW model (Supplementary Table 3). Additional sensitivity analyses using the E-value suggested that the observed associations were at least moderately robust to potential unmeasured confounding. For instance, the estimated E-value of 1.82 for the left fusiform gyrus in sample 1 indicates that in order to explain away the observed effect of HCMV with effect size of SBC=−0.25, a putative unmeasured confounder would need to increase the probability of a subject being HCMV+ and having a smaller fusiform gyrus by 1.82-times each. The E-values estimated for the left fusiform gyrus in sample 2, and the right supramarginal gyri in samples 1 and 2 were 2.56, 1.85, and 2.01 respectively (Supplementary Figure 2).

Main effect of diagnosis and interaction effect

There were four subcortical regions (bilateral caudate and bilateral nucleus accumbens) that significantly differed in MDD versus HC subjects in sample 1. However, none of these regions replicated in sample 2. Conversely, there were three cortical regions (right parahippocampal gyrus, and right pericalcarine cortex) that significantly differed in MDD versus HC subjects in sample 2, but the results did not replicate in sample 1 (Supplementary Figure 3). There was no significant diagnosis-by-HCMV interaction effect in sample 1 although two regions (the right parahippocampal gyrus and the left fusiform gyrus) showed a significant interaction effect in sample 2 (Supplementary Figure 4).

Correlations between HCMV level and CRP

Correlation analyses were performed in the combined MDD/HC HCMV+ samples for the two regions that replicated across sample 1 and sample 2. HCMV IgG level was inversely correlated with GMV in the left fusiform gyrus (r=−0.19, $p_{uncorrected}$=0.049) and the right supramarginal gyrus (r=−0.19, $p_{uncorrected}$=0.043) in sample 1 but not in sample 2 (Figure

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3). Sample 1 results were similar in the MDD group alone: left fusiform gyrus (r=−0.22, $p_{uncorrected}$=0.029); right supramarginal gyrus (r=−0.16, $p_{uncorrected}$=0.122). There were no significant correlations between CRP and HCMV level or CRP and GMVs in either sample.

DISCUSSION

In two independent samples with different inclusion/exclusion criteria we observed a significant main effect of HCMV serostatus: HCMV+ subjects had smaller left fusiform gyri and right supramarginal gyri than HCMV− subjects matched on up to 11 different potential confounding variables. Multiple additional regions were reduced in volume but did not replicate across samples. Although we did not detect a significant interaction between HCMV serostatus and diagnosis, it is clear from Table 2 and Figure 2.C that the significant main effect of HCMV was driven by differences between the HCMV+ and the HCMV− MDD subgroups rather than the HCMV+ and the HCMV− HC groups. This is consistent with our hypothesis that control of HCMV infection is diminished in MDD because of inflammation and impaired viral immunity. Nevertheless, HCs may also experience periods of stress which potentially predispose to viral reactivation, and therefore putative HCMV−mediated changes in brain volume. Thus, because the direction (but not magnitude) of the effect of HCMV on GMV is likely similar in both MDD and HC populations, sample sizes greater than 90 in each subgroup will likely be required to detect significant interaction effects based on the interaction effect size observed in sample 2. Future studies with larger samples in HC subjects are warranted to comfirm the HCMV effect in HC as well as the interaction effect between HCMV and diagnosis.

It is unclear whether the supramarginal gyrus and fusiform gyrus are more vulnerable to HCMV infection than other brain regions. Congenital HCMV infection appears to cause gross structural abnormalities leading to mental retardation, cerebral palsy, and sensorineural hearing loss[4] while HCMV encephalitis can result in destruction of the periventricular regions in HIV patients[66]. While not classically associated with MDD, reductions in thickness of the supramarginal and fusiform gyri have been reported in recent ENIGMA consortium MDD samples[67, 68]. In addition, structural abnormalities of these regions have been observed in the context of viral infection (HIV[69, 70]) and autoimmunity (systemic lupus erythematosus[71, 72]; multiple sclerosis[73, 74]). Further, the supramarginal gyrus has been hypothesized to be particularly vulnerable to neuropathological processes associated with Alzheimer's disease because it is part of the heteromodal cortex that undergoes significant myelination during development[75]. Additional studies are needed to clarify the neuroanatomical specificity of any HCMV−mediated effects. Nevertheless, our results raise the possibility that an attenuated form of HCMV−induced neuropathology may occur in some HCMV+ MDD patients with a mild impairment of anti-viral immunity. This hypothesis receives support from the only other neuroimaging study in a psychiatric population which reported that HCMV+ patients with bipolar disorder and schizophrenia had smaller right hippocampal volumes than HCMV− patients[76].

The mechanisms through which HCMV infection putatively leads to reductions in GMV are unclear but there are at least two possibilities. First, HCMV may directly damage the brain or elicit a microglia-mediated anti-viral immune response. Second, HCMV

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may contribute to systemic inflammation via several mechanisms including the long-term accumulation of cytotoxic CD28⁻ T-cells[18]. Thus, even if HCMV does not always infect the brain, peripheral sites of inflammation could exert systemic effects, impacting GMV. Several studies have reported correlations between circulating inflammatory mediators and reductions in GMV[54, 77]. In this regard, the absence of a significant association between CRP and IgG level is unclear. It is plausible, and perhaps even likely, that the single time point at which CRP was measured did not always overlap with viral shedding since the two studies were not designed to evaluate participants with an active HCMV infection. It is also possible that specific markers of viral infection such as IP-10/CXCL10 or macrophage activation such as sCD14[78] would be more sensitive to HCMV reactivation than CRP.

There was a significant inverse correlation between HCMV IgG level and volume of the left fusiform gyrus and right supramarginal gyrus although these relationships were modest and only present in sample 1. Our results are partly consistent with a previous report of an inverse association between HCMV IgG level and right hippocampal volumes in patients with schizophrenia and bipolar disorder[76]. However, IgG level only provides an approximate measure of HCMV shedding since they have a half-life of <30 days and are also influenced by host factors. Thus, the signal-to-noise ratio of these correlational analyses are likely low.

Given the cross-sectional design, we cannot definitively conclude that HCMV is the cause of the reduction in GMV since an unknown causal factor may be correlated with HCMV serostatus. Nevertheless, we minimized this possibility through careful balancing of potential confounding variables in the HCMV+ and HCMV− groups. In particular, when computing propensity scores, we included childhood trauma which we previously demonstrated to be more prevalent in HCMV+ MDD subjects[79] as well as education level and household income, a surrogate marker for childhood socioeconomic status[80]. Childhood trauma and socioeconomic status have previously been associated with reductions in GMV[81, 82]. Second, we cannot differentiate between the acute and cumulative effects of HCMV on GMV. Longitudinal studies and/or quantification of HCMV−specific T-cell populations are required to address this question. Third, we cannot exclude the possibility that other viral infections that co-occur with HCMV accounted for the reductions in GMV. Nevertheless, HCMV is more strongly linked with congenital brain abnormalities than other herpesviruses and recurrent HCMV reactivation disrupts the balance of the immune system to a greater extent than other herpesviruses[83].

In conclusion, after careful balancing of HCMV+ and HCMV− groups for up to eleven baseline demographic and clinical variables, we found suggestive evidence for an HCMV−associated reduction in GMV in two independent samples. While causal conclusions cannot be drawn, the results raise the possibility that HCMV infection may be a treatable source of structural brain abnormalities in a subset of depressed patients.

Supplementary Material

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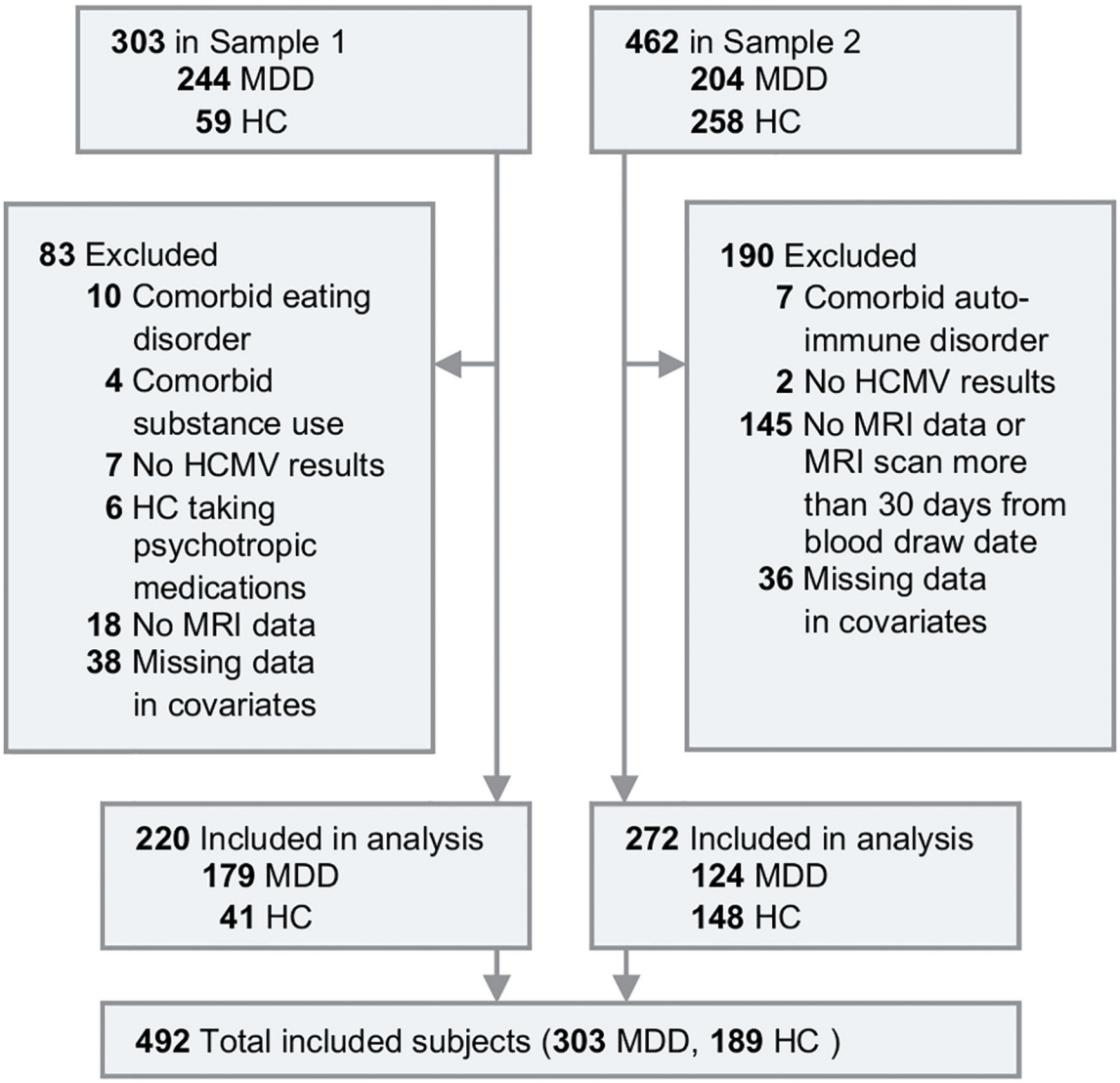

Figure 1.
Flow Diagram of Selection of Participants.

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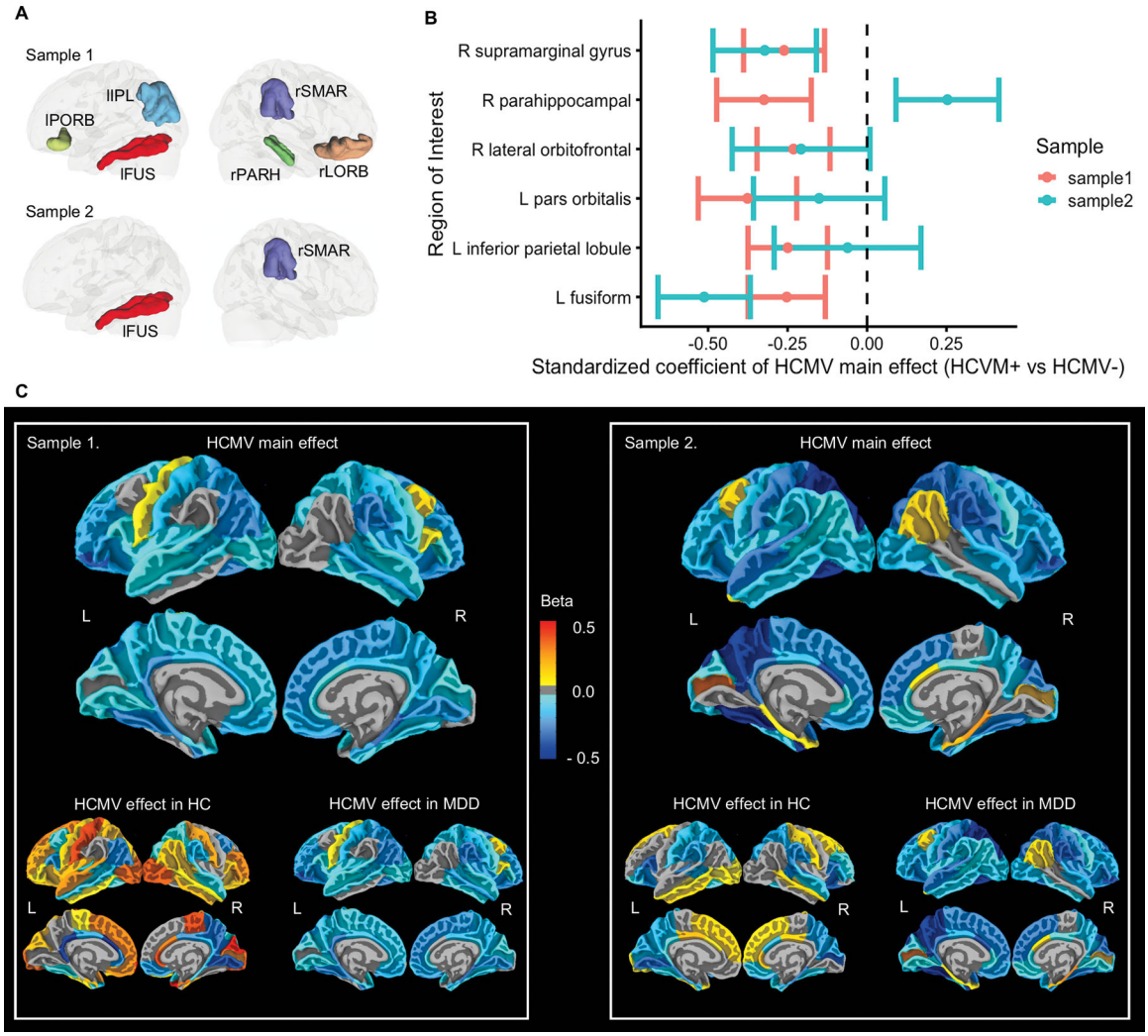

Figure 2.

Regional Effect of HCMV infection on gray matter volumes. (A) Illustration of regions that showed main effect of HCMV. Six regions were significantly smaller in HCMV+ versus HCMV− subjects in sample 1 at $p_{\text{uncorrected}} < 0.05$. Two out of these six regions were also significant decreased in sample 2 at $p_{\text{uncorrected}} < 0.05$. (B) Standardized beta coefficient as effect size with robust standard error as error bar, estimated from IPTW adjusted regression model. (C) Mapping of HCMV main effect, HCMV effect in HC, and the HCMV effect in MDD at all the cortical regions without thresholding. Colors represent the standardized beta coefficients, estimated from IPTW adjusted regression model. They range from −0.5 to 0.5, which means the mean gray matter volume of HCMV+ subgroup in given region increased or decreased by 0.5 standard deviations from HCMV− subgroup. Blue colors represent smaller gray matter volumes in HCMV+ groups, whereas yellow-red colors represent larger gray matter volumes in HCMV+ groups. Relative to HCMV− subjects, HCMV+ subjects showed smaller gray matter volumes across cortical regions in both samples, most prominently in orbitofrontal, temporal and parietal regions. The observed effects of HCMV were mainly driven by the MDD groups. Within the HCs there were less

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consistent differences between HCMV+ and HCMV− subgroups and the effect sizes were generally smaller.

Abbreviations: rSMAR, right supramarginal gyrus; rPARH, right parahippocampal gyrus; rLORB, right lateral orbitofrontal gyrus; lPORB, left pars orbitalis gyrus; lIPL, left inferior parietal lobule; lFUS, left fusiform gyrus.

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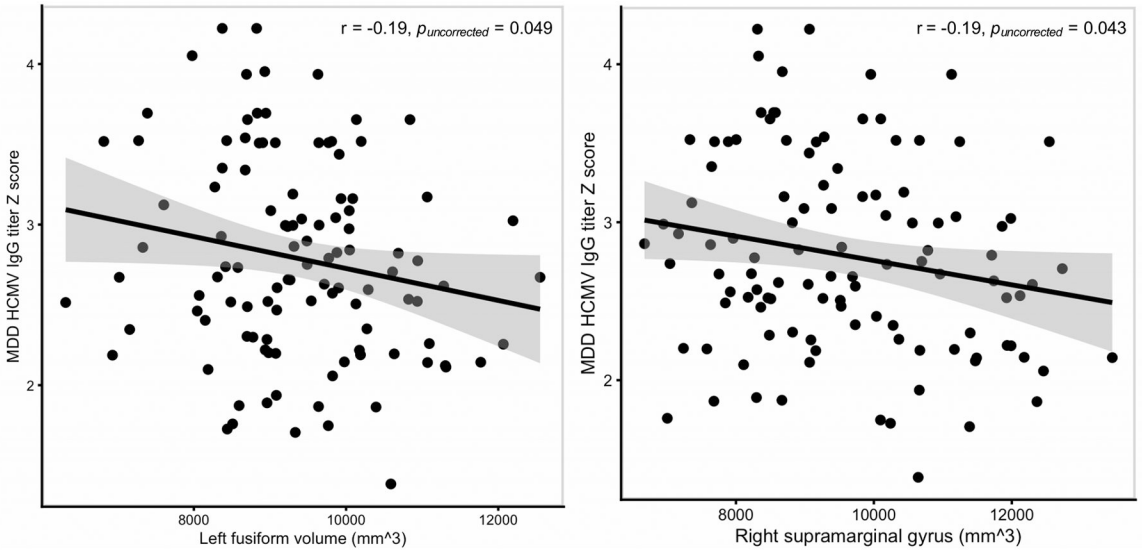

Figure 3.

HCMV IgG level is inversely correlated with gray matter volume in the combined MDD/HC HCMV+ group of sample 1.

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Table 1.

Demographic Characteristics of Study Participants After Application of Applied Inverse Probability of Treatment Weighting

	MDD				HC			
Sample 1	**HCMV−**	**HCMV+**	***P*** [a]	**SMD** [b]	**HCMV−**	**HCMV+**	***P*** [a]	**SMD** [b]
n (weighted population)	78 (75.17)	101 (101.84)			26 (20.67)	15 (9.70)		
Age (mean (SD))	35.40 (11.08)	36.03 (11.02)	0.73	0.06	33.11 (11.35)	30.46 (10.47)	0.55	0.24
Sex (Male (%))	23.9 (31.8)	31.7 (31.1)	0.93	0.02	9.7 (47.0)	4.8 (49.5)	0.90	0.05
BMI (mean (SD))	28.89 (5.57)	29.01 (5.37)	0.89	0.02	26.76 (6.07)	28.82 (4.69)	0.27	0.38
Education (mean (SD)) [c]	6.53 (1.44)	6.45 (1.75)	0.76	0.05	7.02 (1.48)	5.77 (1.01)	<0.01	0.99
Income (mean (SD)) [d]	$50,330 ($67,542)	$45,371 ($39,029)	0.51	0.09	$58,016 ($35,822)	$58,486 ($36,757)	0.97	0.01
Depression severity (mean (SD)) [e]	61.03 (7.10)	61.00 (7.52)	0.98	0.00	44.22 (6.11)	41.31 (6.76)	0.28	0.45
Anxiety severity (mean (SD)) [f]	62.64 (6.90)	62.59 (6.31)	0.96	0.01	46.48 (8.16)	44.01 (6.80)	0.38	0.33
Number of episodes (mean (SD)) [g]	4.27 (3.48)	4.39 (3.49)	0.83	0.04	0.00 (0.00)	0.00 (0.00)	NA	0.00
Un-medicated (%) [h]	21.7 (28.8)	27.7 (27.2)	0.83	0.04	20.7 (100.0)	9.7 (100.0)	NA	0.00
CTQ (mean (SD)) [i]	47.95 (17.06)	49.25 (21.62)	0.67	0.07	31.54 (6.96)	33.68 (11.79)	0.50	0.22
Alcohol use (mean (SD)) [j]	5.22 (2.35)	5.22 (2.62)	1.00	0.00	4.30 (2.82)	3.61 (2.78)	0.57	0.25
HCMV IgG level (mean (SD)) [k]	0.97 (0.15)	2.74 (0.61)	<0.001	3.96	0.97 (0.17)	2.44 (0.68)	<0.001	2.99
CRP (mean (SD)) [l]	0.53 (1.42)	0.74 (1.45)	0.35	0.15	−0.34 (1.25)	−0.05 (1.17)	0.46	0.24
Sample 2								
n (weighted population)	67 (66.6)	57 (57.1)			79 (79.21)	69 (68.47)		
Age (mean (SD))	36.04 (11.09)	35.36 (10.59)	0.76	0.06	31.76 (10.69)	32.06 (9.66)	0.86	0.03
Sex (Male (%))	15.3(23.0)	13.3(23.3)	0.98	0.01	23.0(29.0)	19.3(28.2)	0.92	0.02
BMI (mean (SD))	27.77 (6.09)	28.25 (6.57)	0.72	0.08	26.95 (6.06)	26.90 (5.72)	0.97	0.01
Education (mean (SD)) [b]	5.34 (0.89)	5.32 (0.85)	0.89	0.03	5.36 (0.81)	5.37 (0.87)	0.93	0.02
Depression severity (mean (SD)) [m]	17.94 (11.98)	18.06 (11.88)	0.96	0.01	1.53 (2.11)	1.36 (2.01)	0.67	0.09
Un-medicated (%) [h]	44.4 (66.7)	49.1 (69.2)	0.78	0.05	79.2 (100.0)	68.5 (100.0)	NA	0.00
CTQ (mean (SD)) [i]	51.19 (23.38)	49.05 (20.84)	0.69	0.10	32.83 (11.06)	33.54 (13.66)	0.74	0.06
HCMV IgG level (mean (SD)) [k]	1.24 (0.15)	2.95 (0.68)	<0.001	3.47	1.27 (0.16)	2.73 (0.78)	<0.001	2.60
CRP (mean (SD)) [l]	1.86 (0.85)	1.95 (1.04)	0.62	0.10	1.99 (1.07)	1.89 (0.87)	0.59	0.10

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Abbreviations: MDD, major depressive disorder; HC, healthy control; HCMV, human cytomegalovirus; HCMV−, human cytomegalovirus seronegative; HCMV+, human cytomegalovirus seropositive; SMD, standardized mean difference; BMI, body mass index; CTQ, childhood trauma questionnaire; CRP, C-reactive protein.

[a]Calculated using X^2 test for categorical variables and 2-tailed *t*-test for continuous variables.

[b]The standardized mean differences less than 0.1 reveals a negligible imbalance.

[c]Measured by an ordered categories. Full categories sees Supplementary Table 4.

[d]Annual household income.

[e]PROMIS depression T score was used.

[f]PROMI anxiety T score was used.

[g]Measured by MINI interview. Subjects had over 10 episodes were treated as had 10 episodes.

[h]Un-medicated defined as not taking antipsychotic medication.

[i]Childhood trauma questionnaire total score was used.

[j]Log-transformed lifetime alcohol usage were used. Data obtained from CDDR interview.

[k]HCMV IgG level z score was used.

[l]CRP concentration log transferred.

[m]Montgomery-Asberg Depression Rating Scale (MADRS) score was used.

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Table 2.

Regional Effect of HCMV Infection on Gray Matter Volume

IPTW adjusted	Sample 1			Sample 2		
ROI	**SBC**[a]	**95%CI**[b]	***p***	**SBC**	**95%CI**	***p***
Main effect of HCMV						
L pars orbitalis gyrus	−0.38	−0.68 ~ −0.07	0.02	−0.15	−0.56 ~ 0.25	0.47
L fusiform gyrus	−0.25	−0.49 ~ −0.01	0.04	−0.51	−0.80 ~ −0.23	<0.001 ***
L inferior parietal lobule	−0.25	−0.49 ~ −0.01	0.05	−0.06	−0.51 ~ 0.39	0.79
R lateral orbitofrontal gyrus	−0.23	−0.46 ~ −0.01	0.04	−0.21	−0.63 ~ 0.22	0.34
R parahippocampal gyrus	−0.32	−0.62 ~ −0.03	0.03	0.25	−0.06 ~ 0.57	0.12
R supramarginal gyrus	−0.26	−0.51 ~ −0.01	0.04	−0.32	−0.64 ~ −0.01	<0.05 *
HCMV effect in MDD						
L pars orbitalis gyrus	−0.37	−0.68 ~ −0.07	0.02	−0.16	−0.57 ~ 0.25	0.44
L fusiform gyrus	−0.25	−0.49 ~ −0.02	0.04	−0.50	−0.77 ~ −0.22	<0.001 ***
L inferior parietal lobule	−0.25	−0.50 ~ −0.01	0.05	−0.07	−0.50 ~ 0.36	0.75
R lateral orbitofrontal gyrus	−0.24	−0.47 ~ −0.01	0.04	−0.22	−0.65 ~ 0.21	0.31
R parahippocampal gyrus	−0.33	−0.63 ~ −0.04	0.03	0.29	−0.06 ~ 0.64	0.10
R supramarginal gyrus	−0.27	−0.53 ~ −0.02	0.04	−0.34	−0.67 ~ −0.01	<0.05 *
HCMV effect in HC						
L pars orbitalis gyrus	0.05	−0.51 ~ 0.63	0.86	−0.04	−0.34 ~ 0.27	0.82
L fusiform gyrus	−0.05	−0.52 ~ 0.43	0.84	−0.09	−0.35 ~ 0.18	0.52
L inferior parietal lobule	−0.25	−0.81 ~ 0.31	0.38	−0.02	−0.36 ~ 0.33	0.93
R lateral orbitofrontal gyrus	0.11	−0.32 ~ 0.54	0.61	−0.32	−0.61 ~ −0.03	0.03
R parahippocampal gyrus	−0.11	−0.78 ~ 0.55	0.75	−0.29	−0.62 ~ 0.04	0.08
R supramarginal gyrus	−0.16	−0.79 ~ 0.47	0.62	−0.23	−0.55 ~ 0.09	0.17

Abbreviation: ROI, region of interest; R, right; L, left;

[a]Standardized beta coefficient. SBC of 1 indicates that the mean gray matter volume of the HCMV+ subgroup is 1 standard deviation different from the HCMV− subgroup. A negative value indicates HCMV+ < HCMV−, and a positive value indicates HCMV+ > HCMV−.

[b]95%CI, 95% confidence interval, robust standard errors was used to calculate 95%CI.

*$P_{uncorrected}$ less than 0.05 in both samples and in the same direction.

***$P_{FDR} < 0.01$