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Cytomegalovirus Infection Associated with Smaller Total Cortical Surface Area in Schizophrenia Spectrum Disorders

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Objectives: **Cytomegalovirus (CMV) congenital infection and in immunodeficiency can have deleterious effects on human cortex. In immunocompetent adults, the putative association between CMV infection and cortical measures has not been explored. We hypothesized that CMV exposure is associated with smaller cortical surface area or cortical thinning mainly in patients with schizophrenia spectrum disorders.** ***Study Design:*** **We included 67 adult patients with schizophrenia spectrum disorders and 262 adult healthy controls. We measured circulating CMV IgG antibody concentrations with solid-phase immunoassay techniques. We measured the total cortical surface area, regional cortical surface areas and the overall mean cortical thickness based on T1-weighted MRI scans processed in FreeSurfer v6.0.** ***Study Results:*** **In the whole sample analysis, we found a significant diagnostic group-by-CMV status interaction on the total surface area (P = .020). Among patients, CMV antibody positivity was significantly associated with smaller total surface area (P = .002, partial eta^2 = 0.138) whereas no such association was found in healthy controls (P = .059). Post hoc analysis among patients showed that higher CMV antibody concentrations were also significantly associated with smaller total surface area (P = .038), and that CMV antibody positivity was significantly inversely associated with 14 left and 16 right regional surface areas mainly in the frontal and temporal lobes. CMV infection was not associated with the overall mean cortical thickness.** ***Conclusions:*** **The results are indicative of a cortical surface area vulnerability to CMV infection in patients with schizophrenia spectrum disorders but not in healthy controls. CMV infection may contribute to the established cortical surface area aberrations in schizophrenia.**

Key words: CMV/MRI/cortex/psychosis

Introduction

Human cytomegalovirus (CMV) also known as Human Herpesvirus 5 (HHV-5) is a DNA virus of the Herpesviridae family. Antenatal CMV infection as well as postnatal infection of immunodeficient hosts of any age are both associated with considerable mortality and morbidity.[1] In marked contrast, postnatal CMV infection of immunocompetent hosts is typically either asymptomatic or followed by flu-like symptoms, but cannot be cleared by the host and results in lifetime latency.[1,2] This latent infection has a prominent impact on the adaptive immune system which adjusts its recourses such that a considerable fraction focuses on CMV immunosurveillance.[3] The latent CMV infection can be complicated with periodic reactivations. In immunocompromised hosts, CMV reactivations characteristically lead to apparent illness, but there is evidence that frequent reactivations also occur in immunocompetent hosts, are typically asymptomatic and may be related to chronic disease exacerbation.[4] It is established that hematopoietic cell lineages are main sites of latency.[1] Neural stem cells are the main sites of CMV latency in the brain.[5,6]

Studies on congenital CMV infection as well as infection of immunodeficient individuals have revealed

substantial cerebral cortex involvement. In particular, congenital CMV infection has been associated with MRI aberrations including cortical malformations and enlarged ventricles as well as white matter and hippocampal disturbances.[7] In a neuropathological study of CMV infected human fetal brains, the majority had widespread cortical abnormalities, microcephaly, or hippocampal aberrations.[8] A neuropathological study of AIDS patients with CMV brain infection showed that numerous brain regions had CMV-related abnormalities, and that cerebral cortex was involved in half of the cases.[9] We here hypothesized that such cortical involvement is present even in the latent form of the CMV infection among immunocompetent adults. We base this hypothesis on (a) the apparent CMV neurotropism and the cortical CMV-related aberrations when the human brain is infected prenatally or in the context of immunodeficiency[7–9] and (b) the nonsilent chronic viral latency in CMV-infected immunocompetent individuals with continuous expression of proteins and noncoding RNAs as well as recurrent reactivation events.[1,2] The cortical volume is a product of two components, the cortical surface area (SA) and the cortical thickness (CT) with different developmental courses.[10,11] We hypothesized that circulating CMV immunoglobulin G (IgG) positivity, showing previous CMV infection and current latency, is associated with smaller SA or cortical thinning. To the best of our knowledge, the putative associations between CMV IgG status and cortical measures have not been previously explored in schizophrenia (SCZ).

Immune system disturbances have been repeatedly reported in SCZ[12,13] and may result in a less efficient CMV control. Blood–brain barrier (BBB) hyperpermeability and an inflammatory environment have also been implicated in SCZ.[14,15] Of note, BBB deficiency facilitates CMV brain penetration while inflammation accelerates CMV reactivation rates.[1,16] Furthermore, the female immune system may more efficiently control latent CMV in healthy adults,[17,18] but to our knowledge such studies have not been conducted in SCZ. We therefore hypothesized that a putative association between CMV exposure and cortical measures is greater in patients with SCZ spectrum disorders relative to healthy controls (HC), and that such a putative association may be sex-dependent.

Methods

Participants

We included 67 patients with SCZ spectrum disorders and 262 HC (age range 18–53 years). Specifically, we included 45 patients with SCZ, 5 patients with schizophreniform disorder and 17 patients with schizoaffective disorder. Medical doctors and psychologists evaluated the patients with the Structured Clinical Interview for DSM-IV axis I disorder (SCID-I) module A–E[19] and HC with the Primary Care Evaluation of Mental Disorders (Prime-MD).[20] We recruited the patients from outpatient and inpatient psychiatric units in Oslo, as part of the Thematically Organized Psychosis (TOP) research study, and the HC from the same catchment areas using the national population register. We applied the following exclusion criteria for all participants: previous moderate or severe head injury, a neurological disorder or medical conditions that could affect brain function. We excluded HC with previous or current psychiatric disorders including substance use disorders (including alcohol use disorder) or with first-degree relatives with severe mental illness. The study was approved by the Regional Committee for Medical Research Ethics South East Norway (2009/2485), and was conducted in accordance with the Declaration of Helsinki as revised in 2008. We obtained written informed consent from all participating patients and HC.

Measures and Medication

Education level has been largely used as a socioeconomic status indicator capturing the important shift from parental to own socioeconomic status.[21] We therefore used years of education as proxy indicator for socioeconomic status for all participants. Psychosis can impact patients' education level, and in patient analysis, we also used a categorical maternal education variable (1. primary school; 2. upper secondary school; 3. college/university). In patients, we evaluated alcohol use with the alcohol use disorder identification test (AUDIT)[22] and drug use with the drug use disorder identification test (DUDIT).[23] We further assessed the patients with the Positive and Negative Syndrome Scale (PANSS).[24] We defined the duration of illness (DOI) as the time passed since the first psychotic episode. We finally assessed the current use of antipsychotic medication (binary variable) and we calculated the current chlorpromazine equivalent doses (CPZ) in mg/day.[25]

MRI

We obtained 329 T1-weighted MRI scans with a General Electric 3T Signa HDxt scanner with an 8-channel head coil. A 3D fast spoiled gradient echo (FSPGR) sequence was obtained applying the following parameters: 170 sagittal slices, slice thickness = 1.2 mm, voxel size = 1 × 1 × 1.2 mm, inversion time (TI) = 450 ms, echo time (TE) = MinFull, repetition time (RT) = 7.8 ms, flip angle = 12°. MRI scans were processed using the FreeSurfer v6.0.[26] The total cortical SA was calculated as the sum of the left and right SAs. To calculate the average CT across both hemispheres, we computed a weighted average which takes interhemispheric differences in SA into account, using the following formula: (Mean CT(left) * SA(left)) + (Mean CT(right) * SA(right)) divided by (Sum SA(right + left)). We obtained regional SAs based on the Desikan–Killiany

(DK) FreeSurfer Atlas.[27] Quality inspection and editing was performed by trained research assistants following standard FreeSurfer procedures.[28]

Serology Assessment

Blood samples were drawn from all participants. Serology assessment was performed at the Stanley Neurovirology Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD, USA. CMV IgG antibody concentrations were measured by solid-phase immunoassay techniques and were expressed as continuous (antibody concentrations) and dichotomous measures (seropositivity vs. seronegativity), derived via comparisons of the reactivity generated by the samples in the immunoassay with the optical density generated by standard samples as previously described.[29,30]

Statistics

In the bivariate analysis among all participants (n = 329), we assessed group differences between CMV seropositive (CMV+) and CMV seronegative (CMV−) participants in patient-control status, sex, age, education years and handedness, as well as the correlations between each of these variables and the dependent variables (SA and CT) (table 1). In the bivariate analysis among patients with SCZ spectrum disorders (n = 67), we assessed group differences between CMV+ and CMV− patients in sex, age, education years, maternal education level, daily use of tobacco, handedness, DOI, PANSS total score, the percentage of patients on antipsychotics and the CPZ among patients on antipsychotics, as well as their correlations with SA (table 2). In the bivariate analysis among HC (n = 262), we assessed group differences between CMV+ and CMV− HC in sex, age, education years and handedness, as well as their correlations with SA (table 2).

Among all participants, based on our main hypothesis, we ran two full factorial analyses of covariance (ANCOVAs) investigating main and interaction effects of CMV status (seropositivity/seronegativity), diagnostic group (SCZ spectrum/HC) and sex on (a) SA and (b) CT, whilst controlling for variables that differed between CMV+ and CMV− participants or were correlated with the dependent variables in the bivariate analysis among all participants. As we ran two full factorial ANCOVAs, we accepted statistical significance for main and interaction effects at a Bonferroni corrected alpha level of 0.025 (0.05/2). In the SA ANCOVA among all participants, there was a significant diagnostic group-by-CMV status interaction (as shown in the Results section) which we followed up stratifying by diagnostic group and investigating main CMV effects on SA whilst controlling for variables that differed between CMV+ and CMV− participants or were correlated with SA in the bivariate analysis for the diagnostic group analyzed.

We conducted all the analyses with IBM SPSS Statistics 28.

Results

Patient-control Analysis

To investigate putative patient-control differences in SA and CT, we ran the following ANCOVAs in the whole sample. In the SA ANCOVA (patient-control status, sex, and age on SA) patients had significantly smaller SA than HC, $F(1,325) = 10.102$, $P = .002$; women had smaller SA than men ($P < .001$) and age was inversely associated with SA ($P = .008$). In the CT ANCOVA (patient-control status, sex and age on CT), patients had significantly

Table 1. Group differences between cytomegalovirus (CMV) immunoglobulin G (IgG) seronegative (CMV−) and seropositive (CMV+) participants in patient-control (PC) status, sex, age, education years and handedness. For the whole sample, correlations with the total cortical surface area (SA) and the overall mean cortical thickness (CT) are shown. P values < .05 shown in bold.

	CMV−		CMV+			Correlation with SA		Correlation with CT	
	N[a]	Mean (SD) or %	N[a]	Mean (SD) or %	P value[b]	Direction (+ or −)	P Value[c]	Direction (+ or −)	P value[c]
PC status (% patients)	146	22.6	183	18.6	.368	–	.062	-	.928
Sex (% females)	146	34.9	183	42.6	.156	-[d]	**<.001**	**+**	.665
Age (years)	146	30 (7.5)	183	32.6 (7.9)	**<.001**	−	.380	−	**<.001**
Education years (years)	144	14 (2.6)	183	14.1 (2.3)	.609	+	.363	−	**<.001**
Handedness (% right-handedness)	143	83.9	183	90.2	.091	+	.322	+	.663

Note:
[a]Number of participants with data in each variable.
[b]Chi-square test or t-test.
[c]Point-biserial correlations for PC status, sex and handedness; Spearman's correlations for age and education years.
[d]Women had smaller SA than men.

Table 2. Group differences between cytomegalovirus (CMV) immunoglobulin G (IgG) seronegative (CMV−) and seropositive (CMV+) patients with schizophrenia (SCZ) spectrum disorders in sex, age, education years, maternal education level (primary school/upper secondary school/college or university), daily use of tobacco, handedness (right-handedness vs. left-handedness/ambidexterity), duration of illness (DOI), Positive and Negative Syndrome Scale (PANSS) total score, the percentage of patients on antipsychotics as well as the chlorpromazine equivalent doses (CPZ) among patients on antipsychotics. Group differences between CMV− and CMV+ healthy controls in sex, age, education years and handedness. Separately for all patients and all healthy controls, correlations with the total cortical surface area (SA) are shown. *P* values < .05 shown in bold.

	CMV−		CMV+			Correlation with SA	
	N[a]	Mean (SD) or %	*N*[a]	Mean (SD) or %	*P* value[b]	Direction (+ or -)	P value[c]
Patients with SCZ spectrum disorders							
Sex (% women)	33	30.3	34	41.8	.353	−[d]	**.003**
Age (years)	33	26.8 (6.5)	34	28.6 (8.9)	.347	−	.074
Education years	31	11.7 (2)	34	12.4 (2.6)	.246	−	.058
Maternal education	30	5/7/18	27	5/6/16	1.000[e]		.396[f]
Tobacco use (%)	33	60.6	34	64.7	.729	+	.264
Handedness (% right-handedness)	30	83.3	34	97.1	.090[e]		.629[g]
DOI (years)	33	6.5 (6)	34	6.8 (7.3)	.860	−	**.019**
PANSS total score	33	61.3 (19.2)	34	57.1 (13.3)	.296	+	.120
On antipsychotics (%)	33	90.9	34	97.1	.356[e]	+	.815
CPZ (mg/day)	29	397.6 (274.2)	33	326.5 (214)	.257	+	.632
Healthy Controls							
Sex (% women)	113	36.3	149	43	.275	−[d]	**<.001**
Age (years)	113	30.4 (7.6)	149	33.5 (7.3)	**.001**	−	.620
Education years	113	14.6 (2.4)	149	14.5 (2)	.764	+	.176
Handedness (% right-handedness)	113	84.1	149	86.6	.287		.568[g]

[a]Number of participants with data in each variable.
[b]Chi-square test or *t*-test.
[c]Point-biserial correlations for binary variables; Spearman's correlations for quantitative variables.
[d]Women had smaller SA than men.
[e]Fisher's exact test.
[f]Analysis of variance (ANOVA).
[g]Mann–Whitney *U* test.

thinner cortex than HC, $F(1,325) = 4.601$, $P = .033$; sex was not associated with CT ($P = .968$) whereas age was inversely associated with CT ($P < .001$).

Surface Area Analysis

Whole Sample. The bivariate analysis of the whole sample ($n = 329$) showed that CMV+ participants were older than CMV− participants ($P < .001$), while females had smaller SA than males ($P < .001$) (table 1). In the multivariate model of the whole sample (full factorial ANCOVA), we searched for main and interaction effects of diagnostic group (SCZ spectrum/HC), CMV status (seropositivity/seronegativity) and sex on SA whilst controlling for age. There was no three-way (CMV-by-diagnostic group-by-sex), CMV-by-sex or diagnostic group-by-sex interactions, whereas there was a significant CMV-by-diagnostic group interaction, $F(1,320) = 5.482$, $P = .020$ on SA. Furthermore, there were significant main effects of CMV status, diagnostic group and sex on SA while age was associated with SA. Specifically, CMV+ participants had smaller SA than CMV− participants ($P < .001$), patients had smaller SA than HC ($P = .012$), women had smaller SA than men ($P < .001$) while age was inversely associated with SA ($P = .027$) (table 3). We followed up the statistically significant two-way interaction investigating the CMV-SA association by diagnostic group.

Patients With Schizophrenia Spectrum Disorders. The bivariate analysis of the patient group showed that CMV+ and CMV− patients did not significantly differ in any of the analyzed variables (table 2). Among all patients, women had smaller SA than men ($P = .003$), while DOI was inversely correlated with SA ($P = .019$). Stratifying by CMV status, DOI was significantly inversely correlated with SA among CMV+ ($P = .016$), but not among CMV− patients ($P = .270$).

In the multivariate model (ANCOVA), we searched for main effects of CMV status on SA whilst controlling

Table 3. The results of the full factorial analysis of covariance (ANCOVA) on the total cortical surface area (SA) in the whole sample of patients with schizophrenia (SCZ) spectrum disorders and healthy controls (HC) are presented

	F	*P* value
Whole sample (*n* = 329)		
CMV status (CMV−/CMV+)	13.077	**<.001**
Diagnostic group (SCZ/HC)	6.397	**.012**
Sex	63.095	**<.001**
CMV status-by-diagnostic group-by-sex	0.157	.692
CMV status-by-diagnostic group	5.482	**.020**
CMV status-by-sex	0.174	.677
Diagnostic group-by-sex	3.646	.057
Age	4.948	**.027**
SCZ spectrum (*n* = 67)		
CMV status	10.091	**.002**
Sex	6.892	**.011**
Duration of illness	5.248	.025
HC (*n* = 262)		
CMV status	3.600	.059
Sex	131.667	**<.001**
Age	2.035	.155

Note: There was a significant cytomegalovirus (CMV) status-by-diagnostic group interaction which we followed up stratifying by diagnostic group (we ran an ANCOVA among patients and an ANCOVA among HC). CMV seropositive (CMV+) patients had smaller SA than CMV seronegative (CMV−) patients, whereas CMV+ and CMV- HC did not significantly differ in SA. The selection of covariates in the three ANCOVAs was based on the bivariate analyses.

for sex and DOI. There was a significant main effect of CMV status on SA, $F(1,63) = 10.091$, $P = .002$, partial eta$^2 = 0.138$ (figure 1); as in the bivariate analysis women had smaller SA than men ($P = .011$) while DOI was inversely associated with SA ($P = .025$) (table 3).

Healthy Controls. The bivariate analysis of the HC group showed that CMV+ HC were older than CMV− HC ($P = .001$) but did not significantly differ in sex, education year or handedness (table 2); women had smaller SA than men ($P < .001$) (table 2). In the multivariate analysis of the HC group (ANCOVA), we therefore searched for main effects of CMV status on SA whilst controlling for sex and age. In the multivariate model, there was no significant main effect of CMV status on SA, $F(1,258) = 3.600$, $P = .059$ (figure 1). Furthermore, there was a significant main effect of sex, with women having smaller SA than men ($P < .001$) and no significant association between age and SA ($P = .155$) (table 3).

Post-hoc Analysis Among Patients With Schizophrenia Spectrum Disorders

CMV Antibody Concentrations. The mean (standard deviation) CMV IgG antibody concentration was 2.8 (1.9). As shown in table 2, among patients with SCZ spectrum disorders, sex and DOI were correlated with SA and were therefore included in the multivariate model. We ran a multiple regression to predict the total cortical SA from CMV antibody concentrations, sex and DOI. An increase of CMV IgG concentration by 1 unit was associated with a 1987 mm^2 decrease in SA ($P = .038$), women had 8113 mm^2 smaller SA than men ($P = .035$), and an increase in DOI by one year was associated with 764 mm^2 decrease in SA ($P = .006$) (supplementary table S1).

Extended MRI Analysis. We aimed to investigate the putative associations between CMV status and all left and right regional SAs based on the DK FreeSurfer Atlas.[27] The mean age of the CMV+ and the CMV− patients differed 1.8 years, and further, there were 11% more women among CMV+ patients compared to CMV− patients. Although these differences did not reach statistical significance (table 2), we considered reasonable to include both sex and age in the extended analysis. We ran 34 ANCOVAs by hemisphere investigating CMV IgG status main effect on regional SAs whilst controlling for sex and age. We applied a false discovery rate (FDR) of 5% by hemisphere to correct for multiple testing.[31] CMV seropositivity was significantly inversely associated with 14 left and 16 right regional SAs (figure 2 and supplementary table S3).

Cortical Thickness Analysis

As shown in table 1, CMV+ participants were older than CMV− participants ($P < .001$), while age and education years were both inversely correlated with mean overall CT ($P < .001$ for both). We ran a full factorial ANCOVA searching for main and interaction effects of CMV status (seropositivity/seronegativity), diagnostic group (SCZ spectrum/HC) and sex on CT whilst controlling for age and education years (supplementary table S5). There were no three-way ($P = .653$) or two-way interactions (*P*-values for CMV-by-sex, diagnostic group-by-sex and CMV-by-diagnostic group interactions were 0.514, 0.967 and 0.621, respectively). CMV status ($P = .268$), sex ($P = .928$), diagnostic group ($P = .062$) and education years ($P = .737$) were not associated with CT; age was inversely associated with CT ($P < .001$).

Discussion

We found that among patients with SCZ spectrum disorders, CMV IgG seropositivity was significantly associated with smaller total cortical SA (figure 1). The effect size was large with CMV status explaining 14% of the variation in the total SA. CMV IgG seropositivity indicates previous CMV infection but does not indicate when a person was infected. As the virus is never cleared,[2] CMV IgG seropositivity reflects previous infection and subsequent latency. Post-hoc analysis among patients with SCZ spectrum disorders showed that higher CMV IgG antibody concentrations were also associated with smaller total cortical SA. The biological explanation

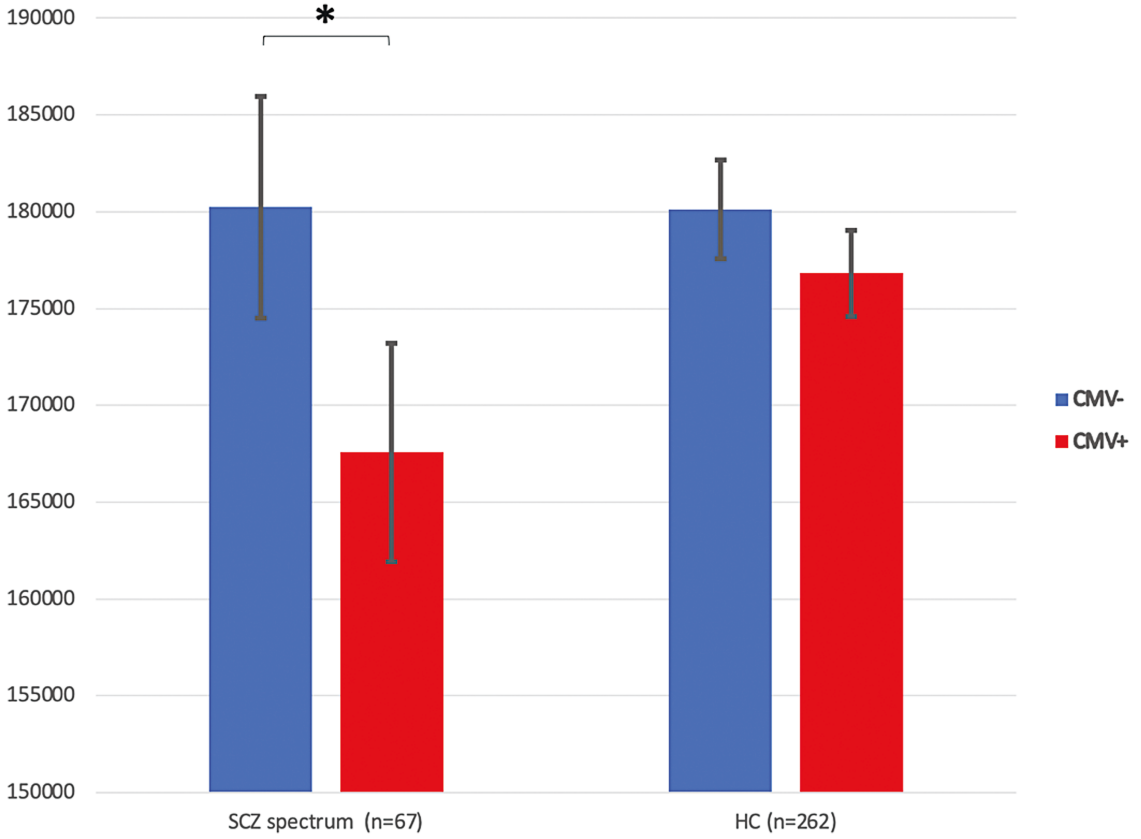

Fig. 1. Total cortical surface area (SA) in mm^2 in cytomegalovirus (CMV) immunoglobulin G (IgG) seronegative (CMV−) and seropositive (CMV+) patients with schizophrenia (SCZ) spectrum disorders and healthy controls (HC). CMV+ patients had significantly smaller total SA than CMV− patients. * $P = .002$.

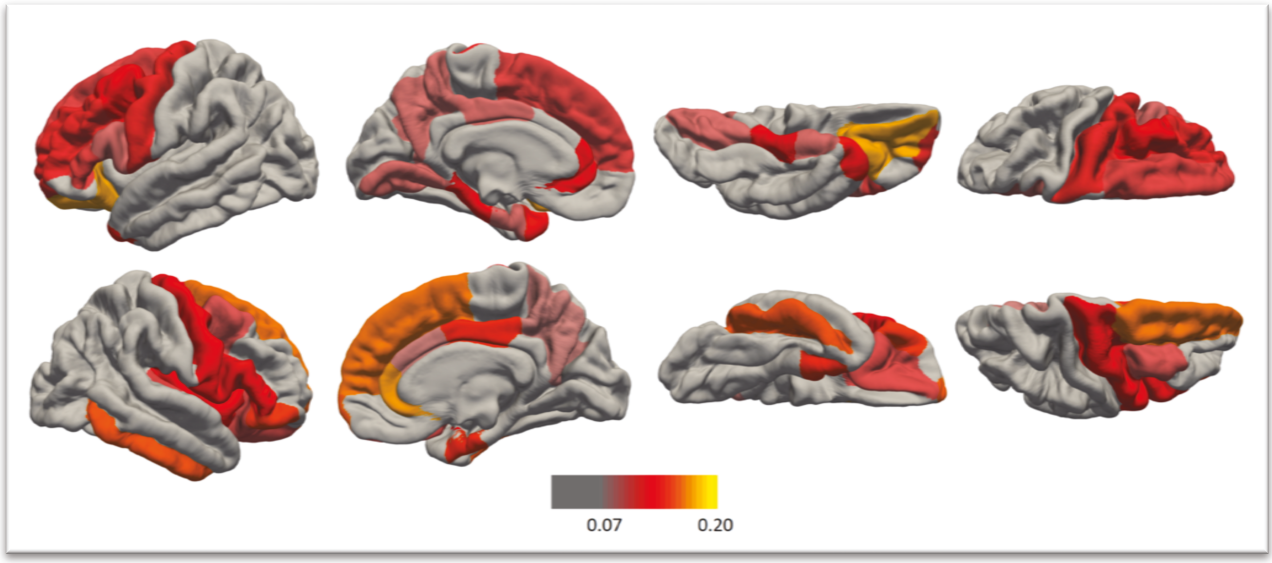

Fig. 2. Cytomegalovirus (CMV) immunoglobulin IgG seropositive (CMV+) patients with schizophrenia spectrum disorders compared to seronegative (CMV−) patients displayed significantly smaller (after false discovery rate correction of 5% by hemisphere) left (L) and right (R) surface areas whilst controlling for age and sex in the following regions: R caudal anterior cingulate, L &R caudal middle frontal, L & R entorhinal, R frontal pole, R inferior temporal, R Insula, L & R lateral orbitofrontal, L lingual, L parahippocampal, L & R pars opercularis, R pars orbitalis, L & R pars triangularis, L & R posterior cingulate, L & R precentral, L & R precuneus, L & R rostral anterior cingulate, L rostral middle frontal, L & R superior frontal, R transverse temporal and L temporal pole. Color bar represents effects sizes: the variation in SAs explained by CMV status (partial eta^2 derived from the analyses of covariance).

of the higher CMV antibody concentrations is not established, but they may indicate more frequent reactivations.[30,32] Not only reactivation rate but also reactivation intensity and duration have been linked to elevated CMV antibody levels.[32] Our results of both seropositivity and antibody concentrations being inversely associated with SA may indicate a SA vulnerability to CMV infection in SCZ especially among patients with more frequent, longer or intense reactivation events. Furthermore, there was no association between HSV1 seropositivity and the total SA indicating a CMV specificity (supplementary material). In addition, we failed to find any associations between CMV status and the mean overall CT. Finally, in the whole sample, patients had smaller SA and thinner cortex than HC, men had larger total SA than women while increasing age was associated with cortical thinning already from young adulthood and with smaller SA after the age of 35 (supplementary material).

CMV seropositivity was associated with significantly smaller regional SAs mainly in the frontal and the temporal lobe regions. Out of the 34 left and 34 right DK atlas SAs, CMV+ patients had significantly smaller SAs ($p_{FDR} < .05$) in 16/34 right and 14/34 left SAs compared with CMV− patients: 10 right and 8 left frontal regions, three right and three left temporal regions, two right and two left parietal regions, one left occipital region and the right insula (figure 2 and supplementary table S3). Furthermore, CMV+ patients had nominally significantly ($p_{uncorrected} < .05$) smaller SAs in totally 19/34 right and 20/34 left regional SA. Interestingly, all 68 DK atlas SAs were smaller in CMV+ patients than CMV− patients (supplementary table S4). In the recent ENIGMA (Enhancing Neuro Imaging Genetics Through Meta Analysis) meta-analysis, patients with SCZ had smaller regional SAs than HC in all DK atlas regional SAs without regional specificity and the largest effect sizes in the frontal and temporal lobes (top 15 SAs were all frontal or temporal).[33] The right superior frontal, left superior frontal and right precentral SA were the frontal SAs with the largest effect sizes in the ENIGMA study, and CMV seropositivity was significantly associated with all three in the present study. Concerning the temporal lobe, the SAs with the largest effect sizes in the ENIGMA were the right middle temporal, right fusiform and right inferior temporal SAs. In the present study, CMV seropositivity was significantly associated with smaller right inferior temporal SA ($p_{FDR} < .05$, partial eta^2 = 0.156), and nonsignificantly associated with the right middle temporal ($p_{uncorrected} = .066$) and the right fusiform ($p_{uncorrected} = .166$) SA. Interestingly, the top regional SA in the ENIGMA was the right superior frontal SA which was one of the top findings in the present study with a large effect size (partial eta^2 = 0.165). Our results may indicate that CMV seropositivity contributes to the widespread smaller regional SAs in some patients with SCZ especially in the frontal lobe.

The observed association between CMV latent infection and cortical SA may be a mild equivalent of the established CMV impact on the human cortex observed in congenital CMV infections and in immunodeficiency.[7,9] Congenital CMV infection is often devastating for the central nervous system, but its incidence is less than 2%,[16] 0.2% in Norway,[34] whereas in our study, approximately 50% of the participating patients have been exposed to CMV. Thus, although we cannot determine from the present results when the primary CMV infection or its hypothesized impact on the cortical SA took place, we can conclude that for the vast majority of the CMV+ patients it occurred postnatally, and we can thereby rule out that the smaller SA in CMV+ patients is related to nonlethal congenital CMV infection. The inverse DOI-SA association also indicates that the smaller SA is distinct from congenital CMV and supports thereby a postnatal CMV involvement. Importantly, normal aging is associated with a declining brain volume which is linked to age-related reductions of both grey and white matter.[35,36] The shrinkage of the cortical grey matter in healthy adults is more prominent in the frontal and parietal lobes,[36] and is linked to cortical volume, cortical SA and CT reductions.[37] Compared with HC patients with SCZ show larger age-related decrement in the whole brain, total and frontal grey matter, and frontal, parietal and temporal white matter[38,39] with the frontal lobes being predominately affected.[39] We are tempted to speculate that CMV is a key environmental factor playing a role in the excess grey matter loss in SCZ. We still cannot know if the smaller SA in CMV+ patients is a result of the primary CMV infection, the CMV reactivations and/or the chronic infection. DOI was linked to smaller SA in CMV+ but not CMV− patients suggestive of a progression of tissue loss with extended illness and CMV seropositivity.

The observed vulnerability of patients with SCZ to CMV infections may be due to genetic variations implicated in both SCZ and CMV infections. The majority of genes involved in CMV infections, including major histocompatibility complex-, interleukin- and tumor necrosis factor-related genes, has also been implicated in SCZ.[40,41] Furthermore, maternal CMV infection interacts with *CTNNA3*, a gene encoding catenin alpha-3 which is a part of a complex that CMV during infection can disconnect disrupting thereby intercellular connections, to predict SCZ risk.[42] Furthermore, CMV latent infection has a surprisingly profound impact on host immune system.[43] It is mainly associated with a decrease of naïve and increase of late-differentiated T-cell subsets.[44] One of the most abundant cytokines that CMV-infected cells secrete is interleukin-6 (Il-6),[45,46] which in turn robustly boosts CMV reactivation rates.[47] Interestingly, increased Il-6 levels have been reported in SCZ[48] and in children at increased risk for psychosis.[49] CMV infection may thereby have a greater impact on hosts with or at risk for psychosis.

It has been shown based on evidence from human and murine CMV that neural stem cells are the main sites of CMV latency in the brain,[5,6] while neural stem cell differentiation to progenitor cells is linked to CMV reactivation.[5] Neural stem and progenitor cells are present in the human subependymal zone (SEZ) in all ages indicating adult neurogenesis.[50] In the SEZ of patients with SCZ, there is a decrease in neurogenesis markers with a parallel increase in inflammation markers.[50] This may be consistent with a CMV infection resulting in inflammation and cell loss. An impaired neurogenesis might contribute to the observed cortical abnormalities not only found in patients with SCZ relative to HC but also in CMV+ patients with SCZ relative to CMV− patients. Interestingly, the hippocampal dentate gyrus is also a major neurogenic niche in the adult mammalian brain with neural stem and progenitor cells giving rise to new neurons.[51–53] In a recent study, among male patients with SCZ spectrum disorders, CMV+ patients had smaller dentate gyrus than CMV− patients.[54]

Finally, even among HC, CMV+ participants had (nonsignificantly, $P = .059$) smaller SA than CMV− participants (figure 1). Considering our directional hypothesis that CMV seropositivity would be associated with smaller SA and dividing thereby the *P*-value by two ($P = .03$) the CMV-SA association in HC may also be considered significant. Still, the significant CMV-by-diagnosis interaction in the whole group indicates that the impact (if we assume causal relationship) of CMV on SA is significantly larger in patients possibly due to resilience in HC as a result of BBB integrity, a noninflammatory environment and an adequate host immune response.

The present study has certain limitations. First, we cannot know when the CMV primary infection and the subsequent CMV reactivations occurred. The study has a cross-sectional design, and long-term course or causal effects cannot be determined. Another limitation of the study is the relatively small number of patients ($n = 67$). However, the patient group was well-characterized and we could therefore take account of possible confounders including medication variables, education variables, DOI and substance use. Furthermore, the patient analysis was the follow-up analysis of the significant two-way (diagnostic group-by-CMV status) interaction from the whole sample analysis, where 329 individuals were included.

To conclude, we have shown that CMV seropositivity, reflecting previous CMV infection and current latency, is associated with smaller total cortical SA and smaller regional SAs mainly in the frontal and temporal lobe in patients with SCZ spectrum disorders. CMV may be an environmental factor with a key role in the established SA aberrations in SCZ.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin* online.

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