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ORIGINAL ARTICLE

Persistent infection with neurotropic herpes viruses and cognitive impairment

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Background. Herpes virus infections can cause cognitive impairment during and after acute encephalitis. Although chronic, latent/persistent infection is considered to be relatively benign, some studies have documented cognitive impairment in exposed persons that is untraceable to encephalitis. These studies were conducted among schizophrenia (SZ) patients or older community dwellers, among whom it is difficult to control for the effects of co-morbid illness and medications. To determine whether the associations can be generalized to other groups, we examined a large sample of younger control individuals, SZ patients and their non-psychotic relatives ($n=1852$).

Method. Using multivariate models, cognitive performance was evaluated in relation to exposures to herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and cytomegalovirus (CMV), controlling for familial and diagnostic status and sociodemographic variables, including occupation and educational status. Composite cognitive measures were derived from nine cognitive domains using principal components of heritability (PCH). Exposure was indexed by antibodies to viral antigens.

Results. PCH1, the most heritable component of cognitive performance, declines with exposure to CMV or HSV-1 regardless of case/relative/control group status ($p=1.09\times10^{-5}$ and 0.01 respectively), with stronger association with exposure to multiple herpes viruses ($\beta=-0.25$, $p=7.28\times10^{-10}$). There were no significant interactions between exposure and group status.

Conclusions. Latent/persistent herpes virus infections can be associated with cognitive impairments regardless of other health status.

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Key words: Cognition, cytomegalovirus, HSV-1, HSV-2, schizophrenia.

Introduction

Herpes viruses are double-stranded DNA viruses, many of which cause lifelong infection, with intermittent latent and reactivation phases (Schmutzhard, 2001). In the majority of individuals, infection is latent and clinically asymptomatic. However, these viruses are not necessarily harmless. DNA from herpes simplex virus type 1 (HSV-1) has been found in the frontotemporal gray matter in the brain among 34% of cases dying from non-neurological diseases, suggesting that the brain may be a favored site for latency (Jamieson *et al*. 1991; Bertrand *et al*. 1993; Baringer & Pisani, 1994). Cytomegalovirus (CMV), another common herpes virus, infects a variety of organs including the brain (Taylor, 2003; Cheeran *et al*. 2009). HSV-1, CMV and herpes simplex virus type 2 (HSV-2), a related herpes virus, can all cause rare and fatal

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encephalitis, particularly in immune-compromised individuals or in neonates (Schmutzhard, 2001; Cheeran *et al*. 2009). Cognitive and behavioral deficits have been observed among survivors of encephalitis (McGrath *et al*. 1997; Borgo *et al*. 2000).

Cognitive impairment traceable to viral exposure can occur even without a history of encephalitis (Prasad *et al*. 2012). An association between HSV-1 exposure and lower total scores on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was noted in a relatively small sample of otherwise healthy individuals (odds ratio 3.2, 95% confidence interval 1.18–8.73, $p < 0.03$, $n = 240$) (Dickerson *et al*. 2008). Another cohort-based study showed elevated rates of cognitive decline over 4 years among elderly Mexican-Americans with elevated CMV antibody titers (Aiello *et al*. 2006). A study of Finnish patients with vascular disease suggested cumulative effects of joint exposure to CMV, HSV-1 and HSV-2 on cognition, but it is difficult to control for the effects of co-morbid diseases, particularly in older individuals (Strandberg *et al*. 2003). Additional risk factors are likely to be involved, as these investigators reported subsequently that the apoliprotein e4 genotype and educational status may act in combination with seropositivity to the herpes viruses (Strandberg *et al*. 2005).

Several prior studies have sought links between exposure to herpes viruses and risk for schizophrenia (SZ) *per se*, but convincing evidence is not presently available (Delisi *et al*. 1986; Brown & Derkits, 2010). Nevertheless, exposure to herpes viruses is consistently associated with cognitive impairment among young or middle-aged SZ patients (Dickerson *et al*. 2003, 2004, 2008; Shirts *et al*. 2008; Schretlen *et al*. 2010). The impairments are particularly noticeable in individuals exposed to HSV-1 (Dickerson *et al*. 2003; Shirts *et al*. 2008; Schretlen *et al*. 2010). The associations have medium effect sizes (Cohen's $d = 0.5$–0.65), after adjusting for age, sex and socio-economic status (SES) (Schretlen *et al*. 2010). Such impairments are more prominently observed in the working memory and executive function domains, and also in psychomotor speed (Shirts *et al*. 2008; Schretlen *et al*. 2010). Among individuals with SZ, exposure to HSV-1 is also associated with decreased gray matter volume in the prefrontal cortex, a region that plays a pivotal role in the regulation of working memory and executive functions (Prasad *et al*. 2007; Schretlen *et al*. 2010). Similar associations between cognitive impairments and exposure to CMV have also been reported (Shirts *et al*. 2008).

Studies evaluating close non-psychotic relatives of SZ individuals typically find that on average they perform better than their ill relatives, but significantly worse than population controls on several cognitive domains (Gur *et al*. 2007; Calkins *et al*. 2010; Toulopoulou *et al*. 2010). These results are notable because the relatives are not exposed to antipsychotic drugs that could arguably impair cognitive performance and thus explain a portion of the impairment among the SZ cases.

To investigate whether the associations between herpes virus exposure and cognitive impairment can be generalized to other groups, particularly seemingly healthy persons, we investigated a large, well-characterized sample. Participants in this study were African-Americans from the Project Among African Americans to Explore Risks for Schizophrenia (PAARTNERS; Aliyu *et al*. 2006; Calkins *et al*. 2010). All participants were evaluated using the Penn Computerized Neurocognitive Battery (CNB), which encompasses several cognitive domains (Gur *et al*. 2001; Calkins *et al*. 2010). Because the cognitive domains are inter-related, we derived a composite measure, the first principal component of heritability (PCH1), using a linear combination of the cognitive domains with maximized heritability (Ott & Rabinowitz, 1999; Klei *et al*. 2008; Wiener *et al*. 2012). Serum samples obtained from participants were tested with immunoassays to indicate prior viral exposure. As there are at least nine viruses within the Herpesviridae family (Schmutzhard, 2001), we selected three agents previously suggested to impact cognitive function: HSV-1, HSV-2 and CMV.

Method

Participants

The investigation was conducted as an ancillary to PAARTNERS, the multi-site African American study. The recruitment and assessment of the sample is described elsewhere (Aliyu *et al*. 2006). The sample included participants with SZ or schizo-affective disorder (SZA), their non-psychotic relatives and screened adult controls. The majority of families were nuclear units with one SZ proband, but some families included more than one SZ patient. After a complete description of the study to the subjects, written informed consent was obtained. All participants completed the Diagnostic Interview for Genetic Studies (DIGS), the Family Interview for Genetic Studies (FIGS) and the CNB (Aliyu *et al*. 2006; Calkins *et al*. 2010). The CNB measures performance accuracy and speed. Because accuracy and speed scores are generally correlated, only accuracy measures were used (Wiener *et al*. 2009). Only participants who had serum available were investigated in this study. The subset of

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the participants who had no CNB data was excluded from the cognitive analysis.

Estimating viral exposure: serological analysis

Serum was obtained from peripheral venous blood samples. A microplate solid-phase enzyme-linked immunosorbent assay (ELISA) was used to measure immunoglobulin G (IgG) antibody levels to CMV, HSV-1 and HSV-2 (Dickerson *et al*. 2003). The glycoproteins used are highly specific and allowed for the differentiation of antibodies to serologically related viruses (Schretlen *et al*. 2010). Reference samples placed on every plate were used to standardize the results. As serum IgG antibody titers for HSV-1 do not differ between humans with frequent and with rare reactivations (McKenna *et al*. 2001), the results were categorized into positive and negative exposure. Positive exposure was defined by comparing signal to cut-off values provided by the manufacturers (Dickerson *et al*. 2003).

Statistical analysis

Principal components of heritability (*PCH*)

PCH1 was derived by a two-step process as described elsewhere (Wiener *et al*. 2012). First, a mixed model was used to estimate the genetic and residual variance components. The components were used to determine the linear combination of the original phenotypes that yielded the PCH in the second step. We estimated the variance components for CNB *Z* scores based on a model that included fixed effects for overall mean, age and sex, and also random effects for individuals and residuals using the average information algorithm for determining restricted maximum likelihood estimates (AIREMLF90; http://nce.ads.uga.edu/~ignacy/newprograms.html). Observed phenotypes were then adjusted for age and sex to obtain residual values. Because PCH analysis requires no missing observations, we predicted missing residuals from the observed ones using the residual co-variance structure. Imputation was not performed when more than two of the nine residuals were missing; instead, such individuals were excluded from analysis. The linear transformation was subsequently used to calculate PCH. The most heritable of the set of PCH was denoted PCH1.

Building the models: initial analyses

Diagnostic groups. To simplify the models, we first sought to reduce the number of diagnostic categories. We noted that individuals with SZ, SZA depressed type and SZA disorder bipolar type did not differ significantly from each other with respect to mean cognitive phenotypes, hence these diagnoses were binned into one group. This reduction allows a simple encoding of three key, mutually exclusive variables in the data set, each with binary outcomes: SZ, which includes SZA for modeling purposes; non-psychotic relatives of an individual diagnosed with SZ/SZA; and control individuals, who have no symptoms of psychosis and no close relative with SZ/SZA. Note that binary encoding of any two of these variables also encodes the third. We chose to fit models with a variable for SZ/SZA and relative. In this scheme, the mean attributable to controls is identified by the model mean. To capture other diagnoses of interest, we included two other indicator variables: (1) the 'mood' group ($n=313$), which encodes major depressive disorder, other non-psychotic mood-related disorders, and a small number of bipolar I disorder cases ($n=23$); and (2) the 'substance use disorders' (SUD) group, which encodes disorders related to alcohol or substance abuse or dependence ($n=429$). These latter two indicators could be + or − for an individual falling into the mutually exclusive sets (SZ, a relative or a control).

Socio-economic indices. Because rates of exposure are elevated in lower social economic groups (Smith & Robinson, 2002), we explored the relationship between virus exposure and two indices of socio-economic status (SES): years of individual education (EDU) and occupation. Conventionally, SES is indicated by a composite variable incorporating EDU and occupation, as in the Hollingshead Redlich Index (HRI). The occupational categories listed in the DIGS do not enable estimation of the HRI, hence EDU and occupation were analyzed separately. The DIGS includes 21 occupational categories that were grouped as follows: (1) managerial and professional specialty (DIGS 01–03), (2) technical, sales and administrative support (DIGS 04–06), (3) service (DIGS 07–09), (4) manual labor (DIGS 10–15), and (5) other (DIGS 16–21). As EDU and occupation can be impacted by the onset of psychotic disorders, they were nested separately within the three main sample groups (EDU|(SZ/SZA, relative, control); occupation|(SZ/SZA, relative, control).

Analyses relating PCH to infectious exposure

PCH1 was analyzed using mixed models with diagnostic (Dx) and sample categories, EDU within sample category (EDU|sample category) and exposure to infectious agents as fixed effects and the individual as a random effect. These models were analyzed using SOLAR, with a modeling procedure in which each effect is tested given that all others are in the model

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Table 1. *Characteristics of participants*

Characteristic	Controls ($n=283$)	Cases ($n=680$)	Relatives ($n=889$)
Age (years), mean (S.D.)	40.8 (14.9)	38.9 (11.2)	45.8[b] (16.4)
Education (years completed), mean (S.D.)	12.8 (2.5)	11.6[c] (2.27)	12.4 (2.56)
Sex (Male), *n* (%)	121 (42.80)	386 (56.8)[d]	288 (32.40)
Exposure to herpes viruses[a], *n* (%)			
CMV	220 (77.70)	480 (70.60)	722 (81.20)
HSV-1	192 (67.80)	503 (74.00)	645 (72.60)
HSV-2	152 (53.70)	302 (44.40)	508 (57.10)
CMV, HSV-1 and HSV-2	88 (31.10)	207 (30.40)	361 (40.60)

CMV, Cytomegalovirus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; S.D., standard deviation.

[a] Exposure is defined by antibody titers above manufacturers' cut-off levels.

[b] Non-psychotic relatives *versus* controls ($t=-4.51$, $p=7.3\times10^{-6}$).

[c] Schizophrenia/schizo-affective (SZ/SZA) cases *versus* controls ($t=7.229$, $p=1.03\times10^{-12}$).

[d] Males significantly over-represented among cases *versus* controls: $\chi^2=15.73$, $p=9.4\times10^{-5}$, cases *versus* relatives: $\chi^2=10.14$, $p=0.002$.

(Almasy & Blangero, 1998). SOLAR also models genetic correlations among family members based on known relationships. The final derived model included only terms with $p<0.10$. Parameter estimates were also generated for non-significant variables for comparison purposes.

Results

Demographic variables

The overall sample included SZ/SZA cases ($n=680$) and non-psychotic relatives ($n=889$). Healthy, screened adult controls unrelated to the cases or the relatives were also analyzed ($n=283$). They were significantly younger than the relatives (Table 1, $t=-4.51$, $p=7.3\times10^{-6}$). Men were significantly over-represented among the cases compared with controls ($\chi^2=15.73$, $p=9.4\times10^{-5}$) or relatives ($\chi^2=10.14$, $p=0.002$). There was significant correlation between the occupational groups and the duration of education (Spearman's $\rho=-0.4$, $p=7.45\times10^{-63}$ among controls and non-psychotic relatives). The negative correlation is consistent with the classification of the occupational categories.

Prevalence of viral exposure: category-wise comparisons of antibody titers

Exposure levels to each virus are presented in Table 1. Exposure status for each virus was used as the outcome in separate analyses, with age, gender, years of education and category (case, relative or control) as covariates. We used the generalized estimating equation (GEE) to account for correlations among family members (Zeger & Liang, 1986) (see online Supplementary Tables S1–S3). The controls had significantly higher rates of exposure to CMV or HSV-2 in comparison with the cases (CMV: $p=0.024$; HSV-2: $p=0.003$). The relatives showed non-significant trends for the same agents in comparison with the controls. No significant group-wise differences were observed for HSV-1.

PCH

Individual virus exposure model

We modeled exposure to individual herpes viruses using a non-exclusive binary classification of exposure and non-exposure ('individual exposure' model). Associations between PCH1 and infectious exposure were assessed after accounting for diagnostic status (SZ/SZA case, relative or control), other psychiatric co-morbidity (mood and substance disorders) and education nested within diagnostic status. Age and gender were not included because they were regressed out in the estimation of PCH1. The mean neurocognitive performance of control individuals was significantly higher as measured by PCH1 (estimated by the overall mean in the model = 1.51, S.E. = 0.13; Table 2) relative to individuals diagnosed with SZ/SZA ($\beta=-1.28$, $p=1.11\times10^{-27}$), and that of the non-psychotic relatives ($\beta=-0.44$, $p=8.22\times10^{-5}$). Individuals who had SUD had modestly enhanced performance ($\beta=0.19$, $p=0.03$). Significant effects are also observed for the nested EDU variable for all three diagnostic categories (EDU|SZ/SZA, $p=1.67\times10^{-10}$; EDU|non-psychotic relatives, $p=3.45\times10^{-20}$; EDU|controls, $p=1.43\times10^{-7}$) in

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Table 2. *Multivariate analysis of the first principal component of heritability (PCH1)*

	Individual viral exposure model			Combined viral exposure model		
	β coefficient	S.E.	p value	β coefficient	S.E.	p value
Overall mean	1.51	0.13		0.96	0.09	
Covariates						
Viral exposure						
CMV	−0.41	0.09	1.09×10^{-5}			
HSV-1	−0.22	0.09	0.01			
HSV-2	−0.14	0.08	0.07			
Combined exposure (CMV, HSV-1, HSV-2)				−0.25	0.04	7.28×10^{-10}
Diagnostic categories						
Schizophrenia[a]	−1.28	0.12	1.11×10^{-27}	−1.28	0.12	1.04×10^{-27}
Mood disorders[b]			0.75			0.77
Substance-related disorders[c]	0.19	0.09	0.03	0.20	0.09	0.02
Non-psychotic relatives	−0.44	0.11	8.22×10^{-5}	−0.44	0.11	8.73×10^{-5}
Nested education variables						
EDU: Schizophrenia[a]	0.18	0.03	1.67×10^{-10}	0.17	0.03	2.22×10^{-10}
EDU: Non-psychotic relative	0.20	0.02	3.45×10^{-20}	0.20	0.02	6.99×10^{-20}
EDU: Control	0.19	0.04	1.43×10^{-7}	0.19	0.04	1.5×10^{-7}
Nested occupation variables						
Occupation: Schizophrenia[a]			0.12			0.10
Occupation: Non-psychotic relative			0.44			0.48
Occupation: Control			0.81			0.84

CMV, Cytomegalovirus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; EDU, individual years of education; S.E., standard error.

[a] Includes schizophrenia and schizo-affective disorder.

[b] Major depressive disorder, other non-psychotic mood-related disorders, and a small number of bipolar disorder 1 cases.

[c] Alcohol and illicit substance abuse and dependence.

relation to PCH1. No significant effects are observed for the nested occupation variable when analyzed in conjunction with educational status (Table 2). These analyses were repeated, incorporating educational status or occupation only, along with the other covariates. The pattern of associations with herpes viral exposure did not differ (data not shown).

After adjusting for the covariates described above, neurocognitive performance, as measured by PCH1, still significantly decreased with exposure to CMV or HSV-1 (CMV: $\beta = -0.41$, $p = 1.09 \times 10^{-5}$; HSV-1: $\beta = -0.22$, $p = 0.01$). A smaller, non-significant decrease in PCH1 was observed for exposure to HSV-2 ($\beta = -0.14$, $p = 0.07$).

Combined viral exposure model

To evaluate the impact of combined (multiple) exposure on cognitive performance, we fit a model that includes a combined herpes virus exposure variable. This variable was encoded such that individuals with no exposure to any of the herpes viruses received a score of zero, exposure to one agent received a score of 1, exposure to any two received a score of 2 and individuals exposed to all three herpes viruses were assigned a score of 3. Covariates described previously were also included in the model, with PCH1 as the outcome variable (Table 2 and Fig. 1). In terms of covariates, the results are consistent with the individual exposure model. The negative impact of multiple viral exposure on PCH1 is more substantive and more significant ($\beta = -0.25$, $p = 7.28 \times 10^{-10}$) than for individual exposure, suggesting that the association with herpes virus exposure is cumulative.

Do exposure and SZ/SZA status act independently?

It is possible that viral exposure has more salience in a subset of the three diagnostic groups, SZ/SZA, their relatives or controls. To evaluate this possibility we fit models including an interaction between exposure and group status. As none of the interactions were significant (data not shown), we conclude that exposure and diagnostic status have independent effects on neurocognitive performance.

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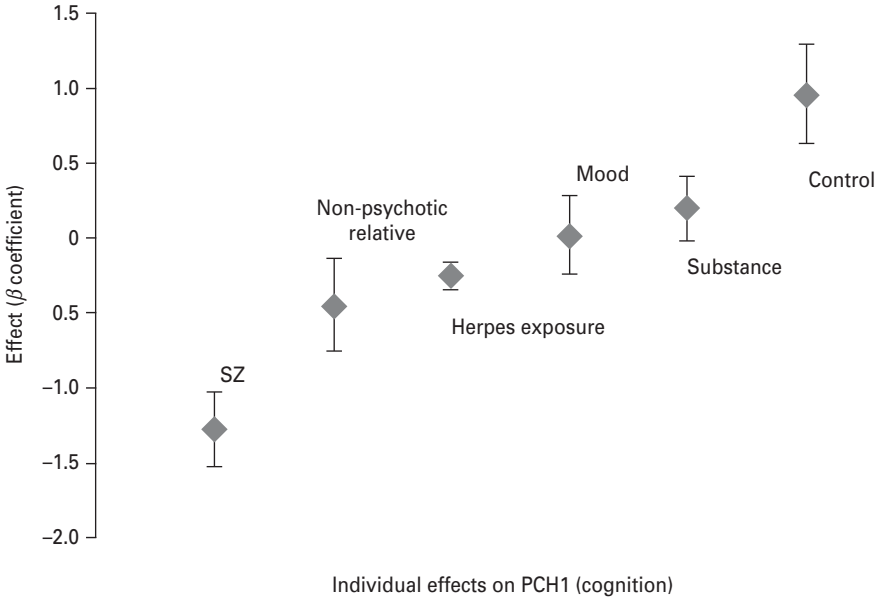

Fig. 1. Variables associated with the first principal component of heritability (PCH1). Covariates and levels of covariates include: SZ, schizophrenia/schizo-affective disorder; herpes virus exposure: exposure to cytomegalovirus (CMV), herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), rated 0–3 for exposure for none, one, two or three herpes viruses; mood disorders: major depressive disorder, depression not otherwise specified and bipolar I disorder; substance, substance use disorders (alcohol or substance abuse or dependence); control, screened adult control participants.

Discussion

Using a highly heritable composite measure derived from representative cognitive domains, we find that exposure to herpes viruses diminishes neurocognitive performance, on average, relative to individuals who are not exposed (Table 2, Fig. 1). This effect is seen even in models that account for several covariates also affecting performance, including duration of education or occupational status. For example, one covariate encodes whether the individual is affected by psychosis (SZ/SZA), is a close relative of an individual affected by psychosis, or a control. As has been observed in several other studies, subjects with SZ/SZA show the greatest mean impairment and the mean performance of their non-psychotic relatives lies between the SZ/SZA subjects and controls. Other psychiatric co-morbidities have a negligible impact. As would be expected, level of educational achievement is substantially correlated with performance.

Although exposure itself is not elevated in the SZ/SZA group, the results from modeling reveal independent effects of viral exposure and affection status on neurocognitive performance. In fact, there is no significant interaction between the covariate accounting for SZ/SZA, relative or control status and exposure status. Thus, our results show that exposure to herpes virus could play an important role in the variability of cognitive performance. This is potentially important from a public health perspective, because exposure to CMV and HSV-1 is very common in the population. The prevalence of HSV-1 infection is less than 1% in US neonates, but rises to approximately 40% among children/teens and 40–70% among middle-aged adults (Smith & Robinson, 2002; www.cdc.gov/nchs/nhanes.htm). Similarly, the overall age-adjusted prevalence of CMV is about 60% (Staras *et al*. 2006). The rates of seropositivity reported here are consistent with those estimates. Thus, a proportion of cognitive impairment in middle-aged and older adults could be attributed to neurotropic viral exposure and may be treatable. It is uncertain how the present studies relate to a substantial literature linking herpes virus exposure to risk for Alzheimer's disease (Itzhaki, 2004; Carter, 2011; Féart *et al*. 2011; Licastro *et al*. 2011; Alvarez *et al*. 2012). Longitudinal follow-up studies could address this question in the present sample.

Several pathological processes could explain the association between viral exposure and cognitive impairment. Although latent infection rarely produces behavioral changes, reactivation leads to cell death in humans (Whitley, 1996) and in animal models (Goodkin *et al*. 2004). These processes occur throughout the lifespan, so their cumulative effects could explain cognitive impairment. In support of this notion, we find that individuals exposed to more than one herpes virus have greater impairment than

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individuals exposed to one agent alone. Peripheral reactivation can also provoke the release of cytokines that could cross the blood brain barrier and damage neuronal membranes or alter neurotransmission (Steiner *et al*. 2007).

It is not currently possible to identify herpes viruses directly in the brain *in vivo*. Although efforts are in progress to use radioactive tracers to detect viral particles, such methods are not widely available at present (Buursma *et al*. 2005). Viral DNA can be detected in the blood or saliva using polymerase chain reaction (PCR) assays, but samples have to be collected repeatedly before exposure can be excluded (Kaufman *et al*. 2005). Moreover, they may not reflect the presence of viral particles in the brain. Therefore, assays that estimate antibodies to the infectious agents are used conventionally to indicate prior exposure. Post-mortem studies of humans and rodents suggest that the HSV-1 antibody titers are correlated with the presence of the infectious agent in the brain (Stevens *et al*. 1988). The assays are highly sensitive and specific but there are some shortcomings: (i) the titers do not indicate duration or timing of exposure; and (ii) antibody titers are elevated after 2 weeks and can remain elevated for prolonged periods; however, if reactivation does not occur for a long time the antibody titers may be lower than the cut-off. Thus, a minority of antibody-negative persons may in fact be exposed; such individuals would reduce the magnitude of the observed associations.

In conclusion, we report that exposure to three herpes viruses diminishes mean cognitive performance in a family-based sample of SZ patients, their unaffected relatives, and relatively young, otherwise healthy community dwellers. Models of the data suggest that the impact of exposure is similar regardless of whether the subject has SZ/SZA, is a relative of an SZ/SZA subject, or a control. We recognize that our results cannot be taken as proof of causality. Nevertheless, because herpes virus infections are common in the population, these observations could have a substantial impact on public health for older adults. Indeed, demonstrating causal links could motivate more aggressive treatment with anti-viral agents.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S003329171200195X.

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Declaration of Interest

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