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# Association between cognitive functioning, exposure to Herpes Simplex Virus type 1, and the COMT Val158Met genetic polymorphism in adults without a psychiatric disorder

Faith Dickerson [a], Cassie Stallings [a], Anne Sullens [a], Andrea Origoni [a], Flora Leister [b], Bogdana Krivogorsky [b], Robert Yolken [b,*]

[a] Stanley Research Program, Sheppard Pratt, Baltimore, MD, USA
[b] Stanley Neurovirology Laboratory, Johns Hopkins School of Medicine, 600 N Wolfe Street, Baltimore, MD 21207, USA

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ABSTRACT

Previous studies have documented that serologic evidence of infection with the neurotropic human herpesvirus Herpes Simplex Virus type 1 (HSV-1) is associated with increased levels of cognitive dysfunction in individuals with schizophrenia or bipolar disorder. The catechol-*o*-methyl transferase (COMT) Val158Met polymorphism has also been associated with cognitive dysfunction in individuals with psychiatric disorders as well as in some control populations. We examined whether these factors are independently associated with cognitive functioning in adults without a history of a psychiatric disorder. A total of 240 individuals were evaluated with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and the Wisconsin Card Sorting Test (WCST). We measured IgG antibodies to HSV-1 by enzyme immunoassay and employed real time PCR to measure COMT Val158Met genotypes. Serological evidence of HSV-1 was significantly associated with a lower RBANS total score independent of demographic factors and the COMT Val158Met genotype. The strongest association between cognitive functioning and serological evidence of HSV-1 infection was with the domain of delayed memory. Serological evidence of HSV-1 infection was associated with an 18-fold increased odds of having a severe impairment in this domain. The Val/Val genotype of the COMT Val158Met polymorphism was also significantly associated with the RBANS total score and with a moderate decrease in the domain of attention. Infections with HSV-1 and the COMT Val158Val genotype are risk factors for cognitive deficits in non-elderly persons without a psychiatric disorder.



## 1. Introduction

The determinants of cognitive functioning in non-elderly adults have not been fully elucidated. Previous studies have identified a number of genetic factors that may contribute including a common polymorphism of the catechol-o-methyl transferase gene that involves the coding of either valine or methionine at position 158 (COMT Val158Met). When present in the homozygous state, the polymorphism encoding valine is associated with an increased rate of dopamine catabolism within the prefrontal cortex of the brain as compared to the polymorphism encoding methionine. This polymorphism has been associated with decreased performance on tasks of working memory in schizophrenia and in controls in some but not all studies (Barnett et al., 2007; Bruder et al., 2005; Diaz-Asper et al., 2008; Egan et al., 2001; Goldberg et al., 2003; Ho et al., 2005; Malhotra et al., 2002; Minzenberg et al., 2006; Szöke et al., 2006; Weinberger et al., 2001). In previous studies, we found that the COMT Val158Val genotype was associated with relative memory impairment in individuals with bipolar disorder but not in individuals with schizophrenia (Dickerson et al., 2006, 2007).

Studies with monozygotic twins have indicated that environmental factors may also contribute to cognitive functioning (Reynolds et al., 2007). Exposure to infectious agents represents one environmental interaction which has been associated with cognitive impairments in some individuals. In particular, the potentially neurotropic human herpesvirus Herpes Simplex Virus type I (HSV-1) has been identified as a cause of deficits in memory and executive functioning following replication within the central nervous system (Barbarotto et al., 1996; Hokkanen and Launes, 2000; Kaplan and Bain, 1999; Pewter et al., 2007; Utley et al., 1997). In addition, serological evidence of infection with HSV-1 has been associated with cognitive impairment in some populations of elderly individuals (Strandberg et al., 2003, 2004). We have previously documented that serological evidence of infection with HSV-1 is associated with impairments in

* Corresponding author. Fax: +1 410 955 3723.
*E-mail address:* rhyolken@aol.com (R. Yolken).

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cognitive functioning in individuals with schizophrenia (Dickerson et al., 2003) and in individuals with bipolar disorder (Dickerson et al., 2004). In this study, we examine the association between serological evidence of HSV-1 infection, the COMT Val158Met polymorphism, and cognitive domains in 240 non-elderly adults without a history of psychiatric disorder.

## 2. Methods

Individuals without a history of psychiatric disorder were recruited from posted announcements and screened to rule out a current or past psychiatric disorder with the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1998). Participants were between the ages of 18 and 65, inclusive and had none of the following conditions: (1) current substance abuse over the past one month or of any history of intravenous substance abuse; (2) mental retardation; (3) medical disorder that would affect cognitive performance such as epilepsy, history of encephalitis or severe head trauma, or any other reported neurological disorder of the central nervous system; and (4) clinically apparent herpesvirus infection or recent treatment with antiviral medications. The sample represents an enlargement of the non-psychiatric sample described previously (Dickerson et al., 2004).

Participants signed an informed consent after the study procedures were explained and were administered the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, Form A) (Randolph, 1998). The RBANS is comprised of 12 subtests that are used to calculate a Total score and 5 index scores. Test indices are Immediate Memory (comprised of List Learning and Story Memory tasks); Visuospatial/Constructional (comprised of Figure Copy and Line Orientation tasks); Language (comprised of Picture Naming and Semantic Fluency tasks); Attention (comprised of Digit Span and Coding tasks); and Delayed Memory (comprised of List Recall, Story Recall, Figure Recall, and List Recognition tasks). Each index score is expressed as an age-adjusted standard score with a mean score of approximately 100 and a standard deviation of approximately 15 based on data reported in the test manual from a normative study group of 540 healthy subjects, ranging in age from 20 to 89, matched to the US Census on gender, ethnicity, and years of education. The index scores are combined to yield a RBANS total score, which is a measure of overall cognitive functioning. Previous studies indicate that the RBANS is highly correlated with more extensive neuropsychological assessment (Hobart et al., 1999). Participants in the current study also were administered the Wisconsin Card Sorting Test (WCST), 64 card version, which is a test of executive functioning, assessing cognitive flexibility and set-shifting (Heaton, 1993). We scored the number of categories completed and the number of perseverative errors.

A venous blood sample of approximately 7 ml was obtained from all of the study participants. Serum was separated from coagulated whole blood by centrifugation. IgG antibodies to the HSV-1 specific glycoprotein gG1 were measured in each serum sample by means of solid phase enzyme immunoassay using commercially available assay reagents (Focus Technologies, Cypress, CA). Details of the assay method have been previously described (Dickerson et al., 2003, 2004). Briefly, the assay consisted of the addition of a 1:101 dilution of serum sample to purified HSV-1 gG1 glycoprotein immobilized on the wells of microtiter plates. Following incubation at 37°, unbound serum was removed by washing and the amount of IgG reactive to the solid phase antigen was quantified by sequential incubations with peroxidase labeled anti-human IgG and tetramethylbenzidine-$H_2O_2$ enzyme–substrate. The optical density of the color generated by the ensuing enzyme–substrate reaction was quantitated by means of a microplate colorimeter at a wavelength of 450 nm. For each sample, the antibody level was expressed in terms of an index value calculated by dividing the optical density generated by the test sample by that of an IgG cutoff calibrator assayed in each test run. An individual whose serum generated an index value of $\geqslant 1.1$ was defined as being seropositive and having serologic evidence of HSV-1 infection. Antibodies to the following additional human herpesviruses were also measured by similar solid phase enzyme immunoassays using previously described methods: Herpes Simplex Virus type 2, Cytomegalovirus, Epstein-Barr Virus, Varicella Zoster Virus, and Human Herpes Virus type 6 (Dickerson et al., 2003, 2004).

From the same blood sample, DNA was extracted from a 0.2 ml aliquot of serum utilizing column chromatography (MinElute Virus Spin Kit, Qiagen Corporation, Valencia, CA). The COMT Val158Met polymorphism was detected using real time polymerase chain reaction, fluorescent labeled hybridization probes and 5′ nucleosidase (Taqman) reactions employing the following reagents: forward primer 5′-CCCAGCGGATGGTGGAT-3; reverse primer 5′-CAGGCATGCACACCTTGTC-3′, VIC labeled minor groove binding probe: 5′-TTCGCTGGCATGAAG-3′, FAM labeled minor groove binding probe 5′-TCGCTGGCGTGAAG-3′. Samples were amplified for 45 cycles at 60 °C prior to fluorescence measurement and genotype determination. The accuracy of the reactions was monitored by the performance of relevant controls. Analyses were performed by comparing individuals with the homozygous COMT 158 Val/Val genotype with individuals who had the COMT 158 Val/Met or the COMT 158 Met/Met genotype.

## 3. Data analyses

We examined the association between HSV-1 seropositivity, the COMT 158 Val/Val genotype, and cognitive scores by performing multiple regression analyses on the RBANS Total and index scores and on WCST number of categories and number of perseverative errors. All analyses included the covariates of age; gender; race (Caucasian vs. non-Caucasian); education; and years of maternal education, as a measure of socioeconomic status; as well as HSV-1 seropositivity and COMT 158 Val/Val genotype.

We also examined the association between HSV-1 seropositivity, COMT 158 Val/Val genotype, and cognitive impairment which was defined as below the 10th percentile in published standardization samples: RBANS index score <80 (Randolph, 1998) and WCST $\geqslant 8$ perseverative errors and $\leqslant 1$ categories completed (Kongs et al., 2000). Multivariate logistic regressions were performed on impaired status for each of the cognitive measures. All analyses included the covariates of age, gender, race (Caucasian vs. non-Caucasian), education, and maternal education as well as HSV-1 serostatus and COMT Val/Val genotype. In cases where HSV-1 serostatus had a significant effect ($p < .05$), additional multiple logistic regression analyses were performed in which the serostatus to other human herpesviruses were analyzed in place of HSV-1. These include Herpes Simplex Virus type 2, Cytomegalovirus, Epstein-Barr virus, Varicella zoster virus and Human Herpes Virus type 6.

## 4. Results

The demographic characteristics of the study population are depicted in Table 1. The sample of $N = 240$ had a mean age of 33.6 (s.d. 11.5) years. A total of 87 (36%) were male and 156 (65%) were Caucasian, 67 (28%) were African American, and 17 (7%) were another race. The mean years education was 15.6 (s.d. 2.1) and the mean years maternal education was 13.3 (s.d. 3.2). Overall a total of 108 (45%) of the individuals had serological evidence of HSV-1 infection and 86 (36%) had the COMT 158 Val/Val genotype. The overall rate of HSV-1 infection did not differ by race ($\chi^2 = 1.9$, $p > .2$ or gender ($\chi^2 = 1.3$, $p > .2$).

As shown in Table 2, both HSV-1 seropositivity and COMT 158 Val/Val status were significantly associated with lower RBANS total score when adjusting for age, gender, race, years education, and years maternal education (overall equation $F(7,232) = 11.28$, $p < .0001$; HSV-1 coefficient = −2.88, $p = .035$; COMT coefficient = −3.07, $p = .032$). There was no significant statistical interaction between HSV-1 and the COMT 158 Val/Val genotype (interaction variable $p > .05$). There was also a significant effect of HSV-1 on RBANS Delayed Memory (coefficient = −3.99, $p = .003$) and of the COMT 158 Val/Val genotype on RBANS Attention (coefficient = −4.48, $p = .024$). There were not any significant associations between either HSV-1 seropositivity or the COMT Val/Val genotype and the mean Wisconsin Card Sorting Test number of categories or number of perseverative errors.

We also examined the independent association between serological evidence of HSV-1 infection and COMT 158 Val/Val genotype and impairment status on the RBANS Total and index scores and the Wisconsin Card Sorting Test (WCST) variables, defined as

**Table 1**
Demographic characteristics of study sample, $N = 240$ non-elderly adults without a history of a psychiatric disorder

| Characteristic | Mean (SD) or number (%) |
|---|---|
| Age years | 33.7 (11.5) |
| Gender—Male | 87 (36%) |
| *Race* | |
| Caucasian | 156 (65%) |
| African-American | 67 (28%) |
| Other | 17 (7%) |
| Years education | 15.6 (2.1) |
| Years maternal education | 13.3 (3.2) |
| HSV-1 seropositivity | 108 (45%) |
| COMT 158 Val/Val genotype | 86 (36%) |