Toxoplasma gondii Immunoglobulin G Antibodies and Nonfatal Suicidal Self-Directed Violence

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ABSTRACT

Objective: The primary aim was to relate *Toxoplasma gondii* seropositivity and serointensity to scores on the self-rated Suicide Assessment Scale (SUAS-S). Another aim was to reevaluate the previously reported positive association between *T gondii* serointensity and a history of nonfatal suicidal self-directed violence.

Method: This cross-sectional, observational study compared *T gondii* serointensity and seropositivity in plasma from 54 adult suicide attempters (inpatients at Lund University Hospital, Lund, Sweden) and 30 adult control subjects (randomly selected from the municipal population register in Lund, Sweden) recruited between 2006 and 2010. The potential of patients and controls for self-directed violence was evaluated with the SUAS-S. Psychiatric diagnoses were made according to *DSM-IV* criteria. Plasma samples were tested for immunoglobulin G antibodies to *T gondii*, cytomegalovirus, and herpes simplex virus type 1. Data were analyzed using multivariable logistic regression to investigate the association between *T gondii* serointensity or seropositivity and a history of nonfatal suicidal self-directed violence; multivariable linear regression was used to explore the relationship between *T gondii* serointensity or seropositivity and the SUAS-S. Both regression models included sex, age, and body mass index as covariates.

Results: Seropositivity of *T gondii* (adjusted odds ratio [OR] = 7.12; 95% CI, 1.66–30.6; $P = .008$) and serointensity of *T gondii* (adjusted OR = 2.01; 95% CI, 1.09–3.71; $P = .03$) were positively associated with a history of nonfatal suicidal self-directed violence. Seropositivity of *T gondii* was associated with higher SUAS-S scores, a relationship significant for the whole sample ($P = .026$), but not for suicide attempters only. No significant associations with other pathogens were identified.

Conclusions: These results are consistent with previous reports on the association between *T gondii* infection and nonfatal suicidal self-directed violence. Confirming these results in future large longitudinal studies and including suicide as an outcome may lead to novel individualized approaches in suicide prevention.

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Suicide is a major public health problem. In the United States, 36,909 suicide deaths were reported in 2009, with 1 death every 14.2 minutes.[1] Globally, suicide accounts for almost 1 million deaths every year.[2] An estimated 90% of people who commit suicide have a diagnosed psychiatric disorder.[3] Furthermore, a history of nonfatal suicidal self-directed violence is one of the most significant risk factors for suicide.[3,4]

Toxoplasma gondii is an intracellular parasite that can reproduce sexually in the lumen of the gut of its definitive host—any member of the cat family. The common route of infection in humans is ingestion of the parasite's oocysts (eg, food or water contaminated by feces of infected cats, and, to some degree, handling of cats' litter) or *T gondii* tissue cysts present in undercooked or raw meat or in raw food (eg, salad) that is contaminated by tools (eg, knives) used to process raw meat. Up to one-third of the world's population is infected with *T gondii*.[5] The prevalence of immunoglobulin G (IgG) antibodies to *T gondii* in the United States is estimated to be 10.8% of the population between the ages of 6 and 49 years,[6,7] and, in Sweden, the prevalence is 23%.[8] The symptoms of the infection depend mainly on the host's immune response. In immunocompromised[9] individuals and fetuses,[10] severe neuropathology (eg, encephalitis), including potential death, has been documented. On the other hand, the far more common chronic toxoplasmosis in immunocompetent hosts is traditionally considered minimally symptomatic[11] or even asymptomatic.[12] However, an emerging body of research has revealed the potential association between latent toxoplasmosis and certain psychiatric conditions. For instance, the prevalence of *T gondii* seropositivity was found to be higher in schizophrenia patients[13] as well as patients with personality disorders,[14] as compared to controls. Additionally, *T gondii* seropositivity has been found to be associated with higher rates of car crashes[15,16] and personality features,[17,18] and its reactivation was proposed to be one of the mechanisms leading to migraine headaches.[19–21]

The first report of an association of *T gondii* with nonfatal suicidal self-directed violence was made by Arling et al[22] in a sample of patients with recurrent mood disorders in the Baltimore, Maryland/Washington, DC area. These results were confirmed in psychiatric patients in a case-control study carried out in Turkey.[23] The association of *T gondii* and nonfatal suicidal self-directed violence was further confirmed in younger patients with schizophrenia in Germany.[24] Additionally, Ling et al[25] found an association between European national rates of *T gondii* infection and suicide in women of menopausal age.

The above studies have focused on nonfatal suicidal self-directed violence, except for the study by Ling et al,[25] which linked *T gondii* to fatalities. However, the Ling et al[25] study can have only restricted impact given the inherent limitations of its ecological design. In the absence of cohorts containing sufficient numbers of suicide cases

with adequate preexisting biological material and funding allowing testing for *T gondii* antibodies, a surrogate method is to use predictors of suicide completion among suicide attempters. Up to this point, there has been no study relating *T gondii* seropositivity to a suicidality severity scale used to predict future suicide. Thus, the primary goal of the current project was to test a hypothesized positive relationship between markers of chronic infection with *T gondii* and suicidality scores on a validated suicidality scale. As a secondary goal, we reevaluated the previously reported association between nonfatal suicidal self-directed violence and *T gondii* IgG antibodies.

Clinical Points

- The study confirms an association between *Toxoplasma gondii* seropositivity and nonfatal suicidal self-directed violence, the most important predictor of fatal suicidal self-directed violence.
- A positive association between *T gondii* seropositivity and higher scores on the self-rated Suicide Assessment Scale, previously reported to predict subsequent suicidal behavior, emphasizes the need for further research to confirm the prognostic role of testing for *T gondii* in patients at risk for suicide and to consider this widespread neurotropic infection, in concert with other suicide risk factors, as a target for prophylaxis and treatment.

METHOD

In this cross-sectional, observational study, we compared *T gondii* antibody titers in plasma from 54 adult suicide attempters and 30 adult control subjects recruited between 2006 and 2010 in Lund, Sweden. The serum samples were collected in Lund, Sweden, with serologic analysis performed blindly at the University of Maryland Institute of Virology, Baltimore, Maryland. The study was approved by the Ethical Review Board for human studies, Lund/Malmoe, Sweden and was exempted by the Institutional Review Board of the University of Maryland, Baltimore.

The participants with a history of self-directed violence were inpatients at the Lund University Hospital and were admitted for suicide attempt, which was a priori defined as "situations in which a person has performed an actually or seemingly life-threatening behavior with the intent of jeopardizing his/her life or to give the appearance of such intent, but which has not resulted in death."[26(p1062)] However, all suicide attempters who were included in the study expressed suicidal intent explicitly. We have converted the terminology to match the recent guidelines of the Centers for Disease Control and Prevention for uniform definitions of self-directed violence.[27]

Control participants were randomly selected from the municipal population register in Lund, Sweden. Exclusion criteria were as follows: previous or ongoing psychiatric or somatic disorder; prior suicide attempts; prior psychiatric treatment, including psychotherapy; ongoing somatic illness or treatment, including painkillers or antibiotics; pregnancy; suicide or nonfatal suicidal self-directed violence in a first-degree relative; and sporadic recent drug use.

Subjects who did not meet any exclusion criteria at the phone interview were further assessed for psychiatric and somatic pathology during an appointment with a research nurse and a resident or specialist in psychiatry. These subjects were checked for alcohol abuse using the Alcohol Use Disorders Identification Test[28]; participants who scored 8 or above were excluded. They were further assessed for possible suicidality as described below. All participants underwent a general physical examination including blood pressure measurement, pulmonary and cardiovascular history and examination, and detailed neurologic examination. The laboratory examination included hemoglobin, white blood cell count, C-reactive protein, blood glucose, thyroid status, blood lipids, and urine sample analysis.

Psychiatric diagnosis was made according to *DSM-IV*, using the Structured Clinical Interview for *DSM-IV* Axis I Disorders[29] and the Structured Clinical Interview for *DSM-IV* Axis II Personality Disorders.[30] Diagnoses among the sample of suicide attempters were as follows: major depressive disorder (n = 7), bipolar I disorder (n = 4), bipolar II disorder (n = 10), dysthymia (n = 2), depression not otherwise specified (n = 3), generalized anxiety disorder (n = 1), anxiety not otherwise specified (n = 4), psychotic disorder not otherwise specified (n = 1), schizoaffective disorder (n = 2), alcohol abuse (n = 5), nonalcohol substance abuse (n = 2), adjustment disorder (n = 4), and personality disorder not otherwise specified (n = 25). Medications used by the suicide attempters included neuroleptics (n = 10), selective serotonin reuptake inhibitors (n = 24), serotonin-norepinephrine reuptake inhibitors (n = 19), antiepileptics (n = 8), hydroxyzine (n = 10), propiomazine (n = 19), and benzodiazepines (n = 34).

The somatic diagnoses included allergy (n = 1), arthrosis (n = 2), asthma (n = 2), diabetes (n = 3), fibromyalgia (n = 1), high blood pressure (n = 2), hyperthyroidism (n = 1), hypothyroidism (n = 1), migraine (n = 3), multiple sclerosis (n = 1), obesity (n = 1), pain syndrome (n = 1), polycystic ovary syndrome (n = 1), and tinnitus (n = 1).

All participants were evaluated using the self-rated version of the Suicide Assessment Scale (SUAS-S), constructed from the original interview-based scale[31]; it consists of 20 items assessing signs and symptoms related to suicidality and has a maximum score of 80.[32] The SUAS is sensitive to change over time.[33] The scale was initially designed to be used in treatment studies, regardless of psychiatric diagnosis. Later studies indicated that high scores might predict future suicidal self-directed violence. For instance, Waern et al[34] showed that high SUAS scores were associated with repetition of nonfatal suicidal self-directed violence, and Holmstrand et al[35] reported that suicide completers had significantly higher prior SUAS scores compared to suicide

Table 1. Characteristics of the Sample of 54 Suicide Attempters and 30 Controls

Variable	Total Sample (N = 84)	Suicide Attempters (n = 54)	Healthy Controls (n = 30)	*P* Value[a]
Sex, %				.60
Male	63.2	42.6	36.7	
Female	36.8	57.4	63.3	
Age, mean ± SD, y	38.9 ± 14.3	38.4 ± 14.4	39.8 ± 14.2	.67
Body mass index, mean ± SD (kg/m^2)	24.7 ± 4.2	25.8 ± 4.4	23.1 ± 3.5	.007
Suicide Assessment Scale score, mean ± SD	26.8 ± 21.5	39.3 ± 16.6	4.7 ± 4.1	< .0001
Age-adjusted *Toxoplasma gondii* titer (on a log scale), mean ± SD	NA	3.0 ± 0.1	2.6 ± 0.2	< .0001
T gondii immunoglobulin G status, n (%)				
Positive	28 (33.3)	22 (40.7)	6 (20.0)	
Negative	56 (66.7)	32 (59.3)	24 (80.0)	
Herpes simplex virus type 1 (HSV-1) titer, mean ± SD	101.9 ± 86.9	107.3 ± 90.9	92.2 ± 79.8	.45
HSV-1 immunoglobulin G status, n[b]				
Positive	65	42	23	
Negative	17	11	6	
Cytomegalovirus (CMV) titer, mean ± SD	96.6 ± 88.3	99.5 ± 86.9	91.3 ± 92.0	.68
CMV immunoglobulin G status, n[b]				
Positive	49	33	16	
Negative	33	20	13	

[a]χ^2 test for categorical variables; *t* test for continuous variables.
[b]The suicide group and the healthy group each had 1 participant with equivocal HSV-1 or CMV seropositivity.
Abbreviation: NA = not applicable.

noncompleters who were matched according to sex, age, and diagnosis.

In addition, the Montgomery-Asberg Depression Rating Scale (MADRS)[36] was administered to all participants. The MADRS consists of 10 items and was derived from the Comprehensive Psychopathological Rating Scale (CPRS). The MADRS has adequate validity with respect to the Hamilton Depression Rating Scale.[37]

Blood Samples

Blood samples were collected between 7:30 AM and 8:00 AM after a night of fasting and bed rest. The same conditions applied to all the samples. The blood was placed on ice and centrifuged (3,000 revolutions per minute at +4°C) within 1 hour. Plasma was collected and stored at −80°C within 1 hour after sampling.

Toxoplasma gondii Analysis

Plasma samples were tested for IgG antibodies to *T gondii* (TOXO), cytomegalovirus (CMV), and herpes simplex virus type 1 (HSV-1) using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Diagnostic Automation Inc, Calabasas, California). The ELISA kits included positive and negative controls; goat antihuman IgG peroxidase conjugate; 3,3′,5,5′-tetramethylbenzidine (TMB); inactivated *Toxoplasma* antigen; inactivated CMV antigen (strain AD169); and inactivated HSV-1 antigen (strain F). Procedures for the 3 ELISAs were identical and are briefly described: 10 µL of plasma was diluted 1:21; calibrators and appropriate positive and negative controls were included; a goat antihuman IgG peroxidase conjugate with TMB substrate was incorporated; and the sample was read spectrophotometrically at 450 nm (spectrophotometer: PowerWave X 340; software: KC junior, version 1.31.5, Rev M; both from BioTek Instruments, Winooski, Vermont). The TOXO test used inactivated *Toxoplasma* antigen, the CMV test used inactivated CMV antigen (strain AD169), and the HSV-1 assay used inactivated HSV-1 antigen (strain F). A threshold optical density reading, determined by the manufacturer and correlated to the calibrator, was used with a correction factor to determine a cutoff value for positive samples and to correct for slight day-to-day variations.

For the TOXO test, optical density ratios were converted to IU/mL by multiplying the optical density ratio by 20, as recommended by the manufacturer. Samples having IU/mL ≥ 22 were considered positive for *T gondii*; the assay is linear and correlates with the World Health Organization's standard between 0 and 35 IU/mL. The personnel involved in manipulating plasma and testing antibodies to neurotropic pathogens were blinded to individual histories of nonfatal suicidal self-directed violence status, suicidal scale measures, diagnoses, and demographic information.

Statistical Analysis

We employed both exploratory and descriptive analyses, including calculating means and standard deviations for continuous variables as well as calculating proportions for categorical variables. The distribution of *T gondii* titer was found to be highly skewed and bimodal. Log-transformation of *T gondii* titer was used to reduce skewness. Suicide attempters and controls were compared on baseline characteristics and on seropositivity and serointensity to *T gondii* using the χ^2 test for categorical variables and the *t* test for continuous variables.

Multivariable logistic regression analyses were performed to examine the association between history of nonfatal suicidal self-directed violence and log-transformed *T gondii* titer (serointensity) as well as *T gondii* seropositivity, with adjustment for sex, age, and body mass index. The same analysis was also applied to CMV and HSV-1. Multivariable linear regression was used to model the relationship between the SUAS-S and *T gondii* serointensity or *T gondii* seropositivity, adjusted for the same variables as described above. The statistical software used was SAS, version 9.2 (SAS Institute Inc, Cary, North Carolina).

RESULTS

Demographic and Clinical Characteristics

Table 1 compares patients with history of nonfatal suicidal self-directed violence and healthy controls on demographic characteristics. The 2 groups did not differ significantly on sex, age, HSV-1 titer, or CMV titer. Crude (unadjusted) differences on seropositivity and age-adjusted log-transformed

Table 2. Adjusted Odds Ratios From Logistic Regression Analysis Comparing Suicide Attempters (n = 54) With Controls (n = 30) on *Toxoplasma gondii* Titer and *T gondii* Seropositivity, Adjusted for Sex, Age, and Body Mass Index

Variable	Adjusted Odds Ratio	95% CI	*P* Value
Log *T gondii* titer, 1 unit increase	2.01	1.09–3.71	.03
Sex, male vs female	0.97	0.32–2.90	.96
Age, 10 years older	0.59	0.35–0.98	.04
Body mass index, 1 unit higher	1.26	1.07–1.49	.006
T gondii, positive vs negative	7.12	1.66–30.6	.008
Sex, male vs female	1.14	0.37–3.45	.82
Age, 10 years older	0.55	0.32–0.93	.02
Body mass index, 1 unit higher	1.27	1.07–1.51	.006

serointensity were of borderline significance ($P = .055$ and $P = .04$, respectively). Patients with nonfatal suicidal self-directed violence had a higher body mass index than the healthy controls ($P = .007$).

Toxoplasma gondii Serointensity and Seropositivity and History of Nonfatal Suicidal Self-Directed Violence

After controlling for sex, age, and body mass index using a multivariable logistic regression model, a strong and significant association was found between *T gondii* seropositivity (adjusted odds ratio [OR] = 7.12; 95% CI, 1.66–30.6; $P = .008$) and history of nonfatal suicidal self-directed violence (Table 2). A somewhat weaker association between *T gondii* serointensity and history of nonfatal suicidal self-directed violence also emerged (adjusted OR = 2.01; 95% CI, 1.09–3.71; $P = .03$).

The SUAS-S and MADRS Rating Scales

In multivariable linear regression models, a significant association of SUAS-S with *T gondii* seropositivity was observed only when the analysis included both groups. Participants with a positive *T gondii* antibody had a mean score 13 units higher than those with a negative *T gondii* antibody ($P = .026$). This association was not significant when the analysis included only the group with nonfatal suicidal self-directed violence ($P = .39$).

There were no significant associations of *T gondii* serointensity with the SUAS-S ($P = .15$ for all participants; $P = .08$ for suicide attempters only). No association was observed between depression symptoms (based on MADRS scores) and either *T gondii* seropositivity ($P = .37$) or *T gondii* serointensity ($P = .41$).

Pathogens Other Than *T gondii*

Seropositivity for CMV (OR = 0.69; 95% CI, 0.25–1.93) or HSV-1 (OR = 1.51; 95% CI, 0.42–5.50) was not significantly associated with history of nonfatal suicidal self-directed violence.

DISCUSSION

The associations of *T gondii* seropositivity and serointensity with history of nonfatal suicidal self-directed violence in this study add to the growing body of evidence linking this protozoan parasite with nonfatal suicidal self-directed violence. Specifically, the current findings are consistent with previous reports showing similar associations in young patients with schizophrenia,[24] patients with mood disorders,[22] and patients with general psychiatric conditions.[23] The consistency of *T gondii*'s association with nonfatal suicidal self-directed violence across studies, regardless of the patient sample and *DSM-IV* diagnostic category, is noteworthy, as it lends further credence to the hypothesis that the link is independent of mental illness specificity or severity. Although the sensitivity for suicide-attempt history of *T gondii* seropositivity in the current study was low (ie, 0.41; 95% CI, 0.28–0.54), the specificity was satisfactory (ie, 0.80; 95% CI, 0.66–0.94), not dissimilar to our previous studies. In Okusaga et al,[24] we obtained a sensitivity of 0.42 (95% CI, 0.36–0.47) and a specificity of 0.62 (95% CI, 0.58–0.66), while, in Arling et al,[22] the sensitivity was 0.15 (95% CI, 0.08–0.22) and the specificity was 0.90 (95% CI, 0.85–0.95). Furthermore, the adjusted OR (7.12; 95% CI, 1.66–30.6) for nonfatal suicidal self-directed violence by seropositivity in this study was much higher than the adjusted OR of 1.62 (95% CI, 0.72–3.65) in Arling et al[22] and 1.57 (95% CI, 1.03–2.38) in Okusaga et al.[24]

These differences may be due to the stricter exclusion and inclusion criteria used in recruitment for the current study and a much more precise selection of suicide attempters (ie, all must have been admitted to an inpatient psychiatric unit for recent nonfatal suicidal self-directed violence—and explicit intent must have been present and confirmed by a clinician using history, interview, and collateral information), in contrast to the Arling et al[22] and Okusaga et al[24] studies, in which, for the majority of those with a history of suicidal self-directed violence, the attempts were in the past, sometimes in the more distant past. Alternatively, however, using nonpsychiatric controls, in fact supercontrols (with no family history of nonfatal suicidal self-directed violence), may have also contributed to the high odds ratios in our current report.

In addition to confirming previous literature, we found for the first time, to our knowledge, that seropositivity (but not serointensity) for *T gondii* was associated with higher scores on the SUAS-S. This result should be interpreted with caution since it was significant only when the analysis included both groups—but not suicide attempters only. However, if replicated in future studies, this result could be meaningful for suicide prediction. The utility of the SUAS-S in predicting repeat nonfatal suicidal self-directed violence and suicide has been validated previously, and higher scores, in combination with other specific patient characteristics, are significant predictors of suicide.[32–34] Since there is a positive relationship between *T gondii* seropositivity and higher SUAS-S scores, it is reasonable to speculate that *T gondii* seropositivity could become a potential predictor for suicide. The association with nonfatal suicidal self-directed violence seems to be specific for *T gondii* infection since we could not detect any association with the serointensity or seropositivity

of other neurotropic pathogens such as CMV or HSV-1. This finding is consistent with the results reported by Okusaga et al,[24] who also observed the association with *T gondii* but not with other neurotropic pathogens. These observations suggest that the mechanism of the association between *T gondii* and nonfatal suicidal self-directed violence is not based on a nonspecific, global immune activation.

How representative was the sample in regard to seropositivity rates in the general population? The percentage of *T gondii* seropositivity in our sample was consistent with the general prevalence in Sweden and was comparable to previous studies. The prevalence of *T gondii* in Sweden is 23%,[8] while, in the United States, the prevalence is estimated to be 10.8%.[6,7] In our sample, the rate of seropositivity for healthy controls was 20%, concordant with the rate (23%) reported by Birgisdóttir et al.[8] In the study by Arling et al,[22] the percentage of seropositivity in individuals without a history of nonfatal suicidal self-directed violence was 11.1%, consistent with the prevalence in the United States.[6,7] In the study by Okusaga et al[24] of schizophrenia patients recruited in the Munich metropolitan area, 37.7% of the patients without a history of nonfatal suicidal self-directed violence were seropositive, concordant with *T gondii* chronic infection prevalence previously reported in Germany.[14]

The pathophysiologic mechanisms mediating the association between *T gondii* infection and nonfatal suicidal self-directed violence have not yet been elucidated. It is unclear whether *T gondii* itself or the immune response to the infection is linked to nonfatal suicidal self-directed violence.

By which mechanisms could *T gondii* induce behavioral and emotional changes? Animal research shows that after infection, *T gondii* eventually localizes in the brain (in addition to muscle), including the prefrontal cortex and the amygdala,[38] which are involved in emotional and behavioral regulation and dysregulation and have shown major histopathological changes in victims of suicide.[39] Brain activity could potentially be altered by *T gondii* via its effect on the bioavailability of dopamine. Tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, is encoded by 2 genes of *T gondii*.[40] The expression of these 2 genes increases dopamine, which may mediate behaviors such as increased aggression and impulsivity that have been conceptualized as intermediate phenotypes for nonfatal suicidal self-directed violence.[41] In addition, intermittent reactivation of latent infection with *T gondii* could be an alternative pathway leading to nonfatal suicidal self-directed violence, as has been previously proposed for psychiatric manifestations in immunosuppressed patients[42,43] as well as for migraine headaches in immunocompetent patients.[19–21]

Nonfatal suicidal self-directed violence, a major risk factor for fatal suicidal self-directed violence (ie, suicide), has been previously associated with markers of immune activation,[44] such as elevation in certain cytokines like tumor necrosis factor–α and interleukin-6 in the plasma[45] and interleukin-6 in the cerebrospinal fluid.[46] Moreover, microglial activation[47] and increased cytokine gene expression in the brain[48] have also been reported in victims of suicide. Certain immunologic parameters related to nonfatal suicidal self-directed violence are among the very ones involved either in protection against *T gondii* (proinflammatory cytokines) or in limiting immune-mediated brain pathology (anti-inflammatory cytokines).[49] In a study on peripartum depression,[50] indeed, the tumor necrosis factor–α level was increased in women who were seropositive for *T gondii*.

Elevation in proinflammatory cytokines may reduce availability of tryptophan—and thus reduce serotonin synthesis—and also increase levels of certain tryptophan metabolites that have potential neurophysiologic altering effects. Under normal conditions, most of the body's tryptophan is metabolized by tryptophan dioxygenase in the liver, but a small portion is degraded by indoleamine 2,3-dioxygenase (IDO) extrahepatically.[51] However, IDO activity increases significantly with the presence of proinflammatory cytokines.[52] After *T gondii* infection, proinflammatory cytokines interferon-γ and interleukin-12 are produced as part of the innate immune response—as well as to eventually mediate the adaptive immune response to effectively control both acute and chronic *T gondii* infection.[53] In addition, the catalysis of tryptophan breakdown by activation of the IDO by these cytokines also deprives *T gondii* of tryptophan, which is essential for its growth.[53,54] Reduction of the availability of tryptophan subsequently decreases synthesis of serotonin. Postmortem, the levels of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) have been previously found to be decreased in victims of suicide.[55,56] Furthermore, lower cerebrospinal fluid levels of 5-HIAA have been associated with suicide attempts,[57–59] although these findings have not been consistently replicated.[60]

In addition, IDO catabolizes tryptophan into kynurenine, which is further metabolized into neuroactive metabolites with opposite effects on the *N*-methyl-D-aspartate receptor: kynurenic acid (antagonist, astrocytic origin, involved in neuroprotection) and quinolinic acid (agonist, glial origin, involved in neurotoxicity), previously implicated in psychiatric psychopathology,[61–69] including histopathologically in victims of suicide with a history of severe depression.[70] More recently, elevated kynurenine level was associated with a history of nonfatal suicidal self-directed violence in patients with major depression.[71]

The observational nature of this study and its cross-sectional design preclude the inference of a causal relationship. Another limitation, in addition to the relatively small sample size, is that we could not control for mental illness, as the study did not include a group of psychiatric patients with no history of nonfatal suicidal self-directed violence. One might argue that mental illness is a confounding factor that mediates the relationship between nonfatal suicidal self-directed violence and *T gondii*. Additionally, the history of suicidal self-directed violence could be understood as a marker for a more severe condition, and, if the severity of illness were to be associated with the infection, then the relationship with suicide attempts could be spurious. However, this possibility

is not likely considering that, in all previous studies[22–24] that had a psychiatric control group, the association between *T gondii* antibodies and nonfatal suicidal self-directed violence was significant independent of psychiatric conditions and even controlling for severity of symptoms. Moreover, in the current study, while seropositivity was related to self-directed violence and the SUAS-S, it was not significantly related to severity of depression as assessed by the MADRS. Nevertheless, there remains a possibility that the high odds ratios from this study are due at least in part to an increased risk of becoming infected with *T gondii* because of (unmeasured) differences in exposure to dust, hygienic food preparation, and proximity to cats between patients with mental illness and healthy controls.

The strengths of this study include its hypothesis-driven approach and the strict inclusion and exclusion criteria. Additionally, researchers who analyzed the blood samples for *T gondii* antibodies were blind to the nonfatal suicidal self-directed violence status information. Also, this study is the first to our knowledge to demonstrate a positive correlation between *T gondii* antibodies and a score on a scale designed to predict nonfatal suicidal self-directed violence.

Future replication of *T gondii*–related nonfatal suicidal self-directed violence in prospective studies could potentially have public health impact. Additionally, studying the outcome of *fatal* self-directed violence in a large cohort, as well as uncovering mechanisms involved in the association with *T gondii*, may have future therapeutic and preventive impact. First of all, people who have elevated suicide risk may be advised to take prophylactic approaches against *T gondii* infection more seriously. Individuals at increased risk for suicide might need routine screening for *T gondii* antibodies. An effective vaccine, chemotherapeutic agents to prevent reactivations, and immune-targeting therapies would be potential options for development after mechanisms mediating a confirmed relationship between *T gondii* and nonfatal suicidal self-directed violence are identified.

CONCLUSIONS

This study replicated the association between *T gondii* IgG antibodies and nonfatal suicidal self-directed violence. In particular, in this attentively screened sample chosen on the basis of a protocol focused specifically on nonfatal suicidal self-directed violence, a 7-fold increase in nonfatal suicidal self-directed violence was found in persons who tested positive for *T gondii* IgG antibodies versus those who tested negative, as compared to the odds ratios of approximately 1.5 reported in previous studies not primarily focused on self-directed violence. This association was specific to *T gondii* antibodies as compared with antibodies to other neurotropic organisms, such as CMV or HSV-1. In the long term, *T gondii* antibodies might become a candidate marker to improve our ability to estimate risk of nonfatal suicidal self-directed violence and to individualize interventions in suicide prevention.

Drug names: hydroxyzine (Vistaril and others).

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REFERENCES

1. American Foundation for Suicide Prevention. Facts and figures from the Centers for Disease Control for the year 2009. http://www.afsp.org/index.cfm?fuseaction=home.viewPage&page_id=04EA1254-BD31-1FA3-C549D77E6CA6AA37. Verified June 7, 2012.
2. World Health Organization. Suicide prevention, 2012. http://www.who.int/mental_health/prevention/suicide/suicideprevent/en/. Verified June 7, 2012.
3. Hawton K, van Heeringen K. Suicide. *Lancet*. 2009;373(9672):1372–1381.
4. Harris EC, Barraclough B. Suicide as an outcome for mental disorders: a meta-analysis. *Br J Psychiatry*. 1997;170(3):205–228.
5. Montoya JG, Liesenfeld O. Toxoplasmosis. *Lancet*. 2004;363(9425):1965–1976.
6. Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. *Int J Parasitol*. 2008;38(11):1257–1278.
7. Jones JL, Kruszon-Moran D, Sanders-Lewis K, et al. *Toxoplasma gondii* infection in the United States, 1999–2004, decline from the prior decade. *Am J Trop Med Hyg*. 2007;77(3):405–410.
8. Birgisdóttir A, Asbjörnsdottir H, Cook E, et al. Seroprevalence of *Toxoplasma gondii* in Sweden, Estonia and Iceland. *Scand J Infect Dis*. 2006;38(8):625–631.
9. Frenkel JK, Escajadillo A. Cyst rupture as a pathogenic mechanism of toxoplasmic encephalitis. *Am J Trop Med Hyg*. 1987;36(3):517–522.
10. Webster JP. Rats, cats, people and parasites: the impact of latent toxoplasmosis on behaviour. *Microbes Infect*. 2001;3(12):1037–1045.
11. Remington JS, Krahenbuhl JL. Immunology of *Toxoplasma gondii*. In: Nahmias A, O'Reilly J, eds. *Immunology of Human Infection, Part 2: Viruses and Parasites; Immunodiagnosis and Prevention of Infectious Diseases*. New York, NY: Plenum; 1982:327–371.
12. Roberts L, Janovy J. *Foundations of Parasitology*. Boston, MA:

McGraw-Hill Companies; 2000.
13. Torrey EF, Bartko JJ, Lun ZR, et al. Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. *Schizophr Bull*. 2007;33(3): 729–736.
14. Hinze-Selch D, Däubener W, Erdag S, et al. The diagnosis of a personality disorder increases the likelihood for seropositivity to *Toxoplasma gondii* in psychiatric patients. *Folia Parasitol (Praha)*. 2010;57(2):129–135.
15. Flegr J, Havlícek J, Kodym P, et al. Increased risk of traffic accidents in subjects with latent toxoplasmosis: a retrospective case-control study. *BMC Infect Dis*. 2002;2(1):11.
16. Yereli K, Balcioğlu IC, Ozbilgin A. Is *Toxoplasma gondii* a potential risk for traffic accidents in Turkey? *Forensic Sci Int*. 2006;163(1–2):34–37.
17. Flegr J, Kodym P, Tolarová V. Correlation of duration of latent *Toxoplasma gondii* infection with personality changes in women. *Biol Psychol*. 2000;53(1):57–68.
18. Novotná M, Hanusova J, Klose J, et al. Probable neuroimmunological link between *Toxoplasma* and cytomegalovirus infections and personality changes in the human host. *BMC Infect Dis*. 2005;5(1):54.
19. Prandota J. Recurrent headache as the main symptom of acquired cerebral toxoplasmosis in nonhuman immunodeficiency virus–infected subjects with no lymphadenopathy: the parasite may be responsible for the neurogenic inflammation postulated as a cause of different types of headaches. *Am J Ther*. 2007;14(1):63–105.
20. Koseoglu E, Yazar S, Koc I. Is *Toxoplasma gondii* a causal agent in migraine? *Am J Med Sci*. 2009;338(2):120–122.
21. Prandota J. Migraine associated with patent foramen ovale may be caused by reactivation of cerebral toxoplasmosis triggered by arterial blood oxygen desaturation. *Int J Neurosci*. 2010;120(2):81–87.
22. Arling TA, Yolken RH, Lapidus M, et al. *Toxoplasma gondii* antibody titers and history of suicide attempts in patients with recurrent mood disorders. *J Nerv Ment Dis*. 2009;197(12):905–908.
23. Yagmur F, Yazar S, Temel HO, et al. May *Toxoplasma gondii* increase suicide attempt–preliminary results in Turkish subjects? *Forensic Sci Int*. 2010;199(1–3):15–17.
24. Okusaga O, Langenberg P, Sleemi A, et al. *Toxoplasma gondii* antibody titers and history of suicide attempts in patients with schizophrenia. *Schizophr Res*. 2011;133(1–3):150–155.
25. Ling VJ, Lester D, Mortensen PB, et al. *Toxoplasma gondii* seropositivity and suicide rates in women. *J Nerv Ment Dis*. 2011;199(7):440–444.
26. Lindqvist D, Isaksson A, Träskman-Bendz L, et al. Salivary cortisol and suicidal behavior—a follow-up study. *Psychoneuroendocrinology.* 2008; 33(8):1061–1068.
27. Crosby AE, Ortega L, Melanson C. Self-Directed Violence Surveillance: Uniform Definitions and Recommended Data Elements, Version 1.0. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, Atlanta, GA. http://www.cdc.gov/violenceprevention/pdf/Self-Directed-Violence-a.pdf. Verified June 5, 2012.
28. Saunders JB, Aasland OG, Babor TF, et al. Development of the Alcohol Use Disorders Identification Test (AUDIT). WHO Collaborative Project on Early Detection of Persons With Harmful Alcohol Consumption, 2. *Addiction*. 1993;88(6):791–804.
29. First MB, Spitzer RL, Gibbon M, et al. *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID I)*. Washington, DC: American Psychiatric Press; 1997.
30. First MB, Gibbon M, Spitzer RL, et al. *Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID II)*. Washington, DC: American Psychiatric Press; 1997.
31. Stanley B, Träskman-Bendz L, Stanley M. The Suicide Assessment Scale: a scale evaluating change in suicidal behavior. *Psychopharmacol Bull*. 1986;22(1):200–205.
32. Niméus A, Hjalmarsson Ståhlfors F, Sunnqvist C, et al. Evaluation of a modified interview version and of a self-rating version of the Suicide Assessment Scale. *Eur Psychiatry*. 2006;21(7):471–477.
33. Niméus A, Alsén M, Träskman-Bendz L. The Suicide Assessment Scale: an instrument assessing suicide risk of suicide attempters. *Eur Psychiatry*. 2000;15(7):416–423.
34. Waern M, Sjöström N, Marlow T, et al. Does the Suicide Assessment Scale predict risk of repetition? a prospective study of suicide attempters at a hospital emergency department. *Eur Psychiatry*. 2010;25(7):421–426.
35. Holmstrand C, Niméus A, Träskman-Bendz L. Risk factors of future suicide in suicide attempters—a comparison between suicides and matched survivors. *Nord J Psychiatry*. 2006;60(2):162–167.
36. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry*. 1979;134(4):382–389.
37. Davidson J, Turnbull CD, Strickland R, et al. The Montgomery-Asberg Depression Scale: reliability and validity. *Acta Psychiatr Scand*. 1986;73(5): 544–548.
38. Vyas A, Kim SK, Giacomini N, et al. Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc Natl Acad Sci U S A*. 2007;104(15):6442–6447.
39. Mann JJ. Neurobiology of suicidal behaviour. *Nat Rev Neurosci*. 2003; 4(10):819–828.
40. Gaskell EA, Smith JE, Pinney JW, et al. A unique dual activity amino acid hydroxylase in *Toxoplasma gondii*. *PLoS ONE*. 2009;4(3):e4801.
41. Mann JJ, Arango VA, Avenevoli S, et al. Candidate endophenotypes for genetic studies of suicidal behavior. *Biol Psychiatry*. 2009;65(7):556–563.
42. Grant IH, Gold JW, Rosenblum M, et al. *Toxoplasma gondii* serology in HIV-infected patients: the development of central nervous system toxoplasmosis in AIDS. *AIDS*. 1990;4(6):519–521.
43. Meers S, Lagrou K, Theunissen K, et al. Myeloablative conditioning predisposes patients for *Toxoplasma gondii* reactivation after allogeneic stem cell transplantation. *Clin Infect Dis*. 2010;50(8):1127–1134.
44. Lindqvist D. *Redefining Suicidal Behaviour—Rating Scales and Biomarkers*. Lund, Sweden: University Press; 2010.
45. Janelidze S, Mattei D, Westrin Å, et al. Cytokine levels in the blood may distinguish suicide attempters from depressed patients. *Brain Behav Immun*. 2011;25(2):335–339.
46. Lindqvist D, Janelidze S, Hagell P, et al. Interleukin-6 is elevated in the cerebrospinal fluid of suicide attempters and related to symptom severity. *Biol Psychiatry*. 2009;66(3):287–292.
47. Steiner J, Bielau H, Brisch R, et al. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J Psychiatr Res*. 2008;42(2):151–157.
48. Tonelli LH, Stiller J, Rujescu D, et al. Elevated cytokine expression in the orbitofrontal cortex of victims of suicide. *Acta Psychiatr Scand*. 2008; 117(3):198–206.
49. Pepper M, Hunter C. Innate recognition and the regulation of protective immunity to *Toxoplasma gondii*. In: Ajioka J, Soldati D, eds. *Toxoplasma: Molecular and Cellular Biology*. Norfolk, UK: Horizon Bioscience; 2007: 111–126.
50. Groër M, Yolken R, Xiao J, et al. Prenatal depression and anxiety in *Toxoplasma gondii*–positive women. *Am J Obstet Gynecol*. 2011; 204(5): 433.
51. Dantzer R, O'Connor JC, Freund GG, et al. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008;9(1):46–56.
52. MacKenzie CR, González RG, Kniep E, et al. Cytokine mediated regulation of interferon-gamma-induced IDO activation. *Adv Exp Med Biol*. 1999;467:533–539.
53. Miller CM, Boulter NR, Ikin RJ, et al. The immunobiology of the innate response to *Toxoplasma gondii*. *Int J Parasitol*. 2009;39(1):23–39.
54. Pfefferkorn ER. Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. *Proc Natl Acad Sci U S A*. 1984;81(3):908–912.
55. Mann JJ. A current perspective of suicide and attempted suicide. *Ann Intern Med*. 2002;136(4):302–311.
56. Mann J, Arango V. The neurobiology of suicidal behavior. In: Jacobs D, ed. *The Harvard Medical School Guide to Suicide Assessment and Intervention*. San Francisco, CA: Jossey-Bass; 1999.
57. Asberg M, Träskman L, Thorén P. 5-HIAA in the cerebrospinal fluid: a biochemical suicide predictor? *Arch Gen Psychiatry*. 1976;33(10): 1193–1197.
58. Träskman L, Asberg M, Bertilsson L, et al. Monoamine metabolites in CSF and suicidal behavior. *Arch Gen Psychiatry*. 1981;38(6):631–636.
59. Lidberg L, Belfrage H, Bertilsson L, et al. Suicide attempts and impulse control disorder are related to low cerebrospinal fluid 5-HIAA in mentally disordered violent offenders. *Acta Psychiatr Scand*. 2000;101(5):395–402.
60. Ernst C, Mechawar N, Turecki G. Suicide neurobiology. *Prog Neurobiol.* 2009;89(4):315–333.
61. Müller N, Myint AM, Schwarz MJ. The impact of neuroimmune dysregulation on neuroprotection and neurotoxicity in psychiatric disorders—relation to drug treatment. *Dialogues Clin Neurosci*. 2009; 11(3):319–332.
62. Guillemin GJ. Quinolinic acid: neurotoxicity. *FEBS J.* 2012;279(8):1355. Epub March 27, 2012.
63. Dantzer R, O'Connor JC, Lawson MA, et al. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology*. 2011;36(3):426–436.
64. O'Connor JC, André C, Wang Y, et al. Interferon-γ and tumor necrosis factor-α mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus

Calmette-Guérin. *J Neurosci*. 2009;29(13):4200–4209.
65. O'Connor JC, Lawson MA, André C, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry*. 2009;14(5):511–522.
66. Raison CL, Dantzer R, Kelley KW, et al. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-α: relationship to CNS immune responses and depression. *Mol Psychiatry*. 2010;15(4):393–403.
67. Sathyasaikumar KV, Stachowski EK, Wonodi I, et al. Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. *Schizophr Bull*. 2011;37(6):1147–1156.
68. Steiner J, Bogerts B, Sarnyai Z, et al. Bridging the gap between the immune and glutamate hypotheses of schizophrenia and major depression: potential role of glial NMDA receptor modulators and impaired blood-brain barrier integrity [published online June 28, 2011]. *World J Biol Psychiatry*. 2011;1–11.
69. Wonodi I, Schwarcz R. Cortical kynurenine pathway metabolism: a novel target for cognitive enhancement in schizophrenia. *Schizophr Bull*. 2010; 36(2):211–218.
70. Steiner J, Walter M, Gos T, et al. Severe depression is associated with increased microglial quinolinic acid in subregions of the anterior cingulate gyrus: evidence for an immune-modulated glutamatergic neurotransmission? *J Neuroinflammation*. 2011;8:94.
71. Sublette ME, Galfalvy HC, Fuchs D, et al. Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder. *Brain Behav Immun*. 2011;25(6):1272–1278.