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Toxoplasma gondii and anxiety disorders in a community-based sample

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ABSTRACT

A growing body of literature suggests that exposure to the neurotropic parasite *Toxoplasma gondii* (*T. gondii*) is associated with increased risk of mental disorders, particularly schizophrenia. However, a potential association between *T. gondii* exposure and anxiety disorders has not been rigorously explored. Here, we examine the association of *T. gondii* infection with both anxiety and mood disorders. Participants ($n = 484$) were drawn from the Detroit Neighborhood Health Study, a population-representative sample of Detroit residents. Logistic regression was used to examine the associations between *T. gondii* exposure (defined by seropositivity and IgG antibody levels) and three mental disorders: generalized anxiety disorder (GAD), posttraumatic stress disorder (PTSD) and depression. We found that *T. gondii* seropositivity was associated with a 2 times greater odds of GAD (odds ratio (OR), 2.25; 95% confidence interval (CI), 1.11–4.53) after adjusting for age, gender, race, income, marital status, and medication. Individuals in the highest antibody level category had more than 3 times higher odds of GAD (OR, 3.35; 95% CI, 1.41–7.97). Neither *T. gondii* seropositivity nor IgG antibody levels was significantly associated with PTSD or depression. Our findings indicate that *T. gondii* infection is strongly and significantly associated with GAD. While prospective confirmation is needed, *T. gondii* infection may play a role in the development of GAD.

1. Introduction

Anxiety and mood disorders contribute substantially to the burden of disease and disability in the United States. A recent national study estimates that generalized anxiety disorder (GAD), posttraumatic stress disorder (PTSD), and major depressive disorder affect 5.7%, 6.8%, and 16.6% of adults in their lifetime, respectively (Kessler et al., 2005). Studies have established a genetic contribution to these mental disorders (Hettema et al., 2001; Sullivan et al., 2000; Xian et al., 2000). Yet, the mapping of direct paths from gene to mental disorders has been slow and inconsistent, as only a few genome-wide association studies have detected risk genes and many putative gene findings have failed replication (Hamer, 2002). More fundamentally, a large proportion of variation in mental health remains unexplained by genetic factors. For these reasons, discovery of new risk factors for mental disorders is crucial.

A growing body of epidemiologic literature has implicated infections as novel risk factors for development of mental disorders (Benros et al., 2013; Dalman et al., 2008). One pathogen of particular interest is the neurotropic parasite *Toxoplasma gondii* (*T. gondii*). *T. gondii* is capable of reproducing asexually within any warm-blooded animal but must return to its definitive host, the cat, to undergo sexual reproduction, develop into infectious oocysts, and return to the environment through fecal shedding (Carruthers and Suzuki, 2007). Infection is transmitted to an intermediate host (e.g., a rodent) or a dead-end host (e.g., a human) via ingestion of tissues cysts in undercooked meat or oocysts in cat feces or contaminated soil, whereupon the parasite progresses to form latent cysts in muscle and neural cells, including neurons, glial cells, and astrocytes (Carruthers and Suzuki, 2007).

As *T. gondii* does not complete its life cycle until passing from its intermediate rodent host to its definitive feline host, the "manipulation hypothesis" posits that the parasite may be under selective

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pressure to influence rodent behavior to promote predation by and transmission to the definitive feline host (Lafferty, 1999). Indeed, *T. gondii* has been shown to profoundly alter anxiety in rodents, as evidenced by increased activity, decreased fear of novel stimuli, and diminished predator vigilance (Berdoy et al., 2000; Webster, 1994; Webster et al., 1994). Meanwhile, a broad range of other behaviors related to learning (Vyas et al., 2007), social status (Berdoy et al., 1995), and olfaction (Vyas et al., 2007) remain unaffected.

While the neurologic effects of toxoplasmosis in congenitally-infected or immunocompromised humans are well-established (e.g., encephalitis in AIDS patients), infection among the immunocompetent is generally considered relatively benign: the parasite is never cleared from the nervous system but cell-mediated immune response suppresses pathogenic activity (Montoya and Liesenfeld, 2004). This "no harm done" assumption is now being reconsidered, as growing evidence links *T. gondii* to several mental disorders (Fekadu et al., 2010). Decades of serological investigations have corroborated a relationship between *T. gondii* and schizophrenia (Torrey et al., 2012). More recently, studies have implicated the infection in mood disorders (e.g., depression, bipolar disease) and suicidal behavior (Fekadu et al., 2010), while a small case-control study suggests an association with obsessive–compulsive disorder (Miman et al., 2010). To our knowledge, no previous study has examined the association between *T. gondii* and either GAD or PTSD, and none has investigated the parasite's association with any diagnosed anxiety disorder among individuals living in the community setting.

To address these gaps in the literature, we used data from the Detroit Neighborhood Health Study (DNHS), a prospective, population-based study of residents of Detroit, Michigan. The purpose of this study was to examine whether *T. gondii* seropositivity and IgG antibody levels were associated with three different mental disorders, GAD, PTSD, and depression, in persons 18 years of age and older living in Detroit, Michigan.

2. Materials and methods

2.1. Study population

The DNHS is a longitudinal, population-based study designed to investigate correlates of mental disorders in the city of Detroit. A probability sample of 1547 individuals (aged $\geqslant$18 years) living within the Detroit city limits participated in a baseline telephone survey in 2008–2009. The DNHS was approved by the institutional review board at the University of Michigan, and all participants provided written, informed consent. Participants were administered a 40 minute assessment via a telephone survey, which included questions on socio-demographic characteristics and a standardized assessment of GAD, PTSD, and depression. Wave 1 survey participants were representative of the Detroit population in terms of age, gender, race, income, and educational attainment (for more detailed information, see Uddin et al., 2010). All respondents were invited during the phone interview to participate in the biospecimen component of the study and 484 (31.3%) participants provided venipuncture blood specimens that were tested for *T. gondii* IgG antibodies. The socio-demographic characteristics of the biospecimen sample were comparable to the overall study sample with the exception of income and education levels, which were lower among those who provided a biospecimen. In addition, past year GAD, PTSD, and depression were statistically significantly more prevalent among those who provided biospecimens tested for *T. gondii*-specific IgG versus those in the overall study sample, where 11.4% vs. 7.7% had GAD ($p = 0.01$), 13.4% vs. 9.4% had PTSD ($p = 0.01$), and 15.8% vs. 11.4% had depression ($p = 0.01$) in the past year at baseline.

2.2. Laboratory analyses

Serum samples were analyzed for *T. gondii* infection by standard procedures. Sera were frozen and stored at −70 °C, then shipped on dry ice (within four weeks) to the Stanley Laboratory of Developmental Neurovirology, Baltimore, Maryland. The presence and quantity of immunoglobulin G (IgG) serum antibodies to *T. gondii* were measured by solid phase enzyme-linked immunosorbent assays and with laboratory personnel unaware of the status of the study participants (Wang et al., 2011; Yolken et al., 2011). Reagents for these assays were obtained from IBL Laboratories, Hamburg, Germany.

2.3. Measures

Participants were categorized in the following manner: (1) Seropositivity: participants with *T. gondii* IgG values <10 International Units (IU) were dichotomized as seronegative and those with IgG values $\geqslant$10 IU were categorized as seropositive; (2) Serointensity: continuous IgG antibody levels were standardized such that a one unit increase in *T. gondii* IgG antibody level represents the effect of 1 standard deviation change in *T. gondii* IgG antibody level; and (3) Antibody level category: IgG antibody level was categorized as high level ($\geqslant$20.2 IU), low level (10–20.2 IU), or seronegative (<10.0 IU).

History of GAD, PTSD, and depression during the past year was assessed during the baseline telephone survey with validated instruments based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (American Psychiatric Association, 2000) as previously described (Uddin et al., 2010). Briefly, past-year GAD was assessed using the seven-item generalized anxiety disorder scale (GAD-7) (Spitzer et al., 2006). Each of the seven symptoms was scored from 0 (not at all) to 3 (nearly every day), with total scores ranging from 0 to 21. Respondents who scored $\geqslant$10 were categorized as having past-year GAD. Past-year PTSD was assessed using a modified version of the PTSD checklist (PCL-C), a 17-item measure of DSM-IV symptoms of PTSD (Weathers, 1996). Participants identified past exposure to 19 potential traumatic events (PTE) and described PTSD symptoms related to two traumatic events: (1) the event identified by the participant as the most traumatic and (2) a randomly selected PTE experienced by the participant. PTSD was considered present if all six DSM-IV criteria were met in reference to either the worst event or the random event. Past-year depression was assessed with the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001). The nine items on the PHQ-9 were scored from 0 (not at all) to 3 (nearly every day), with total scores ranging from 0 to 27. Past-year depression was considered present if participants reported depressed mood or anhedonia and the co-occurrence of at least one additional symptom for "more than half the days" in a 2-week period over the past year. One symptom, "thoughts that you would be better off dead or of hurting yourself in some way," was included in the depression score if present, regardless of symptom duration. A clinical reappraisal study ($n = 51$) demonstrated that the identification of individuals with GAD, PTSD, and depression by the survey screening scales displayed high concordance for diagnoses of GAD, PTSD, and depression obtained via in-person clinical interviews (Uddin et al., 2010).

Covariates: Age in years was self-reported and treated as a continuous variable. Race was self-reported and individuals were categorized as White, African-American, and Hispanic/Other. Gender was dichotomized as female and male. Household income was self-reported as pre-tax family income and was categorized as (1) less than $25,000, (2) $25,000–$50,000, or (3) greater than $50,000. Marital status was categorized as married, divorced, separated, widowed, or never married. Medications were classified according to the Center for Disease Control and Prevention Ambu-