Journal of Affective Disorders 148 (2013) 444–448

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Brief report

Relationship between *Toxoplasma gondii* infection and bipolar disorder in a French sample

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ARTICLE INFO

Article history:
Received 2 September 2012
Received in revised form
16 November 2012
Accepted 16 November 2012
Available online 27 December 2012

Keywords:
Toxoplasma gondii
Bipolar disorder
Neuro-immunology
IL6

ABSTRACT

Background: Prenatal exposure to viruses or parasites with tropism for the central nervous system is one of the risk factors for psychotic disorders. However, the relationship between past exposure to *Toxoplasma gondii* (*T. gondii*) and incidence of bipolar disorders (BD) is poorly documented across populations.
Methods: We explored the potential association between *T. gondii* exposure and BD in France, a country of high prevalence of *Toxoplasmosis*, comparing the prevalence of serological markers (IgG/IgM class antibodies) for *T. gondii* infection in 110 BD patients and 106 healthy controls all living in France. In a subgroup of 42 patients and 42 controls we also evaluated the levels of interleukin 6 (IL-6) transcripts, an adjunct marker of inflammation.
Results: We found that the sero-positive group for IgG antibodies to *T. gondii* had a 2.7 fold odds of having BD as compared to the sero-negative group (OR=2.17 CI 95%=1.09–4.36, $p=0.028$). Despite the fact that BD patients had significantly higher levels of IL-6 than the non-patient controls, no notable association between *T. gondii* status and IL-6 transcript levels was found. We did not find any clinical or demographic correlates of *Toxoplasma* exposure in the study population.
Limitations: Our results are to be interpreted with caution because of our small sample size.
Results: We confirm the association between seropositive status to *T. gondii* and bipolar disorders reported in other populations and extend it to French patients. Our data strengthen the importance of early detection of *T. gondii* infected patients in order to propose specific and adequate treatments.

1. Introduction

The implication of infectious events in the development of major psychosis has recently gained increasing attention (see for review, Brown, 2011; Brown and Derkits, 2010; Arias et al., 2011). Rubella, herpes simplex virus (HSV), cytomegalovirus (CMV), *Toxoplasma gondii* (*T. gondii*), and other infections have been

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http://dx.doi.org/10.1016/j.jad.2012.11.034

shown to be potent disrupters of fetal neurodevelopment leading to abnormalities of brain and behavior, including psychiatric disorders. In this context, the most studied link between a pathogen and psychiatric disorders concerns the association between *T. gondii* and schizophrenia (Torrey et al., 2007).

T. gondii is an intracellular protozoan parasite which infects around one-third of the human population and resides encysted in the brain of immunocompetent hosts. In humans, *T. gondii* infection can be contracted from ingestion of raw or undercooked meat containing tissue cysts, from unpasteurized goat cheese (frequently consumed in France), from raw fruits, vegetables, soil, and water contaminated with sporulated oocysts from the feces of infected cats (Gilbert et al., 1993; Dubey, 2008). In terms of epidemiology, large population-based studies showed that the *Toxoplasma* infection is widespread and it is generally assumed that approximately 25 to 30% of the world's human population is infected by the parasite (Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012). Actually, the prevalence vary widely between countries (from 10 to 80%) (Robert-Gangneux and Dardé, 2012). For example, low seroprevalence (10 to 30%) have been observed in North America, in South East Asia and in Northern Europe, while high prevalence have been found in South America and in tropical African countries. In rural France it is estimated around 47% (Fromont et al., 2009A; Robert-Gangneux and Dardé, 2012)

The high infection rate in these countries has been attributed to some life style factors such as consumption of undercooked meat and a high level of water contamination. The contextual link between *Toxoplasma* infection and psychiatric disorders (in particular schizophrenia) can be summarized as follows: (i) proven *T. gondii's* neurotropism and its impact on dopamine pathway (Prandovszky et al., 2011), (ii) shared epidemiological characteristics between *Toxoplasma* exposure and schizophrenia such as urban living, (iii) anti-parasite effect of antipsychotic drugs, (iv) parallel increase in *T. gondii* infection and incidence of psychosis (especially schizophrenia) in various populations (USA, Turkey, Iran) (Torrey and Yolken, 2003, 2001; Arias et al., 2011), (v) a significantly high levels of antibodies to *T. gondii* in maternal sera whose offspring(s) subsequently develop schizophrenia later in life (Wang et al., 2006) and (vi) a 2.73 fold increase in overall odds of *T. gondii* seropositivity for schizophrenia measured in a meta analysis (Torrey et al., 2007).

However, the relationship between *T. gondii* infection and bipolar disorders (BD) is less documented due to paucity of information. Nevertheless, (i) a first study using a toxoplasmin intradermal test showed that high grade positivity was observed among patients with manic-depressive illness (Delgado García and Rodríguez Perdomo, 1980), (ii) a recent Ethiopian case-control study revealed a significantly higher seroprevalence of *T. gondii* infection in BD patients compared to healthy controls (Tedla et al., 2011) and (iii) a large cross-sectional survey in the general population showed a specific association between seroprevalence and type I BD, not found with other affective disorders (Pearce et al., 2012). Some of the reported discrepant data (Hinze-Selch et al., 2010) may be related either to the fact that studies involved multiple psychiatric disorders and were not based on clearly defined bipolar samples or were explained by geographical differences in the exposure to *T. gondii* infection.

In order to document the potential association between *T. gondii* exposure and BD in France, a country of high prevalence of *Toxoplasmosis*, we performed a case-control sero-prevalence study involving 110 BD patients and 106 healthy controls all living in France. We also evaluated the transcript level of interleukin-6 (IL-6), an adjunct marker of inflammation in bipolar disorders, in a randomly taken subset of patients and controls. We studied the expression status of IL-6, a hallmark of inflammatory status, because IL-6 is also involved in the development of protective immunity (Silver et al., 2011) and is a major regulator of NK cell activation and IL-17 production during *Toxoplasmosis* (Passos et al., 2010).

2. Materials and methods

2.1. Patients and controls

One hundred and ten BD patients (type I and II) meeting DSM-IV criteria consecutively admitted as in- ($N=50$) or out- ($N=60$) patients in two university-affiliated psychiatric departments in France (Mondor hospital, Créteil, University Paris-Est and Fernand Widal hospital, Paris, University Paris Diderot), were included in this study after approval by local institutional ethical committee and written informed consent for their participation. Patients were interviewed with the French version of the "Diagnostic Interview for Genetic Studies" (DIGS) for the assessment of lifetime clinical characteristics of bipolar disorder as well as for demographic characteristics [i.e., number of years of education, working status, season of birth, birth place (inside or outside France)]. Ongoing treatments as well as hospitalization status were recorded. Manic symptoms were assessed with the Young Mania Rating Scale (Young et al., 1978) and depressive symptoms with the Depression Rating Scale. Current smoking status was recorded using the Fagerström scale (Heatherton et al., 1991).

One hundred and six controls were enrolled through a clinical investigation center (Center for Biological Resources, Mondor hospital, Créteil, France). Only those, with neither personal nor family history (first degree) of psychiatric disorders, affective disorders, addictive or suicidal behaviour and nor personal or family history of auto-immune diseases, were included. Patients and controls were also excluded if they were (or have been treated) by immunosuppressive treatment, had a recent infection or an inflammatory disease and or neurological disease. All subjects were submitted to serological screening and were negative for HIV1/2, Hepatitis A, B and C with no known inflammatory, auto-immune or neurological disorders.

2.2. Serological analysis

Solid phase-enzyme microplate immunoassay methods were used to measure IgG and IgM class antibodies to *T. gondii* in blood sample using previously described methods (Yolken et al., 2001). Assay reagents were obtained from IBL America (Minneapolis, Minnesota, USA). The results were quantified by calculating a ratio between the reactivity of the samples and a standard sample run on each microplate. Seropositivity was defined as a *T. gondii* IgG ratio ≥ 0.8, equivalent to $>=10$ international units. All antibody measurements were carried out at the Stanley Laboratory of Developmental Neurovirology, Baltimore, Maryland, USA. All samples were analyzed under code by the laboratory not having access to diagnostic or clinical information.

2.3. IL-6 transcript analysis

Forty-two BD patients and forty-two controls were randomly tested for IL-6 transcript levels. The cDNA product (after RNA extraction from PBMCs and reverse transcription) was amplified with IL-6 sequence-specific primer pairs in duplicates. The primers used were as follows: 5′AATTCGGTACATCCTCGACGG3′ (forward) and 5′GGTTGTTTTCTGCCAGTGCC3′ (reverse). Amplification kinetics was monitored using SYBRGreen chemistry on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Values were normalized with simultaneously derived GAPDH