Toxoplasma gondii as a Risk Factor for Early-Onset Schizophrenia: Analysis of Filter Paper Blood Samples Obtained at Birth

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Background: Infections during fetal life or neonatal period, including infections with *Toxoplasma gondii,* may be associated with a risk for schizophrenia and other mental disorders. The objectives of this study were to study the association between serological markers for maternal and neonatal infection and the risk for schizophrenia, related psychoses, and affective disorders in a national cohort of newborns.

Methods: This study was a cohort-based, case-control study combining data from national population registers and patient registers and a national neonatal screening biobank in Denmark. Patients included persons born in Denmark in 1981 or later followed up through 1999 with respect to inpatient or outpatient treatment for schizophrenia or related disorders (ICD-10 F2) or affective disorders (ICD-10 F3).

Results: *Toxoplasma gondii* immunoglobulin G (IgG) levels corresponding to the upper quartile among control subjects were significantly associated with schizophrenia risk (odds ratio [OR] = 1.79, p = .045) after adjustment for urbanicity of place of birth, year of birth, gender, and psychiatric diagnoses among first-degree relatives. There was no significant association between any marker of infection and other schizophrenia-like disorders or affective disorders.

Conclusions: Our study supports an association between *Toxoplasma gondii* and early-onset schizophrenia. Further studies are needed to establish if the association is causal and if it generalizes to cases with onset after age 18.

Key Words: Affective disorder, neonatal, psychosis, schizophrenia, *Toxoplasma gondii*

The fact that infectious agents can cause psychiatric syndromes when they infect adults is well known (Caroff et al 2001; Torrey 1986). Of greater interest is whether infectious agents, when they infect a developing fetus, newborn, or young child, can cause psychiatric syndromes later in life.

Three approaches to this problem have been utilized. The first consists of temporal-association studies in which the births of individuals with a psychiatric syndrome, such as schizophrenia, are temporally linked to a known past disease outbreak, such as the 1957 influenza pandemic. Approximately 20 studies of schizophrenia and influenza have been published, almost evenly split between those reporting a significant association and those reporting no association (Barr et al 1990; Mednick et al 1994). Other temporal-association studies have linked schizophrenia to polio (Suvisaari et al 1999) and to measles and varicella-zoster virus (Torrey et al 1988).

A second approach to the problem is to follow up the offspring of women who are clinically thought to have actually been infected during pregnancy; this contrasts with temporal association studies, in which it is known that the women were pregnant at the time of the outbreak but not whether they were infected. Examples of this approach are studies linking maternal respiratory infections to an increased incidence of schizophrenia spectrum disorders in the offspring (Brown et al 2000b).

The third and most direct approach to linking infections and subsequent psychiatric syndromes is to assess antibodies to infectious agents in pregnant women or their offspring. For example, a study following offspring of women examined serologically for rubella has related this infection to schizophrenia and related disorders in the offspring (Brown et al 2000a). Recent studies using mothers' third-trimester stored sera have linked the presence of maternal antibodies to influenza A virus (Brown et al 2004), herpes simplex virus type 2 (HSV-2) (Buka et al 2001), and the protozoan *Toxoplasma gondii* (Brown et al 2005) to the subsequent development of schizophrenia spectrum disorders.

There may be a latent period of many years between the original in utero infection and the subsequent development of psychiatric syndromes, making it very difficult to establish causality. Stored serological samples are both rare and precious. Because of this and the relatively low incidence of schizophrenia, studies using stored maternal sera have been few and relatively small, including 63 cases (association with influenza and *T gondii*) (Brown et al 2004, 2005) and 27 cases (association with HSV-2) (Buka et al 2001) of schizophrenia or related disorders, respectively. No study to date has examined the specificity of infections as risk factors for schizophrenia compared with affective disorders.

We investigated an alternate source of stored blood, i.e., neonatal heel stick blood samples stored on filter paper. These samples are generally sent to a central laboratory for testing for phenylketonuria (PKU) and other metabolic abnormalities and then stored at −20°C in a desiccated state. Such testing has been widespread since the mid 1970s, and in some states and countries the filter papers are stored for future reference. Previous studies have indicated that immunoglobulin G (IgG) class antibodies can be stored on desiccated filter papers with little loss of

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activity. We hypothesized that samples stored in this way could be used to measure antibodies at the time of birth for individuals known to have later developed severe psychiatric disorders in early adulthood.

We capitalized on the nationwide Danish PKU biobank and the linkage of these samples through the unique Danish identifier to other registers, thereby increasing the sample size and reducing possible bias due to loss of follow-up. Our aims were to investigate possible associations between schizophrenia and specific infections, to ascertain if such findings are specific for schizophrenia, and to investigate if such findings could be ascribed to confounding factors associated with schizophrenia risk, i.e., family history for psychiatric disorders and urbanicity of place of birth.

Methods and Materials

The study was based on Danish population-based registers as well as the Danish PKU biobank (Nørgaard-Pedersen and Simonsen 1999). The design was a population-based case-control study. Potential cases consisted of all persons born in 1981 or later who, as of October 1999, had been registered in the Danish Psychiatric Case Register (Munk-Jørgensen and Mortensen 1997) with a diagnosis of schizophrenia, schizoaffective disorder, or other schizotypal or delusional disorders (ICD-10 F20–29) or bipolar affective disorder, recurrent depressive disorder, or other mood disorders (ICD-10 F30–39). Using the Central Population Register (CPR) (Malig 1996), the identity of the mothers of these cases was established, and using the identity of the mother and the child who later developed psychoses, it was possible to retrieve the biological samples from the PKU databank for a total of 186 cases who had been diagnosed with schizophrenia or related psychoses (ICD-10 F20–29) and 258 cases who had been diagnosed with bipolar affective disorder or other affective disorders (ICD-10 F30–39). Thirty-one individuals had been diagnosed in both categories, meaning that a total of 413 cases were included. Based on the psychiatric register data, it had originally been expected that the procedure would yield approximately 620 cases; however, a large number of cases could not be retrieved because of difficulties in retrieving the paper files that linked the unique person identifier, i.e., the CPR number, to the biological samples. For each case, two control subjects were chosen by selecting the samples in the biobank immediately before and after the sample chosen. The identities of the control subjects and their mothers, fathers, and siblings were then established by going back through the paper records to the Central Population Register. The cases were also linked to datasets created for other studies that yielded information regarding family history of mental disorders, sibship size, birth order, parental age, urbanicity of place of birth, and other potential risk factors; these were then added to the analyses.

The data were analyzed by logistic regression, using STATA 7.0 (StataCorp LD, College Station, Texas). Because PKU samples are typically sent in small batches by the midwives, it was assumed that cases and control subjects would at least be crudely matched for period and hospital of birth. Therefore, the year of birth (1981, 1982, 1983, or later), place of birth (in five categories according to urbanicity), and gender were included as obligate confounders in all analyses. In addition, the family history for psychiatric hospitalization of the father, the mother, and any siblings; maternal age; and sibship size (categorized as no siblings vs. one or more siblings) was entered into the analyses. Eligible control subjects had to have survived the study period without having had any psychiatric admissions.

All analyses of the blood samples were done blind to the case-control status of the individuals from whom the samples came. The analyses were performed on an eluate from 3.2 mm filter paper discs. Analyses included total IgG, total immunoglobulin M (IgM), IgG, and IgM antibodies specific for *T gondii* and IgG antibodies against herpes simplex virus type 1 (HSV-1) and HSV- 2 measured by enzyme immunoassays as previously described (Buka et al 2001).

Levels of antibodies were included in the analyses when relevant, both as continuous variables and as discrete variables comparing levels above and below the 75th percentile based on the distribution among control subjects. The 75th percentile was chosen, both as a way of excluding the effect of outlying values of IgG levels and because this would roughly correspond to the definition of seropositivity used in a *Toxoplasma* screening study of the prevalence and rate of seroconversion during pregnancy in about 90,000 Danish women (Lebech et al 1999). In their study, they found that 27.8% of women were seropositive, based on IgG levels from the same source of filter papers.

Data were analyzed for four diagnostic outcomes based on the Danish Psychiatric Case Register: schizophrenia (ICD-10 F20), the broader corresponding category of schizophrenia and related disorders (ICD-10 F20–29), bipolar affective disorder (ICD 10 F30–31), and the broader category of affective disorders (ICD-10 F30–39).

The project was approved by the Danish Data Protection Agency and the Danish Scientific Ethical Committees (approval no. 1999-1.4).

Results

Neonatal blood samples that had been stored on filter papers were retrieved from the following cases and control subjects: 71 persons who had been diagnosed with schizophrenia (ICD-10 F20); 186 persons who had been diagnosed with schizophrenia or related disorders (ICD-10 F20–29); 26 persons who had been diagnosed with bipolar affective disorder (ICD-10 F30–31); and 258 persons who had been diagnosed with any affective disorder (F30–39), including bipolar affective disorder. Among these, 33 had been diagnosed with both a schizophrenia-like disorder (F20–29) and an affective disorder (F30–39) on separate occasions; these were included in both categories in the analyses. It was possible to identify 684 control subjects and to link 682 of them to population data on parents and siblings.

Samples from individuals who were later diagnosed with schizophrenia had significantly higher levels than control subjects of IgG antibodies against *T gondii* (one-way analysis of variance $p < .003$). The distribution of cases of schizophrenia, schizophrenia-like disorders, and affective disorders and control subjects according to exposure is shown in Table 1. When dichotomized at the 75th and 90th percentile, respectively, there was also a significantly elevated risk in individuals with higher levels of antibodies. However, this association was confounded by the fact that population levels of IgG antibodies against *T gondii* were higher in samples from newborns in 1981 compared with the other years, and more individuals born that year had been hospitalized, simply because they were older than the rest of the study population. It is therefore more relevant to focus on the results when year of birth and other confounders have been taken into account (Tables 2 and 3).

The association was still statistically significant for the 75th percentile dichotomy (odds ratio [OR] = 1.79, 95% confidence