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# Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: Implications for genesis of neurodevelopmental disorders

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**Abstract**

Prenatal viral infection has been associated with development of schizophrenia and autism. Our laboratory has previously shown that viral infection causes deleterious effects on brain structure and function in mouse offspring following late first trimester (E9) administration of influenza virus. We hypothesized that late second trimester infection (E18) in mice may lead to a different pattern of brain gene expression and structural defects in the developing offspring.

C57BL6J mice were infected on E18 with a sublethal dose of human influenza virus or sham-infected using vehicle solution. Male offsping of the infected mice were collected at P0, P14, P35 and P56, their brains removed and prefrontal cortex, hippocampus and cerebellum dissected and flash frozen. Microarray, qRT-PCR, DTI and MRI scanning, western blotting and neurochemical analysis were performed to detect differences in gene expression and brain atrophy. Expression of several genes associated with schizophrenia or autism including Sema3a, Trfr2 and Vldlr were found to be altered as were protein levels of Foxp2. E18 infection of C57BL6J mice

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with a sublethal dose of human influenza virus led to significant gene alterations in frontal, hippocampal and cerebellar cortices of developing mouse progeny. Brain imaging revealed significant atrophy in several brain areas and white matter thinning in corpus callosum. Finally, neurochemical analysis revealed significantly altered levels of serotonin (P14, P35), 5-Hydroxyindoleacetic acid (P14) and taurine (P35). We propose that maternal infection in mouse provides an heuristic animal model for studying the environmental contributions to genesis of schizophrenia and autism, two important examples of neurodevelopmental disorders.


*Keywords:* Schizophrenia; Autism; Viral model; Mouse; DNA microarray; Brain

## 1. Introduction

Schizophrenia is a major debilitating disease with a lifetime prevalence of 1% throughout the world (American Psychiatric Association, 1994). The familial transmission of this disorder is multifactorial and heterogeneous (Asherson et al., 1994). Not all cases of schizophrenia can be accounted for by genetic causes. There is robust epidemiologic evidence indicating that environmental contributions, such as infections prenatally, may lead to genesis of schizophrenia (Fatemi, 2005).

Recent serologic evidence also points to multiple prenatal exposures to various viruses as causative factors in rise of schizophrenic births (Brown et al., 2004). Several groups, including our laboratory, have shown evidence for viral infections and/or immune challenges being responsible for production of abnormal brain structure and function in rodents where mothers were exposed to viral insults throughout pregnancy (Fatemi et al., 2005a; Meyer et al., 2006).

Recent evidence has shown that the time of prenatal insult may provide distinct changes in the exposed offspring. In a recent series of experiments by Meyer et al. (2006) using the viral mimic polyribocytidilic acid (PolyI:C) at E9 (which corresponds to mid-pregnancy) and E17 (which corresponds to late pregnancy) there were distinct behavioral deficits, neuropathological differences and acute cytokine responses (Meyer et al., 2006). Adult mice that were exposed on E9 displayed reduced exploratory behavior while those exposed on E17 displayed perseverative behavior (Meyer et al., 2006). At P24, mice that were exposed on E9 displayed a more pronounced reduction of Reelin immunoreactivity in hippocampus than mice exposed at E17 (Meyer et al., 2006). In contrast, mice exposed at E17 displayed an increase in apoptosis as visualized by immunoreactivity of caspase-3, a key enzyme involved in apoptosis (Rami et al., 2003), in the dorsal dentate gyrus (Meyer et al., 2006). Finally, Meyer et al. (2006) found that late gestational immune challenge uniquely stimulated increased IL-10 and TNF-α in fetal brain (Meyer et al., 2006). Taken together, these results provide evidence that the time of prenatal insult results in important differences that are persistent through adulthood.

Previous work by our group showed that influenza infection at E9 of pregnancy in mice leads to abnormal corticogenesis, pyramidal cell atrophy, and alterations in levels of several neuroregulatory proteins, such as Reelin, and GFAP, in the exposed mouse progeny (Fatemi et al., 1999, 2002a,b; Shi et al., 2003). We hypothesized that late second trimester infection (E18) in mice may lead to a different pattern of brain gene expression and structural defects in the developing offspring. Here, we present extensive genetic, imaging, and neurochemical data showing that a similar sublethal dose of human influenza virus (H1N1) in C57BL6J mice at E18, also leads to altered expression of many brain genes which may underlie brain atrophy and neurochemical dysregulation at puberty in the exposed mouse offspring.

## 2. Methods

### 2.1. Viral infection

All experimental protocols used in this study were approved by the Institute for Animal Care and Use and Institutional Biosafety Committees at the University of Minnesota. Influenza A/NWS/33 (H1N1) virus was obtained from R.W. Cochran, University of Michigan (Ann Arbor). A virus pool was prepared in Maden Darby canine kidney (MDCK) cells; the virus was ampuled and frozen at −80 °C until used. Data were expressed as $\log_{10}$ cell culture infectious doses ($CCID_{50}$)/ml by the method of Reed and Muench (1938). By this titration, it was determined that at a dilution of $10^{-4.5}$, none of the mice died of the infection but displayed a mean lung consolidation scores and mean lung weights similar to those obtained by Fatemi et al. (2002b) and had a mean virus titer of $10^{5.25}$ $CCID_{50}$/ml, indicating that a moderate but sublethal infection had been induced. This was the virus dose selected for use in the pregnant mouse study. On day 18 of pregnancy, C57BL6J mice (Charles River, Wilmington, MA) were anesthetized using 200 μl isoflurane, and intranasally (i.n.) administered a dilution