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Host-parasite interaction associated with major mental illness

Shin-ichi Kano[1], **Colin A. Hodgkinson**[4], **Lorraine Jones-Brando**[3], **Sharon Eastwood**[5], **Koko Ishizuka**[1], **Minae Niwa**[1], **Eric Y. Choi**[1], **Daniel J. Chang**[1], **Yian Chen**[1], **Swetha D. Velivela**[1], **Flora Leister**[3], **Joel Wood**[9], **Kodavali Chowdari**[9], **Francesca Ducci**[4], **Daniel A Caycedo**[4], **Elizabeth Heinz**[4], **Emily R. Newman**[4], **Nicola Cascella**[1], **Preben B. Mortensen**[6], **Peter P. Zandi**[1,7], **Faith Dickerson**[8], **Vishwajit Nimgaonkar**[9], **David Goldman**[4], **Paul J. Harrison**[5], **Robert Yolken**[3], **Akira Sawa**[1,2,7,*]

[1]Departments of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

[2]Departments of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

[3]Stanley Division of Developmental Neurovirology, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

[4]Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892, USA

[5]Department of Psychiatry, University of Oxford, Oxford, OX3 7JX, United Kingdom

[6]National Centre for Register-Based Research, University of Aarhus, Aarhus, 8000, Denmark

[7]Department of Mental Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, USA

[8]Stanley Research Program, Sheppard Pratt Health System, Baltimore, MD 21204, USA

[9]Departments of Psychiatry and Human Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

Abstract

Clinical studies frequently report that patients with major mental illness such as schizophrenia and bipolar disorder have co-morbid physical conditions, suggesting that systemic alterations affecting both brain and peripheral tissues might underlie the disorders. Numerous studies have reported elevated levels of anti-*T. gondii* antibodies in patients with major mental illnesses, but the underlying mechanism was unclear. Using multidisciplinary epidemiological, cell biological, and gene expression profiling approaches, we report here multiple lines of evidence suggesting that a major mental illness-related susceptibility factor, Disrupted in schizophrenia (DISC1), is involved in altered host immune responses against *Toxoplasma gondii (T. gondii)* infection. Specifically,

*Correspondence: asawa1@jhmi.edu.

Conflict of Interests

There are no competing financial interests in relation to the current work.

our cell biology and gene expression studies have revealed that DISC1 Leu607Phe variation, which changes DISC1 interaction with activating transcription factor 4 (ATF4), modifies gene expression patterns upon *T. gondii* infection. Our epidemiological data have also shown that DISC1 607 Phe/Phe genotype was associated with higher *T. gondii* antibody levels in sera. Although further studies are required, our study provides mechanistic insight into one of the few well-replicated serological observations in major mental illness.

Introduction

Mechanism(s) underlying many mental illnesses are poorly understood [1], in contrast to physical disorders, such as diabetes and cancer. Although the biology of mental illnesses tends to be discussed only in the context of brain dysfunction, recent epidemiological studies have indicated shorter lifespans and increased mortality in patients with schizophrenia and bipolar disorder [2]. Many factors, such as differences in lifestyle, adverse effects of medication, and unnatural death can influence lifespan and mortality [3]; however, beyond these confounding factors, it is possible that intrinsic factors associated with mental illness might underlie both mental and physical susceptibility.

The *Disrupted in schizophrenia* (*DISC1*) gene was originally reported in a Scottish pedigree, in which major mental illnesses (e.g., depression and schizophrenia) were clearly segregated with a hereditary chromosomal translocation disrupting this gene [4, 5]. Although the genetic association of *DISC1* polymorphisms with major mental illness *per se* is under debate, biological studies clearly demonstrate the significance of DISC1 protein as a “hub” molecule in neurobiology related to mental conditions [5–12]. In addition, in the general population, the *DISC1* Leu607Phe (rs6675281) and Ser704Cys (rs821616) polymorphisms have been reported to be associated with a wide range of mental conditions, and to influence brain morphology and connectivity [13–22]. Recent genetic association studies from the Psychiatry Genomic Consortium (PGC) highlight the link between schizophrenia and genes related to immune function [23]. These results suggest that aberrant immune and inflammatory responses may underlie the pathophysiology of schizophrenia and related disorders. Given that the etiology of these mental disorders includes significant influences of environmental stressors [24], intrinsic susceptibility in immune/inflammatory responses to environmental stressors may play a role in the pathophysiology of the disease.

Toxoplasma gondii (T. gondii) is a protozoan parasite that infects about one-third of the human population worldwide [25–29]. The parasite’s natural life cycle in the feline definitive host encompasses three stages: oocysts, tachyzoites, and bradyzoites, with the last stage found in tissue cysts [25–29]. Although *T. gondii* infection is usually asymptomatic, it can cause cervical lymphadenopathy and chorioretinitis in immunocompetent individuals [29]. Furthermore, in immunocompromised individuals, this infection can result in life-threatening conditions, including encephalitis and pneumonia [26, 29].

Epidemiological and clinical studies have suggested an involvement of infection by pathogenic microorganisms in the pathology of devastating major mental disorders [30–35]. In particular, elevated levels of serum antibodies against *T. gondii* have been reproducibly

reported in schizophrenia and bipolar disorder, and some studies show its association with more severe positive psychopathology [30, 33–38].

Host genetic variations play a major role in determining susceptibility to various infectious diseases [39, 40] and thus could explain altered host responses against *T. gondii* in a specific subgroup of the general population. Nonetheless, the influence of host genetic variations relevant to major mental illness on *T. gondii* infection has not yet been investigated [41, 42]. Our recent study using a *DISC1* animal model shows that behavioral abnormalities appear only in the presence of psychosocial isolation stress [43], suggesting that genetic variation at *DISC1* might participate in the pathophysiology or biological processes underlying mental illness in the context of host susceptibility to environmental stressors.

In the present study, we hypothesized that variation at *DISC1* may underlie the host response to *T. gondii* infection, possibly associated with major mental illness. We have observed that a *DISC1* low frequency variant that alters the encoded DISC1 protein (Leu607Phe) influences host immune responses against *T. gondii*. Our data also show that anti-*T. gondii* IgG serum levels are elevated in subjects homozygous for the DISC1 607 Phe/Phe variant in a cohort in which the majority of subjects are Caucasian.

Materials and Methods

Epidemiological study

Two independent cohorts from Baltimore (n=650) and Pittsburgh (n=652) were used in this study (Supplementary Table 1). The Baltimore cohort was recruited from a clinical program affiliated with the Sheppard Pratt Health System and other agencies in central Maryland, with inclusion/exclusion criteria as previously described [44]. Participants included individuals with schizophrenia and related (schizophrenia), bipolar disorder, or recent onset psychosis, and individuals with no history of a mental illness. The Pittsburgh cohort was recruited at the University of Pittsburgh as part of a schizophrenia genetics research study; individuals were assessed using semi-structured interviews and consensus diagnoses assigned using DSM-IV criteria as described [45]. Participants included individuals with schizophrenia and related (schizophrenia and schizoaffective disorder), major depression, substance abuse/dependence (alcohol, nicotine, cannabis), and those with no diagnosis or condition on Axis II. Sera were collected and stored at −80ºC until tested. Levels of anti-*T. gondii* IgG and antibodies against other pathogens were measured by enzyme immunoassay (EIA) as previously described [46–49]. Genotypes for [DISC1 Leu607Phe (rs6675281), Ser704Cys (rs821616), COMT Val158Met (rs4680), BDNF Val66Met (rs6265), and GRM3 (rs6465084)] were determined TaqMan SNP genotyping assays (Life technologies) as previously reported [50, 51]. This study was approved by Institutional Review Boards at Johns Hopkins University.

Cell biology experiments

***In vitro T. gondii* infection:** Epstein Barr Virus (EBV)-transformed B lymphoblastoid cells were obtained from the cohorts that were reported in previous studies [52, 53] and are independent of the two cohorts (Baltimore and Pittsburgh) used in the current

epidemiological study. DISC1 607 Leu/Leu and Phe/Phe genotyping was performed as previously described [51]. Individual profiles are provided in Supplementary Table 2. Primary mouse glial cell cultures were prepared from the cortices of postnatal day 3 (P3) pups of C57BL/6 mice as described previously [54–56]. Human lymphoblastoid cells (Leu/Leu, n=8; Phe/Phe, n=7) and mouse glial cells (two independent cultures) were infected with tachyzoites of *T. gondii* strain 2F [RH-2F], which constitutively expresses cytoplasmic β-galactosidase [57], at a multiplicity of infection (MOI) of 1, and then harvested for RNA collection at 48 h. *T. gondii* tachyzoite growth was determined by CPRG (chlorophenol red-β-D-galactopyranoside) assay as previously reported [57] and described in Supplementary Methods.

Gene expression assay: Total RNA was purified using RNeasy kit (Qiagen) from lymphoblastoid cells (Leu/Leu, n=3; Phe/Phe, n=3) with or without 48 h infection with *T. gondii*. Gene expression levels were determined using Illumina Human HT-12 V4 BeadChip arrays (Illumina) at the Microarray Core Facility at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University. Data were extracted using GenomeStutio v1.0 Expression Module and statistical analysis was performed using GeneSpring GX 11.0.2. Because of the small sample size, we used unadjusted $p < 0.05$ as cut-off for differential gene expression. Gene ontology analysis of overrepresented gene classes was performed with the DAVID (Database for Annotation, Visualization and Integrated Discovery) software (http://david.abcc.ncifcrf.gov). Quantitative real-time PCR was carried out with SYBR GreenER reagent (Invitrogen) on an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems) following a standard protocol [58, 59]. qPCR data were normalized against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression levels. Gene-specific primers are shown in Supplementary Methods.

Statistical analysis

Statistical analysis was performed by two-tailed Student's *t*-test, one-way ANOVA, Kruskal-Wallis test, and simple χ^2 test. *Post-hoc* multiple correction was performed with Bonferroni's method. GraphPad Prism 6 (GraphPad) and R were used for these analyses.

Results

Altered host responses and *T. gondii* growth in DISC1 Phe607 homozygotes exposed to *T. gondii*

To address whether DISC1 is involved in immune responses against *T. gondii*, we studied primary cortical glial cell culture from mice in which the major isoforms of the *Disc1* gene are genetically depleted (*Disc1* LI homozygous mice) [60]. Glial cells were used because they play a critical role in brain immune responses against *T. gondii* infection [61] and because it has been suggested that chronic infection of *Toxoplasma* affecting the brain is linked to the development and severity of psychiatric disorders [47, 62, 63]. Our data showed that reduced DISC1 levels in primary mouse cortical glial cells resulted in an enhancement of *Tnf* mRNA induction in response to *T. gondii* infection (Figure 1A, B). In contrast, *Disc1* LI homozygous mice did not show any obvious immune/inflammation-related phenotypes or

loss in the subsets of T, B, CD11b$^+$, CD11c$^+$, and NK cells under a resting condition (Supplementary Figure 1).

Based on this observation that DISC1 levels altered glial response to *T. gondii* infection, we investigated whether the DISC1 Leu607Phe low frequency variant might influence human immune responses to *T. gondii*. This SNP was selected because it is located at the interaction domain of DISC1 with activating transcription factor 4 (ATF4), a transcription factor controlling the expression of genes related to cellular stress and inflammation [64–66]. Indeed, our two-hybrid and co-immunoprecipitation experiments revealed that DISC1-ATF4 interaction was attenuated in the presence of 607Phe variation (Figure 2A, B). In addition, over-expression of DISC1 in cultured cells showed that the nuclear localization of DISC1–607Phe was markedly reduced compared with that of DISC1–607Leu (Figure 2C), consistent with findings from the Millar/Porteous lab [67]. Thus, together with our previous observation of the role of DISC1 in gene transcription [68, 69], we hypothesized that cellular responses against *T. gondii* infection are dysregulated in the cells from individuals with DISC1 607Phe/Phe (homozygous for low frequency variants) via ATF4-related mechanisms.

We utilized EBV-transformed lymphoblastoid cells as surrogate cells because previous studies successfully used these cells to address the role of genetic variations in immune responses against pathogen infections in humans [70, 71]. These lymphoblastoid cells readily expressed DISC1 protein and the genotype did not affect the expression level of DISC1 proteins (Supplementary Figure 2A). We infected lymphoblastoid cells from individuals with DISC1 607 Leu/Leu (homozygous for high frequency variant) or Phe/Phe *in vitro* with *T. gondii* tachyzoites for 48 h. As expected, DISC1 607 genotype influenced DISC1-ATF4 protein binding (Supplementary Figure 2B).; DISC1 607 Phe/Phe genotype reduced endogenous DISC1-ATF4 binding. In contrast, *T. gondii* infection did not cause a marked change in DISC1-ATF4 interaction across the genotypes.

Microarray-based gene expression profiling (n=3 for Leu/Leu, n=3 for Phe/Phe) of *T. gondii*-infected cells revealed that 38 and 46 genes were changed more than 1.5-fold in DISC1 607 Leu/Leu and Phe/Phe cells respectively, with 71 genes showing differential expression patterns and 13 genes showing similar expression changes between the two genotypes (Figure 3A, B). Notably, 61 genes out of 71 genes that were differentially expressed between DISC1 607 Leu/Leu and Phe/Phe cells by *T. gondii* infection contained predicted cAMP response element (CRE)-binding in their promoters (Supplementary Table 3). These findings suggest that DISC1 607 variation alters gene expression changes in response to *T. gondii* infection by modifying ATF4-mediated transcriptional mechanisms through CRE-binding sites in the promoters. Gene ontology analysis revealed several functional gene groups that are uniquely overrepresented in either Leu/Leu or Phe/Phe cells upon infection (Figure 3C). For example, the groups for “regulation of programmed cell death” and “transcription factor binding” were specifically underscored in cells with DISC1 Leu/Leu, whereas the groups for “plasma membrane” and “defense response” were specific to cells with DISC1 Phe/Phe. In contrast, groups for “cytokine-cytokine receptor interaction” and “immune responses” were represented similarly in cells of both genotypes.

To overcome the limitations of using microarray with such a small sample size (n=3 per group), we confirmed the findings using quantitative reverse-transcription PCR (qRT-PCR) with a larger number of samples (n=7–8 per group). We observed that the extent of mRNA upregulation for *JUNB* and *BATF3*, both of which encode transcription factors known to be involved in the host response to *T. gondii* infection [72, 73], were significantly smaller in Phe/Phe cells in response to *T. gondii* infection than in Leu/Leu cells (Figure 4A). The increase of *JUNB* mRNA induction was significantly higher in Leu/Leu cells than Phe/Phe cells after *T. gondii* infection. In the case of *BATF3* mRNA, there was no significant increase in Phe/Phe cells after *T. gondii* infection, in contrast to significant increases in Leu/Leu cells. Both of these genes contain CRE-binding sites in their promoter, and therefore represent potential targets of ATF4 regulation (Supplementary Figure 3).

To further link DISC1 Leu607Phe variation and altered responses of lymphoblastoid cells to *T. gondii* infection, we examined the growth of *T. gondii* tachyzoites after infection of DISC1 607 Leu/Leu or Phe/Phe lymphoblastoid cells. Because *T. gondii* tachyzoites rely on arginine for their growth [74], limiting arginine availability can enhance host resistance to the parasite and result in lower growth rates and higher conversion into bradyzoites. We therefore tested the growth of *T. gondii* under both normal and arginine-free conditions. Under normal conditions DISC1 genotype did not significantly alter tachyzoite growth, however, we observed a significant decrease in the growth of *T. gondii* tachyzoites in DISC1 607 Phe/Phe cells when cells were cultured under arginine-free medium (Figure 4B).

Effects of DISC1 Leu607Phe variation on anti-*T. gondii* IgG level

We then examined the effects of the DISC1 607Phe variant on the levels of anti-*T. gondii* IgG in serum in two different cohorts of human subjects. In the first cohort (n=650) (Supplementary Table 1), we found that the 607Phe variant is associated with the levels of anti-*T. gondii* IgG in serum; individuals homozygous for the DISC1 Phe607 variant (DISC1 607 Phe/Phe) showed a >3-fold elevation in serum anti-*T. gondii* IgG levels compared to carriers of the DISC1 Leu607 variant (DISC1 607 Leu/Leu and Leu/Phe) (Figures 5A, 5B). Notably, *T. gondii* IgG levels were associated with DISC1 607 Phe/Phe by logistic regression analysis (p=0.01) adjusting for age, gender, race, and clinical diagnosis (Table 1). We did not observe any influence of the Ser704Cys variation on the serum levels of anti-*T. gondii* IgG (Figure 5C). We also examined *T. gondii* seropositivity among these cohorts, but high seropositivity for anti-*T. gondii* IgG was observed at trend levels both for individuals with the 607Phe and Ser704Cys variants (Figures 5D, E**)**. Variations in the genes coding for catechol-O-methyltransferase (COMT at Val158Met: rs4680), metabotropic glutamate receptor 3 (GRM3: rs6465084), and brain-derived neurotrophic factor (BDNF at Val66Met: rs6265), all of which are relevant to major mental illness [45, 75–78], did not show any association with *T. gondii* seropositivity (Supplementary Figures 4A-4C). Finally, we found that the association of DISC1 Leu607Phe variation to serum IgG levels is specific to *T. gondii*, as the same variation did not show any significant differences in serum levels of antibodies against other infectious agents, including cytomegalovirus (CMV), varicella zoster virus (VZV), and herpes simplex virus type1 (HSV-1) (Supplementary Figure 4D-4F).

We examined the DISC1 Leu607Phe variation in an independent sample (n=652) (Supplementary Table 1), but no seropositive subjects among those with DISC1 607 Phe/Phe genotype having *T. gondii* seropositivity were found in this cohort. Thus, we were unable to evaluate changes in the seropositivity for anti-*T. gondii* IgG in individuals with DISC1 607 Phe/Phe in the Pittsburgh cohort. Indeed, with the current sample size, the data showed no significance (Figure 5F). Notably, these two cohorts were significantly different in terms of age, sex, race, clinical diagnosis, and *T. gondii* seropositivity (Supplementary Table 1). In summary, although the data from one cohort clearly showed that the DISC1 607Phe variation is specifically associated with elevated serum anti-*T. gondii* IgG levels, the lack of *T. gondii* seropositive individuals with DISC1 607 Phe/Phe genotype in another independent cohort prevented us from conducting confirmation studies.

Discussion

In this study, we have provided evidence that variation at *DISC1* can regulate immune responses. Our data suggest that impaired DISC1 function can modify host immune responses against *T. gondii* infection. To our knowledge, no previous study has addressed the involvement of DISC1 in host-pathogen interactions. Although our findings in cell biology do not directly address either the mechanisms underlying elevated anti-*T. gondii* IgG or a potential causal role of *T. gondii* infection in psychiatric disorders, this study provides an important first step to investigate the biological link between psychiatric genetics and epidemiological findings. The current study needs to be followed up by multi-institutional larger studies in the near future.

With respect to mechanisms, the Leu607Phe variation is likely to modify ATF4-mediated gene transcription upon *T. gondii* infection by altering nuclear targeting of DISC1 and thereby DISC1-ATF4 interaction. Indeed, recent studies provide evidence that DISC1 modulates ATF4-dependent gene transcription [67–69]. ATF4 is a key transcription factor that regulates genes carrying CRE binding sites in their promoters and is known to regulate the unfolded protein response (UPR) and, more recently, immune responses [64–66]. Thus, DISC1/ATF4-mediated modulation of immune/stress gene expression provides a promising working hypothesis to link DISC1 607 variation and host immune responses to *T. gondii* infection. Loss of DISC1-ATF4 interaction in cells of DISC1 607 Phe/Phe genotype or *Disc1* LI mouse cell culture may result in enhanced innate immune responses, which can decrease *T. gondii* tachyzoite growth by forcing their conversion into bradyzoites and simultaneously induce strong T and B cell-mediated adaptive immune responses, including higher IgG production. This scenario may be supported by our *Disc1* LI mouse cell culture data, gene expression and cell biology findings in DISC1 607 Phe/Phe cells upon *T. gondii* infection, and epidemiological findings on the association of higher anti-*T. gondii* IgG levels with DISC1 607 Phe/Phe genotype, but further mechanistic studies are essential to reinforce the hypothesis.

Intriguingly, accumulating evidence suggests that UPR-related molecules are involved in host responses against pathogens [79–81]. Although the role of ATF4 in *T. gondii* infection is not yet known, mice with a genetic deficiency of ATF6β, one of the UPR initiators, showed altered immune responses against *T. gondii* infection via altered function of dendritic cells

[81]. This implicated a potential role of ATF4 and UPR in antigen presentation and cytokine production in dendritic cells, macrophages, and B lymphocytes against *T. gondii* infection. Although DISC1/ATF4-mediated modulation of immune responses is a promising hypothesis, we acknowledge that DISC1 has many binding partners [5] and even the Leu607Phe variation affects its interaction with another protein, FEZ1 [68]. Despite the paucity of evidence supporting the role of FEZ1 in immune responses, further studies are necessary to determine the immune functions of DISC1 through other binding partners.

DISC1 expression is found in both neurons and glia in the brain and also in peripheral non-neuronal cells, including lymphocytes [82, 83]. Therefore, cell populations that are mainly affected by DISC1 607 SNP need to be identified. Although it is beyond the scope of this study, once induced pluripotent stem cells from subjects carrying DISC1 607Phe/Phe and 607Leu/Leu are established, such cells can be further differentiated into neurons and/or glia for further validation of the working hypothesis proposed in the present study.

A limitation of our findings was that only one cohort revealed the effects of DISC1 607 variation on the levels of anti-*T. gondii* IgG in serum, the other cohort lacking any seropositive DISC1 607 Phe/Phe homozygotes, which prevented replication. These cohorts represent two independent populations with distinct features (Supplementary Table 1), and such differences in demographic background (e.g., gender, race and genetic background, and disease profiles) may underlie the different patterns of association in the results between the two cohorts. Furthermore, the diversity of *T. gondii* infection in different geographical locations may affect the results, because infection is influenced by lifestyle. Thus, we have estimated the sample size that can overcome the heterogeneity of cohorts. Our power analysis suggests that we need at least 8,091 samples to obtain a significant difference (α=0.05) in the levels of anti-*T. gondii* IgG between individuals with DISC1 607 Phe/Phe and others (DISC1 607 Leu/Leu and Leu/Phe) with a power of 80%. This number is very large, given that the study requires simultaneous measurement of the levels of proteins in sera and SNP genotypes. We hope that the present study will serve as a platform to design larger comprehensive studies in the near future. Alternatively, by forming multi-institutional consortium for larger sample studies in the near future, we might be able to identify another cohort where sufficient DISC1 607 Phe/Phe individuals with high *T. gondii* IgG levels are available. The concept of interactions between host and infectious agents for mental illness may be extended not only to *T. gondii,* but also more generally to other infectious and non-infectious agents such as bacteria, parasites, and intestinal microbiomes. Future studies will also address the link between the levels of anti-*T. gondii* IgG and disease severity, which requires a larger sample size.

Cell biology and molecular findings of this study are limited by the small sample size and the heterogeneous demographic data of EBV-transformed lymphoblastoid cells from individuals with DISC1 607 Leu/Leu and Phe/Phe genotypes. At this moment, demographically-matched lymphoblastoid cells are not available for both DISC1 607 Leu/Leu and Phe/Phe genotypes. In the current study, many of the cells with DISC1 607 Phe/Phe genotype were derived from individuals with substance abuse. Thus, unclear differences in exposure to substance may have affected our findings. In future studies where a larger cohort will be used, we expect to recruit these cells from individuals with DISC1

607 Phe/Phe genotype and no psychiatric history. Another limitation of cell biology studies is that the data has been compared only between cells with DISC1 Leu/Leu and Phe/Phe genotype. The effects of DISC1 607 variation on anti-*T. gondii* immune responses may be recessive at least in our epidemiology data (Figure 5B). Analysis of EBV-transformed lymphoblastoid cells from individuals with DISC1 607 Leu/Phe on matched demographic information would be important to address this points in the future. Our study is also limited in that we could only examine primary infection of *T. gondii*. Clinical findings related to psychiatric disorders have suggested that either chronic infection or reactivation of a latent, quiescent infection plays a role in brain dysfunction and behavioral changes [47, 62, 63]. Currently, cell culture models cannot recapitulate these important biological phenomena. Recent advances in human brain organoids [84–86] may be useful in addressing these more clinically related questions in future studies.

Recent clinico-epidemiological studies indicate a high rate of comorbid physical conditions (e.g., autoimmune diseases and metabolic disorders) as well as shorter lifespans and increased mortality in patients with schizophrenia and bipolar disorder [66, 67]. Here we have provided a strategy to address these issues by utilizing human cells from genetically defined populations in the context of gene and environmental interactions. This approach may open a window for further validation of the concept that schizophrenia and bipolar disorder are systemic disorders involving immune alterations. This novel concept, if true, would dramatically change future strategies for drug discovery and help overcome the social stigma that accompanies mental illness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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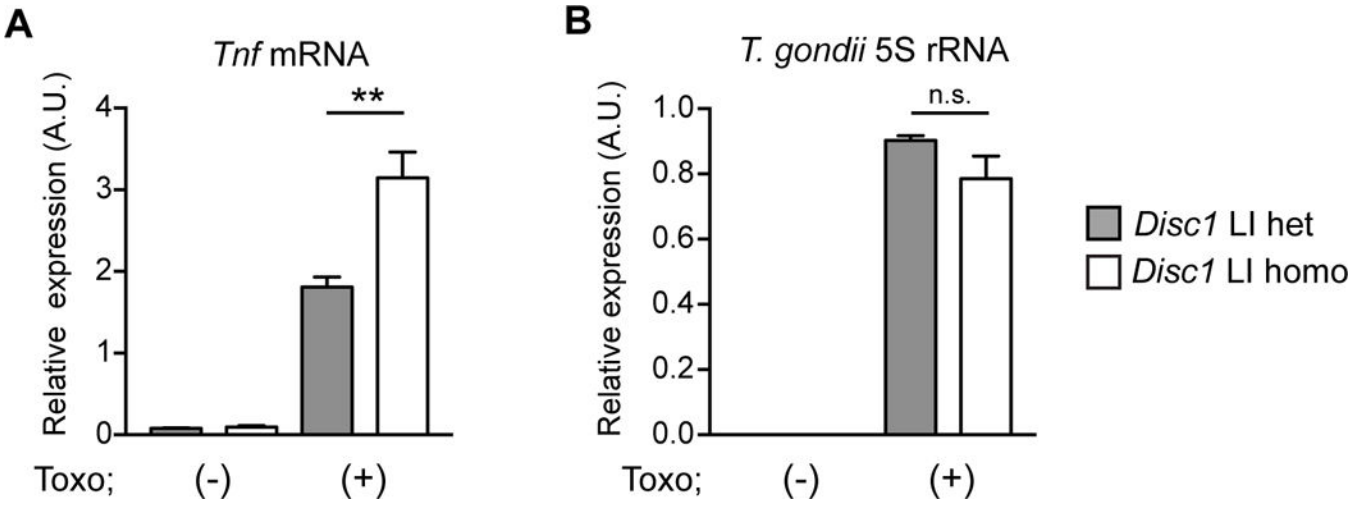


Figure 1: Increased anti-*T. gondii* immune response in primary cortical glial cells from mice with loss of function of *Disc1* gene.

Primary cortical glial cells were prepared from postnatal day 2 (P2) pups of mating pairs of *Disc1* LI heterozygous mice. *Disc1* LI heterozygous and homozygous pups of the same litters were used for the study. On day 10, glial cells were infected with *T. gondii* tachyzoites and harvested at 48 h post-infection for qRT-PCR analysis. **A.** Enhanced induction of *Tnf* mRNA in *Disc1* LI homozygous glial cells compared to *Disc1* LI heterozygous glial cells. **B.** No difference in the amount of intracellular *T. gondii* between *Disc1* LI mice and their heterozygous littermates. *T. gondii* 5S rRNA levels were measured by qRT-PCR in glial cell lysates to estimate the number of *T. gondii*. **p<0.01. n.s., not significant. Error bars show s.e.m.

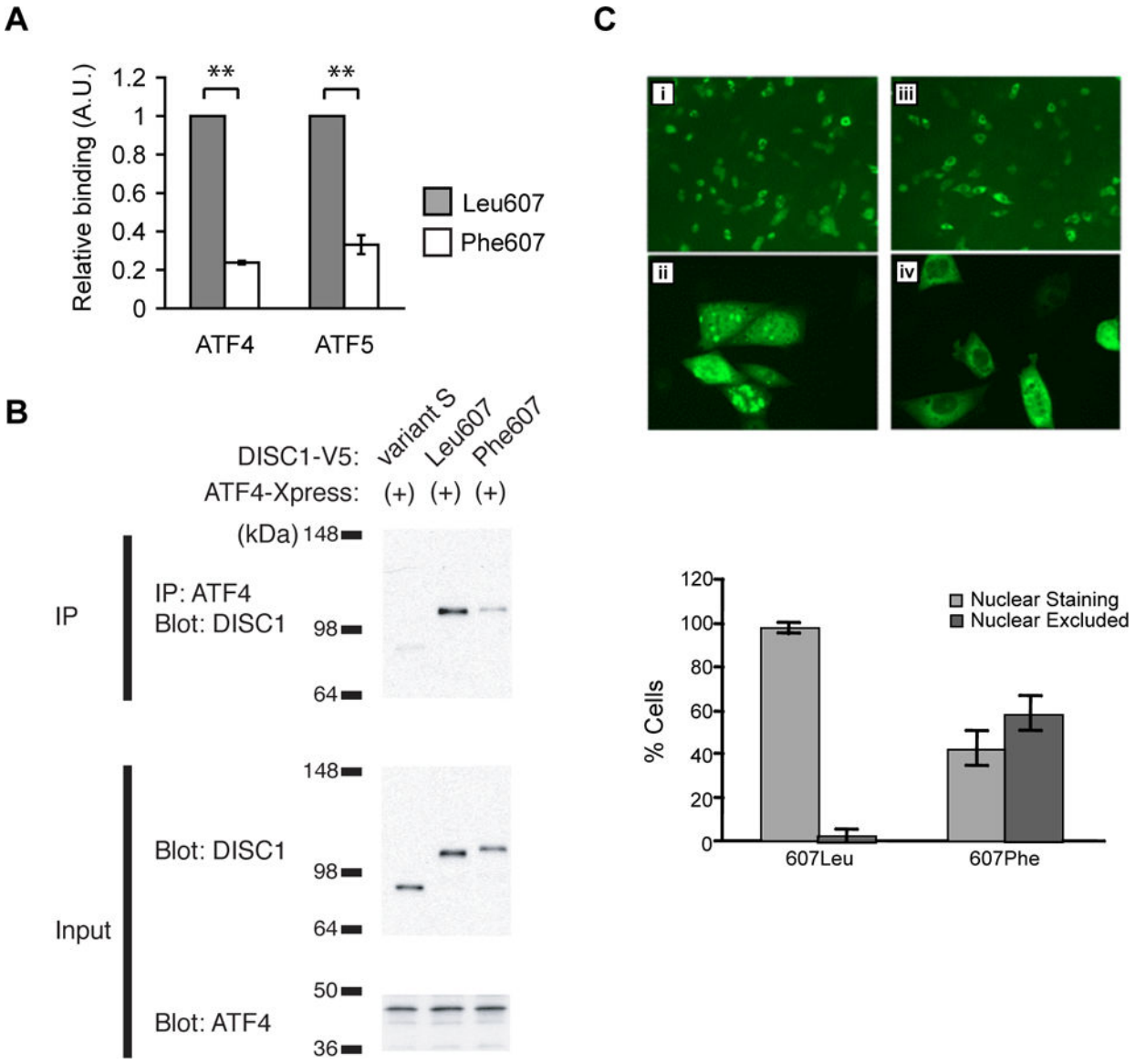
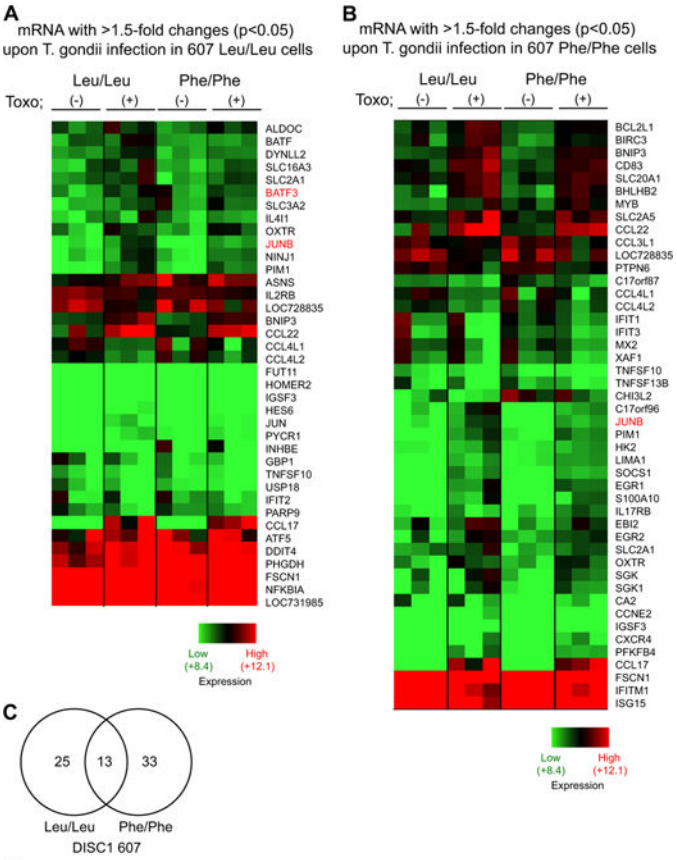


Figure 2: Altered DISC1 function by the 607Phe variation.

A. Reduced interaction of DISC1 607Phe variant to ATF4 in mammalian two-hybrid assay. CHO cells were transfected with pBIND-DISC1 together with pACT-prey and pGL5luc. Twenty-four hours after transfection, the cells were harvested for luciferase assays. Binding activity of DISC1 to ATF4 and ATF5 was severely impaired by DISC1 607Phe construct compared to that of the 607Leu. Experiments were performed in triplicate and data from four independent transfections were combined for analysis. Error bars show s.e.m. **ATF4, $p=8.42\times10^{-12}$; ATF5, $p=1.74\times10^{-7}$ (one-tailed *t*-test). **B.** Decreased binding of ATF4 to DISC1 607Phe in co-immunoprecipitation experiment. COS-7 cells were co-transfected with plasmids expressing V5-tagged DISC1 and ATF4. Twenty to thirty hours later, cell lysates were immunoprecipitated with anti-ATF4 antibody and analyzed by Western blot using anti-V5 antibody. ATF4 did not efficiently precipitate DISC1 607Phe compared to DISC1 607Leu. **C.** Reduced nuclear distribution of DISC1 protein by the 607Phe variant.

CHOk1 cells transfected with the 607Phe variant showed reduced nuclear DISC1 protein (**iii** and **iv**) compared to cells transfected with the 607Leu variant (**i** and **ii**). Bar graph shows a reduction in the percentage of cells with nuclear DISC1 localization in 607Phe-transfected cells. Error bars show s.e.m. Difference in intracellular localization of DISC1 between 607Leu and 607 Phe variant was significant at $p< 2.2\times10^{-16}$ (Fisher's Exact test).



Annotation Term	Leu/Leu		Phe/Phe	
	Count	p-value	Count	p-value
GO:0043067~regulation of programmed cell death	8	0.00202		
GO:0010941~regulation of cell death	8	0.00206		
GO:0008134~transcription factor binding	7	0.00098		
GO:0046983~protein dimerization activity	7	0.00130		
GO:0005886~plasma membrane			16	0.02852
GO:0006952~defense response			9	0.00011
GO:0005615~extracellular space			9	0.00022
GO:0044421~extracellular region part			9	0.00204
GO:0042981~regulation of apoptosis	8	0.00191	6	0.04873
hsa04060:Cytokine-cytokine receptor interaction	6	0.00095	8	0.00008
GO:0006915~apoptosis	6	0.01116	8	0.00063
GO:0012501~programmed cell death	6	0.01185	8	0.00069
GO:0006955~immune response	6	0.01920	9	0.00024
GO:0008219~cell death	6	0.02253	8	0.00178
GO:0010033~response to organic substance	6	0.02277	8	0.00181
GO:0016265~death	6	0.02314	8	0.00185
GO:0007610~behavior	5	0.02139	8	0.00014

Figure 3: Altered gene expression patterns in cells with DISC1 607 Phe/Phe variant during *T. gondii* infection.

A. Heat map of the mRNAs that were changed more than 1.5-fold upon *T. gondii* infection in DISC1 607 Leu/Leu cells. **B.** Heat map of the mRNAs that were changed more than 1.5-fold upon *T. gondii* infection in DISC1 607 Phe/Phe lymphoblastoid cells. Data were normalized and plotted on a log2 color scale. Welch t-test (unpaired and unequal variance) was used to select differentially expressed genes between two groups with $p<0.05$. Expression pattern similarity was visualized by hierarchical clustering with Euclidean as

distance metric and Centroid as linkage rule. **C.** Venn diagram indicating overlap of gene expression changes upon *T. gondii* infection in DISC1 607 Leu/Leu and Phe/Phe lymphoblastoid cells. **D.** Ontology profiling of genes that are changed upon *T. gondii* infection in DISC1 607 Leu/Leu and Phe/Phe lymphoblastoid cells. Genes listed in **A** and **B** were analyzed using DAVID Bioinformatics Database (http://david.abcc.ncifcrf.gov/).

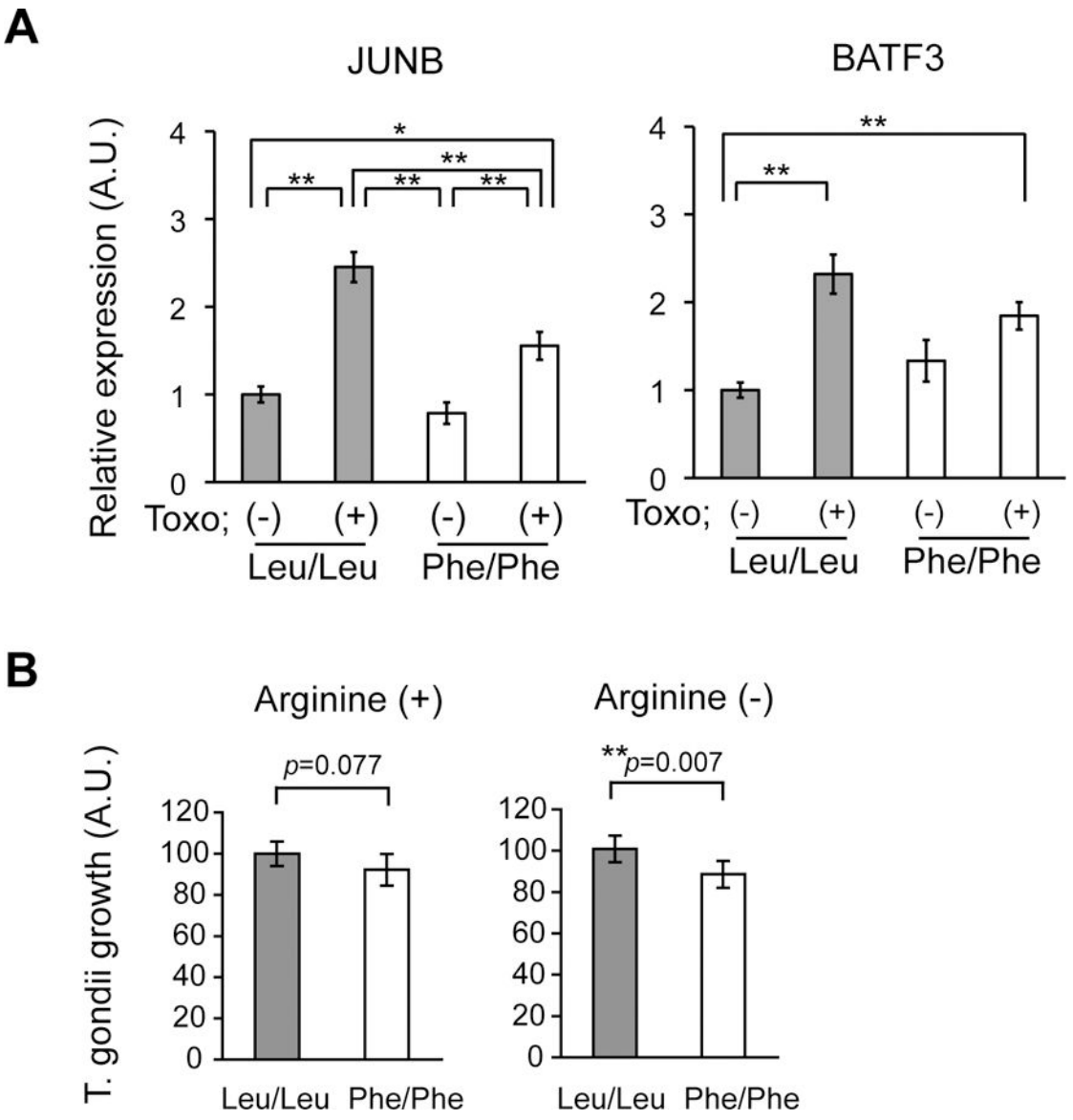
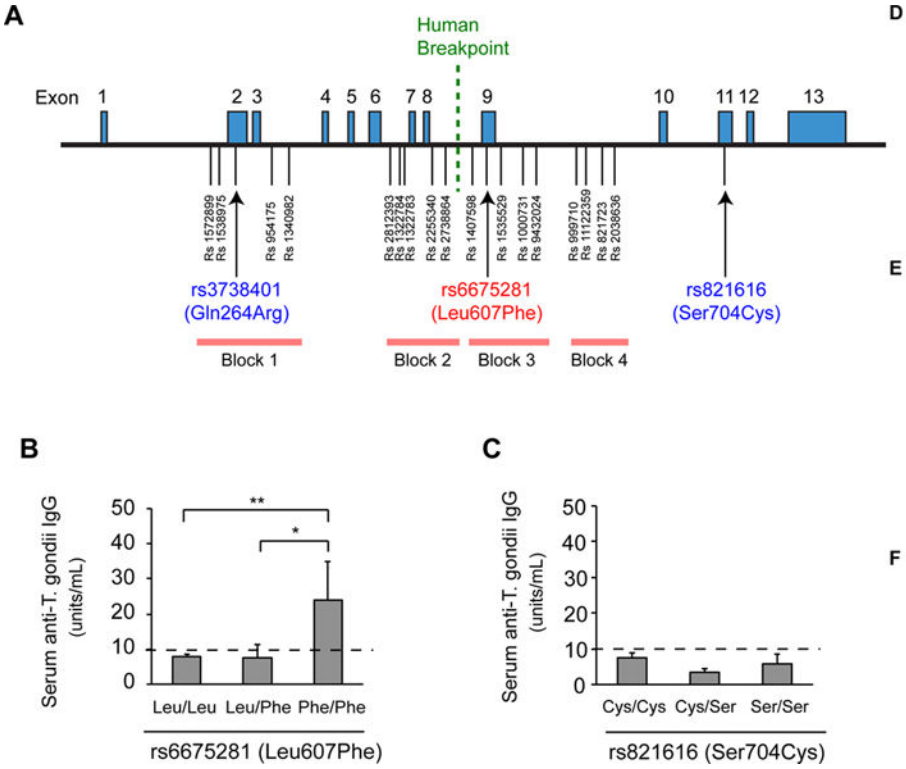


Figure 4: Altered response of DISC1 607 Phe/Phe cells to *T. gondii* infection.

A. qRT-PCR analysis of *JUNB* and *BATF3* mRNA expression before and after *T. gondii* infection in DISC1 607 Leu/Leu and Phe/Phe lymphoblastoid cells. **p<0.01, *p<0.05. **B.** Reduced growth of *T. gondii* tachyzoites in DISC1 607 Phe/Phe lymphoblastoid cells. **p<0.01. Error bars show s.e.m.



D

Baltimore cohort	DISC1 607 Leu/Leu	Leu/Phe	Phe/Phe	Total
Positive	69	21	6	96
	71.88	21.88	6.25	100.00
	14.08	14.69	35.29	14.54
Negative	421	122	11	554
	75.99	22.02	1.99	100.00
	85.92	85.31	64.71	85.46
Total	490	143	17	650
	75.38	22.00	2.62	100.00
	100.00	100.00	100.00	100.00

χ^2= 5.87 p=0.053

E

Baltimore cohort	DISC1 704 Cys/Cys	Cys/Ser	Ser/Ser	Total
Positive	34	13	2	49
	69.39	26.53	4.08	100.00
	11.89	6.57	4	9.18
Negative	252	185	48	485
	51.96	38.14	9.90	100.00
	88.11	93.43	96.00	90.82
Total	286	198	50	534
	53.56	37.09	9.36	100.00
	100.00	100.00	100.00	100.00

χ^2= 5.75 p=0.056

F

Pittsburgh cohort	DISC1 607 Leu/Leu	Leu/Phe	Phe/Phe	Total
Positive	58	20	0	78
	74.36	25.64	0.00	100.00
	12.01	12.66	0.00	11.96
Negative	425	138	11	574
	74.04	24.04	1.92	100.00
	87.99	87.34	100.00	88.04
Total	483	158	11	652
	74.08	24.23	1.69	100.00
	100.00	100.00	100.00	100.00

χ^2= 1.57 p=0.456

Figure 5: Levels of anti-*T. gondii* IgG in serum from individuals with specific DISC1 SNP genotypes.

A. Human *DISC1* gene and known single nucleotide polymorphisms (SNPs). Human *DISC1* gene covering exons 1 to 13 is shown, with the positions of SNPs and the haplotype blocks defined in a previous study [68, 69, 72, 73, 75]. **B.** Increase in serum anti-*T. gondii* IgG levels in individuals with DISC1 607 Phe/Phe (n=17) compared to those with DISC1 607 Leu/Leu (n=490) or Leu/Phe genotype (n=143). Anti-*T. gondii* IgG levels in the serum were measured by ELISA. Error bars show s.e.m. *p<0.05, **p<0.01. **C.** No difference in serum anti-*T. gondii* IgG level among individuals with DISC1 704 Ser/Ser, Ser/Cys and Cys/Cys. **D.** Higher *T. gondii* seropositivity (antibody levels ≥10 units/mL) rate in individuals with DISC1 607 Phe/Phe genotype compared to those with DISC1 607 Leu/Leu. Note that *T. gondii* IgG levels were well predicted by DISC1 Phe/Phe in multinomial logistic regression analysis with age, gender, race, and disease diagnosis as variables (p=0.010) (Table 1). **E.** No difference in *T. gondii* seropositivity (antibody levels ≥10 units/mL) among individuals with DISC1 704 Ser/Ser, Ser/Cys and Cys/Cys. **F.** *T. gondii* seropositivity rate in another cohort (n=652). No individuals with DISC1 607 Phe/Phe (n=11) showed increase in serum anti-*T. gondii* IgG levels.

Table 1:

Multinominal logistic regression analysis for Baltimore sample

DISC1 607 variant	Variable	Coefficiency	Standard Errors	z-score	P>|z|	[95% Confidence Interval]	
Leu/Leu		(base outcome)					
Leu/Phe	T. gondii IgG (units)	−0.0007434	0.0045311	−0.16	0.870	0.0096241	0.0081373
	Age	0.0011233	0.0083343	0.13	0.893	0.0152116	0.0174582
	Gender	0.1421207	0.1986375	0.72	0.474	0.2472018	0.5314431
	Race	−0.3015441	0.1943543	−1.55	0.121	0.6824716	0.0793834
	Diagnosis						
	Schizophrenia	0.2269713	0.2337690	0.97	0.332	0.2312076	0.6851503
	Bipolar Disorder	−0.0570001	0.2885047	−0.20	0.843	0.6224589	0.5084587
	Recent Onset Psychosis	0.0182294	0.5322108	0.03	0.973	1.024885	1.061343
	Cons	−1.0899000	0.5290639	−2.06	0.039	2.126847	−0.0529542
Phe/Phe	T. gondii IgG (units)	0.0169319	0.0065574	2.58	0.010	0.0040796	0.0297842
	Age	−0.0166023	0.0217365	−0.76	0.445	0.0592051	0.0260004
	Gender	0.5696324	0.5437728	1.05	0.295	0.4961427	1.635408
	Race	−0.5836219	0.5036877	−1.16	0.247	1.570832	0.4035879
	Diagnosis						
	Schizophrenia	0.6237704	0.5954931	1.05	0.295	0.5433746	1.790916
	Bipolar Disorder	−13.89627	658.48050	−0.02	0.983	1304.494	1276.702
	Recent Onset Psychosis	0.5512231	1.1538960	0.48	0.633	1.710371	2.812818
	Cons	−3.1783410	1.4435280	−2.20	0.028	6.007604	−0.3490788