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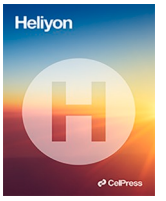

Research article

Toxoplasma gondii infection and brain inflammation: A two-sample mendelian randomization analysis

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ABSTRACT

Background: *Toxoplasma gondii* is an opportunistic parasitic protozoan that can cause highly fatal toxoplasmic encephalitis when the host immune system is compromised. However, the transition from chronic to acute infection remains poorly understood. In this study, we conducted a 180-day observation of tissue damage and inflammation in the brains of mice infected with *T. gondii*. Subsequently, we investigated the inflammatory factors that *T. gondii* infection may alter using two-sample Mendelian randomization (MR) analysis.

Methods: We first established a mouse model of *T. gondii* infection. Subsequently, the mice were euthanized, the brain tissue collected, and immunohistochemistry and hematoxylin and eosin staining performed to observe tissue damage and inflammatory conditions at various time points. Our study also included a published large-scale genome-wide association study meta-analysis that encompassed the circulating concentrations of 41 cytokines. This dataset included 8293 individuals from three independent population cohorts in Finland. Genetic association data for *T. gondii* were sourced from the Integrative Epidemiology Unit and European Bioinformatics Institute datasets, which included 5010 and 559 individuals of European ancestry, respectively. To assess the causal relationship between *T. gondii* infection and inflammatory biomarkers, we applied a two-sample MR.

Results: Inflammation and damage resulting from *T. gondii* infection varied among the distinct regions of the mouse brain. Based on the MR analysis results, three inflammatory biomarkers were chemically assigned to Chemokines and Others, including IP10 (interferon gamma inducible protein-10), MCP1 (monocyte chemoattractant protein-1), and TRAIL (TNF-related apoptosis-inducing ligand).

Conclusion: Our study commenced with the assessment of tissue damage and progression of inflammation in distinct regions of the mouse brain after *T. gondii* infection. Subsequently, using MR analysis, we detected potential alterations in inflammatory factors associated with this infection. These findings offer valuable insights into the mechanisms underlying toxoplasmic encephalitis and suggest directions for the prevention and treatment of *T. gondii* infections.

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1. Introduction

Toxoplasma gondii is an obligate intracellular parasite with a complex life cycle that affects almost all warm-blooded animals, including humans [1]. *T. gondii* is widely distributed worldwide because it can infect the nucleated cells of most mammals and humans. Approximately one-third of the global population is estimated to harbor latent toxoplasmosis [2]. The severity of *T. gondii* infection varies, and the normal host presents with only mild symptoms, such as swollen lymph nodes and fever. Nevertheless, individuals with compromised immune systems frequently experience systemic symptoms, including toxoplasmic encephalitis (TE), which is common among patients with AIDS, and ocular toxoplasmosis [3]. TE is one of the most severe clinical complications of toxoplasmosis and is recognized as the primary cause of death associated with this infection.

The overall inflammatory response mechanism in TE is highly complex because of the unique anatomical structure of the brain [4]. The blood-brain barrier (BBB) limits the passage of blood and lymphatic components, thus minimizing the potential for inflammation. Nevertheless, brain infections are not rare and necessitate a carefully orchestrated reaction that addresses the invading pathogens while preserving normal brain functionality. A proinflammatory response is necessary to prevent clinical illnesses caused by the reactivation of *T. gondii*. Patients with different immune functions exhibit varying symptoms of *T. gondii* infection. Individuals with healthy immune systems tend to display persistent low-level inflammation, whereas immunocompromised patients may develop TE [5]. To prevent the development of highly lethal TE, it is essential to promote the passage of T cells across the BBB during the inflammatory damage phase. This requirement involves a coordinated effort in antigen presentation, synthesis of proinflammatory mediators, and establishment of chemokine gradients [6]. The role of cytokines in the outcome of *T. gondii* infection is supported by studies involving the systemic administration of cytokines or antibodies targeting cytokines. These interventions lead to alterations in infection severity [7]. The significance of different cytokines in combating *T. gondii* infection has been gradually recognized, such as interferon (IFN)-γ [8–10] and tumor necrosis factor (TNF)-α [11]. However, the relationship between additional inflammatory biomarkers and *T. gondii* infection needs to be elucidated. A comprehensive understanding of these mechanisms will contribute to the diagnosis and treatment of *T. gondii* infections.

Mendelian randomization (MR) is an analytical approach that employs genetic variants as instrumental variables (IVs) to produce more dependable causal estimations of the enduring impact of risk factors on disease outcomes [12]. This strategy capitalizes on the inherent random assignment of alleles during conception [13]. Consequently, the magnitude of a particular exposure typically remains unaffected by other exposures and is not influenced by the presence of the disease [14]. Hence, MR can address the limitations associated with residual confounders and reverse causation in observational studies [15]. Here, we conducted continuous pathological examinations of mice infected with *T. gondii* and found that at different time points, inflammation appeared in various parts of the mouse brain. We investigated the initiation of the cytokine responses. Consequently, we conducted a two-sample MR analysis to examine alterations in all forty-one types of inflammatory factors following *T. gondii* infection. This study aimed to elucidate the mechanisms underlying the induction of brain inflammation by *T. gondii* infection.

2. Materials and methods

2.1. Cells and parasites

We isolated the *T. gondii* Wh6 (avirulent) strain belonging to the Chinese 1 genotype (ToxoDB#9) as previously described [16]. Cysts were retained in the brains of chronically infected mice for in vivo infection. The brains of infected mice were mechanically homogenized in 1 ml of sterile phosphate-buffered saline to collect the cysts. The cyst count was determined in a 10 μl brain suspension under a light microscope [17].

2.2. Mice and infection

The mice were categorized into eight groups, each containing 13 mice: control (non-infection group), 10 days, 30 days, 40 days, 90 days, 120 days, 160 days, and 180 days groups. Female BALB/c mice, aged 7 weeks, were intragastrically administered 30 Wh6 strain cysts. After reaching the designated infection time points, the eight groups of mice were euthanized to collect the brain tissue for subsequent experiments. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Anhui Medical University.

2.3. Hematoxylin-eosin (H&E) staining

After fixing with 4 % paraformaldehyde, brain tissues obtained from mice that had been infected with *T. gondii* were dried and embedded in paraffin. Each piece was a serial portion sliced into four. First, slides were dewaxed with xylene and treated with different concentrations of ethyl alcohol (100, 95, 80, and 70 %). The sections were stained with Harris H&E and the slides sealed with neutral balata. The sections were then examined microscopically.

2.4. Immunohistochemistry (IHC)

The dewaxed sections were placed in xylene I (60 min), 100 % alcohol I (5 min), 100 % alcohol II (5 min), 90 % alcohol (5 min), 80 % alcohol (5 min), 70 % alcohol (5 min), and pure water (3 min). The slides were then incubated with 3 % hydrogen peroxide (H_2O_2)

for 10 min. Antigen retrieval was then carried out by incubating mouse brain tissue slides in citrate buffer (11.48 g citric acid, 16.75 g trisodium citrate, and 100 ml ddH2O, pH = 6.0) for 30 min; the microwave oven was heated for 8 min, cooled for 2 min, boiled for 2 min, repaired for 5 min, and cooled naturally to room temperature or cooled completely. The slides were treated with *T. gondii* antibody (East Coast Bio, USA) overnight after pre-incubation with 0.3 % H_2O_2 and blocked with 5 % goat serum. DBA (3, 3′-diaminobenzidine tetrahydrochloride) staining was used to visualize the results. The slides were then re-stained with hematoxylin for 5 min. The immune complexes were observed under a microscope after cleaning, drying, becoming transparent, and fixing using a gel. Three replicates were performed for each experiment.

2.5. Quantitative real-time PCR assay

RNA was isolated from the brains of infected mice using the TRIzol reagent (Tiangen Biotech, China) according to the manufacturer's guidelines. The concentration of the obtained RNA was assessed using a NanoDrop 2000c (ThermoFisher, USA). Subsequently, 1 μg of total RNA was reverse-transcribed into cDNA using a reverse transcription kit (TaKaRa, Japan). Quantitative real-time PCR (qPCR) was conducted using a QuantStudio R6 Flex real-time PCR system (Applied Biosystems, USA) using SYBR™ Green qPCR Master Mix (ThermoFisher, USA). To determine gene expression levels, normalization to β-tubulin levels was performed utilizing the $2^{-\Delta\Delta Ct}$ method. The primers used in this study are listed in Supplementary Table 1.

2.6. MR study design

MR was based on three main assumptions (Fig. 1) [18]. Genetic variants chosen for instrumental purposes must exhibit a strong and reliable connection with the risk factor. Additionally, there should be no correlation between the genetic variants and confounding variables. Finally, the influence of genetic variants on the outcome risk should occur specifically through the risk factor itself, excluding alternative pathways.

2.7. Data sources

In this study, we used a published large-scale genome-wide association study (GWAS) meta-analysis that focused on the circulating concentrations of 41 cytokines. The analysis encompassed data from 8293 individuals in Finland drawn from three independent population cohorts: the Cardiovascular Risk in Young Finns Study (YFS), FINRISK1997, and FINRISK2002 [19]. We acquired genetic association data for *T. gondii* from both the Integrative Epidemiology Unit (IEU) and European Bioinformatics Institute (EBI) datasets, encompassing 5010 and 559 individuals of European ancestry, respectively. Further information regarding the genetic data of these datasets can be retrieved by searching for GWAS ID: ieu-b-4910 and ebi-a-GCST006349 on the website https://gwas.mrcieu.ac.uk/datasets/.

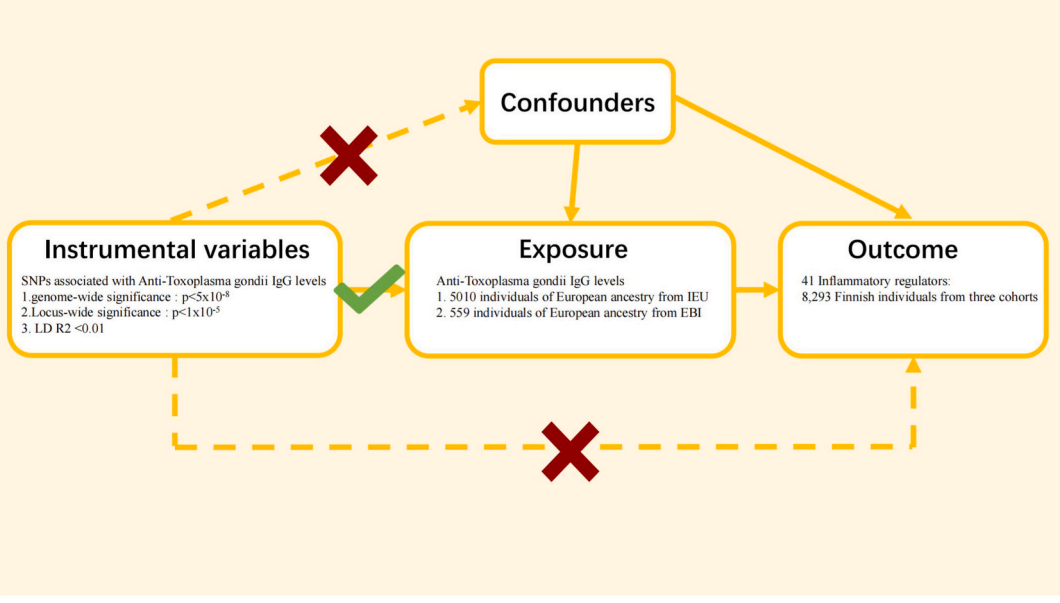

Fig. 1. Key assumptions underlying Mendelian randomization study design.

2.8. Selection of genetic IVs

In this study, we conducted a two-sample MR to evaluate the causal association between inflammatory factors and *T. gondii* infection. Several quality control steps were taken in our analysis to select qualified IVs closely associated with inflammatory factors. First, we identified the single nucleotide polymorphisms (SNPs) linked to the studied exposure using a genome-wide significance threshold ($p < 5 \times 10^{-8}$). These SNPs were treated as IVs. Second, we employed a linkage disequilibrium distance threshold of 500 kb and restricted the correlation coefficient (r^2) to less than 0.01 [20] to ensure independence. To maintain consistency in the allele associations between exposure and outcome, palindromic SNPs were excluded from the analysis. The strength of the association of each IV with exposure was quantified using the F-statistic [21]. The formula for F is $F = \frac{R^2(n-k-1)}{(1-R^2)k}$. where R^2 denotes the variance in exposure explained by IVs, n is the sample size, and k is the number of instrumental variables. Only IVs with F statistics greater than 10 were retained [22].

2.9. Statistical analyses

To assess the potential causal influence of exposure on the outcome, we conducted MR analyses using five different methods: inverse variance weighting (IVW) [23], weighted median [24], MR-Egger [25], weighted mode, and MR pleiotropic residuals and outliers (MR-PRESSO) [26]. Heterogeneity in causal estimates among IVs indicates potential deviations from the assumptions of the MR analysis. Cochran's Q test was used to identify such variations, using both fixed-effects IVW estimates and MR-Egger regression. Heterogeneity was considered significant if Cochran's Q test yielded a p-value of <0.05. To assess the potential pleiotropic effects of IVs, we used the MR-Egger regression, wherein the intercept term indicates directional horizontal pleiotropy in the causal estimates. Moreover, a leave-one-out analysis was performed, systematically excluding each SNP to detect possible outlying instrumental variables. The results are presented as odds ratios (ORs) and corresponding 95 % confidence intervals (CIs). Statistical significance was determined as a two-sided p-value of <0.05. The TwoSampleMR and MR-PRESSO packages were used in R version 4.2.2.

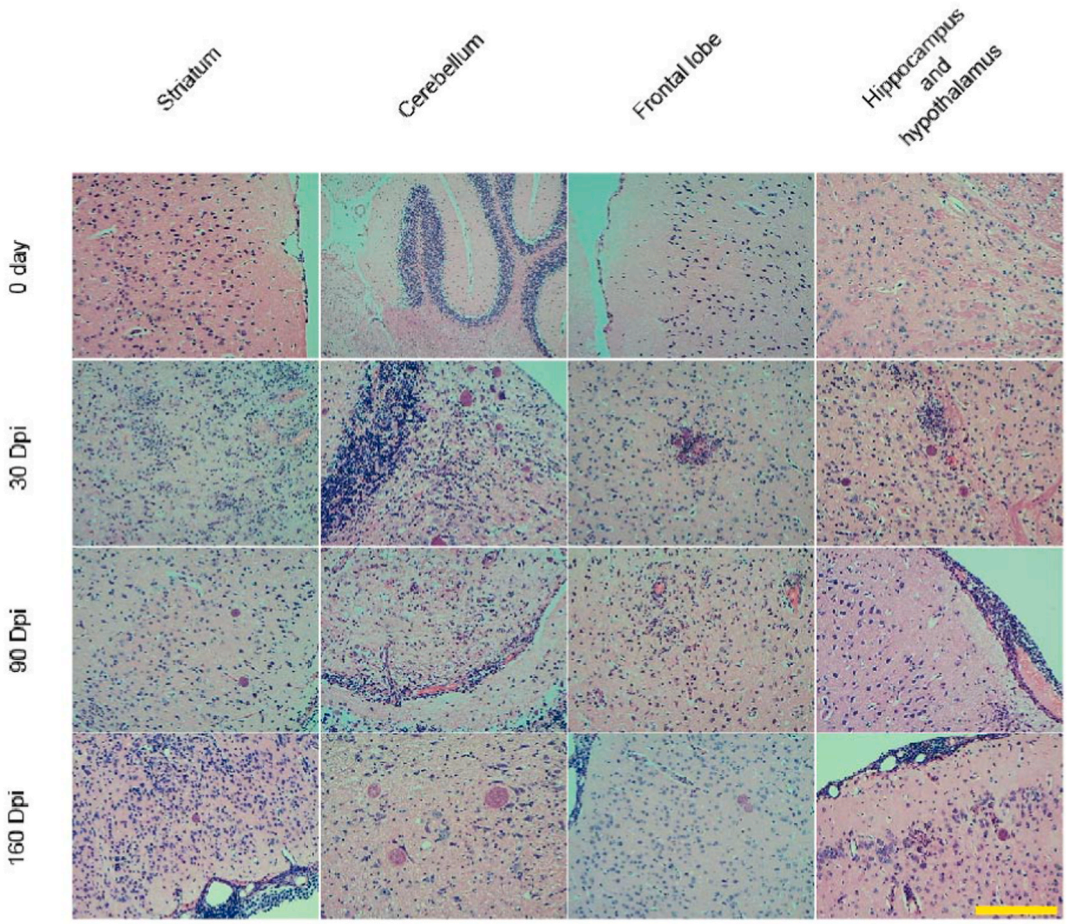

Fig. 2. Hematoxylin and eosin staining to detect the brain tissues (striatum, cerebellum, frontal lobe, and hippocampus and hypothalamus) of infected mice on day 0, and 30, 90, and 160 days post infection. Scale bar, 50 μm.

3. Results

3.1. The stage-by-stage pathological diagnosis of T. gondii infected mouse brain tissues

Once the mouse model was established, we performed a follow-up pathological examination of different parts of the mouse brain (striatum, cerebellum, frontal lobe, and hippocampus and hypothalamus). *T. gondii* cysts are distributed in all parts of the brain; however, there are still many commonalities throughout the process of infection. The results showed the presence of tissue cysts in the brains of mice 30 days post infection (dpi). As shown in Figs. 2 and 3, during 0–30 dpi, inflammatory cells (lymphocytes, plasma cells, macrophages, and neutrophils) are present in the brain and the brain shows edema. Vasodilation and lymphocyte infiltration were also observed. Inflammatory cells exudate from the surface of the brain and meningeal epithelial cell proliferation occurs. Between 90 and 160 dpi, no obvious abnormalities were observed in brain tissue structure. The number of pseudocysts of *T. gondii* in the brain tissue was lower than before. The infiltration of inflammatory cells was also lower. A few abscesses formed, and vasculitis was observed. More inflammatory cells exuded from the surface of the brain tissue, and the edema of the cells was relieved. Throughout the 180-day experiment, we collected data at eight time points (0, 10, 30, 40, 90, 120, 160, and 180 dpi), with the aforementioned four representing the time points at which significant changes were observed. In the Supplementary Materials, we opted for another format to present the changes in the brains of infected mice. The brains were segregated into four distinct regions (Fig. S1: frontal lobe; Fig. S2: cerebellum; Fig. S3: striatum; Fig. S4: hippocampus and hypothalamus), and pathological examinations were conducted at eight

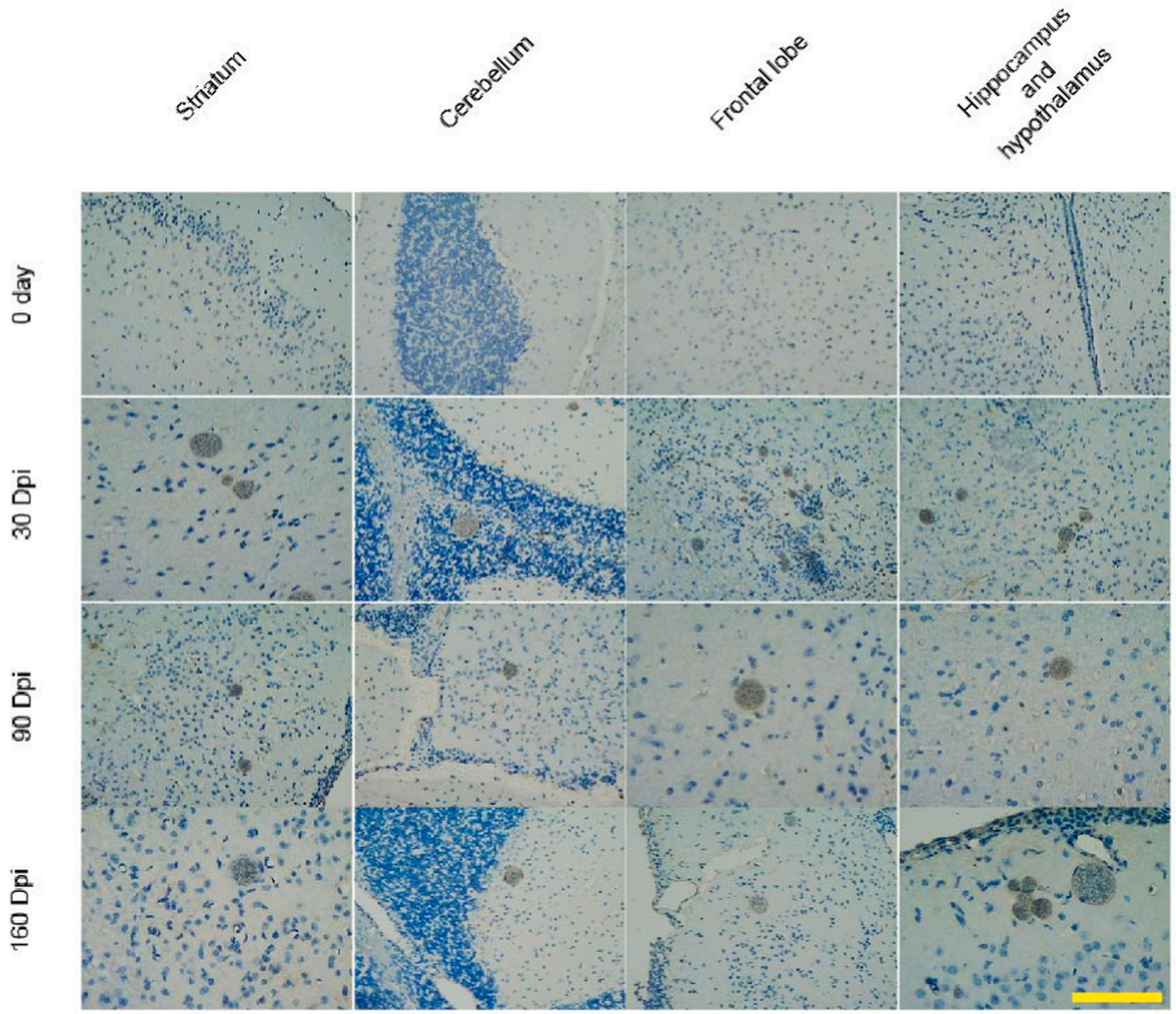

Fig. 3. The different regions of the infected mouse brain (striatum, cerebellum, frontal lobe, and hippocampus and hypothalamus) observed via immunohistochemistry on day 0, and 30, 90, and 160 days post infection to confirm the presence of *Toxoplasma* cysts, indicating the infection status. Scale bar, 50 μm.

different time points to illustrate the progressive changes in inflammation in each region throughout the course of infection. We have included H&E and IHC images corresponding to the time points in the Supplementary Materials, along with descriptions of the respective pathological conditions. In summary, *T. gondii* infection led to severe inflammation and tissue damage in various parts of the mouse brain.

3.2. Selection of IVs related to T. gondii infection

After stringent quality control of IVs, we identified 20 significant SNPs as IVs for *T. gondii* infection, with the significance threshold set at $p < 1 \times 10^{-5}$. Notably, all SNPs associated with inflammatory biomarkers exhibited F statistics exceeding 10, indicating robust IV strength. Thus, compelling IVs were obtained.

3.3. Results of MR analysis

Specific information on the 41 inflammatory factors is presented in Table 1. Fig. 4A depicts the association between the 41 inflammatory biomarkers and *T. gondii* infection. IVW analysis was conducted to identify the three inflammatory biomarkers that may have causal effects on *T. gondii* infection. As shown in Fig. 4B, the three inflammatory biomarkers were chemically assigned to Chemokines and Others, including interferon gamma inducible protein-10 (IP10) (Beta = −0.15; Se = 0.06; OR = 0.86; 95 % CI, 0.77–0.96; $p = 8.75 \times 10^{-3}$) and monocyte chemoattractant protein-1 (MCP1) (Beta = −0.07; Se = 0.04; OR = 0.93; 95 % CI, 0.87–1.00; $p = 4.47 \times 10^{-2}$), and TNF-related apoptosis-inducing ligand (TRAIL) (Beta = −0.08; Se = 0.04; OR = 0.93; 95 % CI, 0.86–1.00; $p = 3.71 \times 10^{-2}$). In summary, the IVW estimates were statistically significant ($p < 0.05$), and the directions and

Table 1
The sample size for each cytokine analyzed in this study acquired from the GWAS.

Cytokines	Abbreviation	Sample size	Number	Relationship
Cutaneous T-cell attracting (CCL27)	CTACK	3631	GCST004420	No significant
Beta nerve growth factor	βNGF	3531	GCST004421	No significant
Vascular endothelial growth factor	VEGF	7118	GCST004422	No significant
Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	MIF	3494	GCST004423	No significant
TNF-related apoptosis inducing ligand	TRAIL	8186	GCST004424	Significant
Tumor necrosis factor-beta	TNFβ	1559	GCST004425	No significant
Tumor necrosis factor-alpha	TNFα	3454	GCST004426	No significant
Stromal cell-derived factor-1 alpha (CXCL12)	SDF1α	5998	GCST004427	No significant
Stem cell growth factor beta	SCGFβ	3682	GCST004428	No significant
Stem cell factor	SCF	8290	GCST004429	No significant
Interleukin-16	IL-16	3483	GCST004430	No significant
Regulated on Activation, Normal T Cell Expressed and Secreted (CCL5)	RANTES	3421	GCST004431	No significant
Platelet derived growth factor BB	PDGFbb	8293	GCST004432	No significant
Macrophage inflammatory protein-1β (CCL4)	MIP1β	8243	GCST004433	No significant
Macrophage inflammatory protein-1α (CCL3)	MIP1α	3522	GCST004434	No significant
Monokine induced by interferon-gamma (CXCL9)	MIG	3685	GCST004435	No significant
Macrophage colony-stimulating factor	MCSF	840	GCST004436	No significant
Monocyte specific chemokine 3 (CCL7)	MCP3	843	GCST004437	No significant
Monocyte chemotactic protein-1 (CCL2)	MCP1	8293	GCST004438	Significant
Interleukin-12p70	IL-12p70	8270	GCST004439	No significant
Interferon gamma-induced protein 10 (CXCL10)	IP10	3685	GCST004440	Significant
Interleukin-18	IL-18	3636	GCST004441	No significant
Interleukin-17	IL-17	7760	GCST004442	No significant
Interleukin-13	IL-13	3557	GCST004443	No significant
Interleukin-10	IL-10	7681	GCST004444	No significant
Interleukin-8 (CXCL8)	IL-8	3526	GCST004445	No significant
Interleukin-6	IL-6	8189	GCST004446	No significant
Interleukin-1 receptor antagonist	IL1ra	3638	GCST004447	No significant
Interleukin-1-beta	IL-1β	3309	GCST004448	No significant
Hepatocyte growth factor	HGF	8292	GCST004449	No significant
Interleukin-9	IL-9	3634	GCST004450	No significant
Interleukin-7	IL-7	3409	GCST004451	No significant
Interleukin-5	IL-5	3364	GCST004452	No significant
Interleukin-4	IL-4	8124	GCST004453	No significant
Interleukin-2 receptor, alpha subunit	IL2rα	3677	GCST004454	No significant
Interleukin-2	IL-2	3475	GCST004455	No significant
Interferon-gamma	IFN-γ	7701	GCST004456	No significant
Growth regulated oncogene-α (CXCL1)	GROα	3505	GCST004457	No significant
Granulocyte colony-stimulating factor	GCSF	7904	GCST004458	No significant
Basic fibroblast growth factor	bFGF	7565	GCST004459	No significant
Eotaxin (CCL11)	Eotaxin	8153	GCST004460	No significant

Sample size: The number of individuals included in the GWAS study for each of the 41 inflammatory factors; Relationship: The relationship between *Toxoplasma gondii* infection and inflammatory factors, Significant: $p < 0.05$.

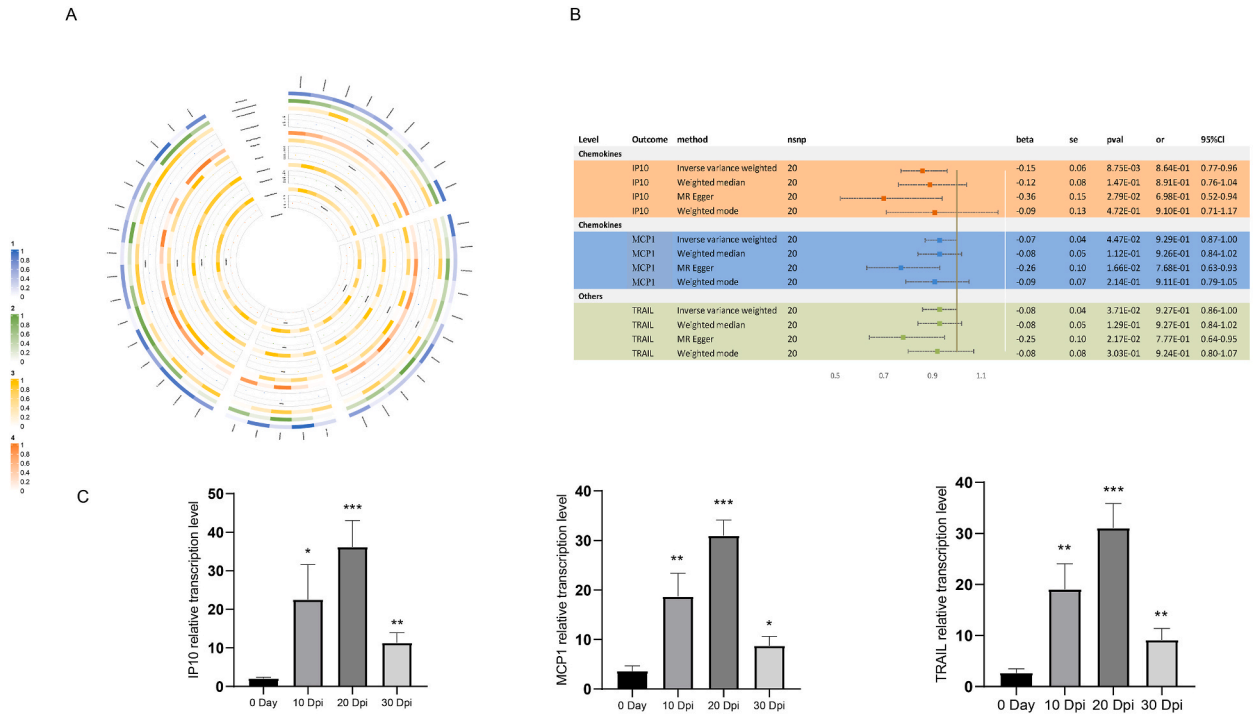

Fig. 4. Causal analysis of *Toxoplasma gondii* infection and inflammatory biomarkers. (**A**) Results of Mendelian randomization (MR) analysis and sensitivity analysis between *T. gondii* infection and inflammatory biomarkers; (**B**) MR results of *T. gondii* infection with a causal relationship to inflammatory biomarkers. (**C**) Relative mRNA expression level of three inflammatory factors in infected mouse brain tissues at different time points.

magnitudes of the IVW, MR-Egger, weighted median, and weighted mode estimates were consistent. Additional information is detailed in Table 2.

To further validate the reliability of the MR analysis results, we used qPCR to detect the expression of the three inflammatory factors in the brain tissues of mice during the acute infection period. As shown in Fig. 4C, following *T. gondii* infection, the transcript levels of IP10, MCP1, and TRAIL increased. While statistically higher than that in the control group, there was substantial variation between individuals, leading to significant data dispersion.

3.4. Sensitivity analysis

To ensure the accuracy of our results, we validated the impact of accurate MR results on sensitivity analysis. There was no heterogeneity detected by Cochran's Q test (IP10-IVW, Q = 19.853, p = 0.403; IP10-MR-Egger, Q = 17.531, p = 0.487; MCP1-IVW, Q = 16.903, p = 0.596; MCP1-MR-Egger, Q = 12.703, p = 0.809; TRAIL-IVW, Q = 16.368, p = 0.633; TRAIL-MR-Egger, Q = 12.819, p = 0.802). All Egger regression tests yielded negative results for IP10 (intercept = 0.032, p = 0.145), MCP1 (intercept = 0.029, p = 0.055), and TRAIL (intercept = 0.026, p = 0.076). The MR-PRESSO global test also indicated the absence of horizontal pleiotropy effects for

Table 2
MR results between Toxoplasma gondii infection and inflammatory biomarkers.

Outcome	Exposure	Method	nsnp	Beta	SE	Pval	OR	OR_lci95	OR_uci95
IP10	ieu-b-4910	Inverse variance weighted	20	−0.15	0.06	0.01	0.86	0.77	0.96
IP10	ieu-b-4910	Weighted median	20	−0.12	0.08	0.15	0.89	0.76	1.04
IP10	ieu-b-4910	MR Egger	20	−0.36	0.15	0.03	0.70	0.52	0.94
IP10	ieu-b-4910	Weighted mode	20	−0.09	0.13	0.47	0.91	0.71	1.17
MCP1	ieu-b-4910	Inverse variance weighted	20	−0.07	0.04	0.04	0.93	0.87	1.00
MCP1	ieu-b-4910	Weighted median	20	−0.08	0.05	0.11	0.93	0.84	1.02
MCP1	ieu-b-4910	MR Egger	20	−0.26	0.10	0.02	0.77	0.63	0.93
MCP1	ieu-b-4910	Weighted mode	20	−0.09	0.07	0.21	0.91	0.79	1.05
TRAIL	ieu-b-4910	Inverse variance weighted	20	−0.08	0.04	0.04	0.93	0.86	1.00
TRAIL	ieu-b-4910	Weighted median	20	−0.08	0.05	0.13	0.93	0.84	1.02
TRAIL	ieu-b-4910	MR Egger	20	−0.25	0.10	0.02	0.78	0.64	0.95
TRAIL	ieu-b-4910	Weighted mode	20	−0.08	0.08	0.30	0.92	0.80	1.07

nsnp: Number of SNP; Pvaul: P value.

the biomarkers IP10 (p = 0.385), MCP1 (p = 0.640), and TRAIL (p = 0.658). This confirms that our MR results remained unaffected by horizontal pleiotropy. Additional information is detailed in Table 3. Meta-analyses, funnel plots, leave-one-out plots, scatter plots, and forest plots were used to further verify the reliability of the results regarding the relationship between *T. gondii* infection and inflammatory factors (Fig. S5: IP10; Fig. S6: MCP1; Fig. S7: TRAIL).

3.5. Replication and meta-analysis

To bolster the credibility of our estimates, we replicated the MR analysis using different GWAS datasets (ieu-b-4910 and ebi-a-GCST006349) for *T. gondii* infection. As anticipated, we observed similar trends in inflammatory biomarkers in this alternative GWAS dataset for *T. gondii* infection. However, the results were not significant, mainly because of the substantial disparity in sample sizes. The sample size for ieu-b-4910 was 5010 while the sample size of ebi-a-GCST006349 was 559. In detail, IP10 (OR 0.90, 95 % CI: 0.82–0.98, p = 0.02); MCP1 (OR 0.92, 95 % CI: 0.86–0.97, p = 0.0038); TRAIL (OR 0.93, 95 % CI: 0.88–0.99, p = 0.02). This study demonstrates a high level of credibility.

4. Discussion

In the present study, we observed cysts at various time points and locations in the brains of mice infected with *T. gondii*. Furthermore, distinct regions of the mouse brain exhibited varying degrees of inflammation severity. We performed a two-sample MR analysis of the inflammatory factors that can be influenced by *T. gondii* infection. Employing the largest publicly accessible GWAS dataset, we observed that *T. gondii* infection downregulated the expression of MCP1, IP10, and TRAIL. This implies that the absence of these three inflammatory factors might explain why immunocompromised patients do not exhibit sustained low-level inflammation after *T. gondii* infection, leading to the occurrence of TE.

The incidence of human *T. gondii* infections is high [27–29]. Individuals with healthy immune systems typically eliminate most parasites during the acute stages of infection. Parasites that survive the transition to the bradyzoite state are characterized by slow growth, which leads to the formation of tissue cysts. These cysts are particularly prevalent in tissues with limited immune surveillance such as the brain, eyes, heart, and skeletal muscles [30]. Throughout chronic infections, the central nervous system (CNS) has the highest parasite density per gram of tissue. The potential impact of neural infection on host behavior and internal equilibrium has sparked significant interest in understanding the biology of *T. gondii* infection within the brain. Therefore, this study aimed to provide a comprehensive perspective on cerebral *T. gondii* infections. We focused on the striatum, cerebellum, frontal lobe, hippocampus, and the hypothalamus. Using IHC and H&E staining, we observed the timing of cyst appearance in these distinct regions along with the corresponding inflammatory conditions. Integrating tissue structural changes will contribute to a deeper understanding of the life stages of *T. gondii* in the brain. The immune reaction against *T. gondii* remains active during chronic infection, as indicated by increased *T. gondii*–specific IgG and IFN-γ levels in the blood, both of which play a crucial role in limiting parasite growth [31]. In cases such as chemotherapy, organ transplantation, or AIDS, where the immune system is suppressed, *T. gondii* can transition back to tachyzoite replication [2,32]. When this phenomenon occurs in the CNS, it manifests as the highly lethal TE. However, the specific stages and mechanisms underlying this process remain unclear. Numerous unanswered questions remain, including the mechanism that how chronic *T. gondii* infection ensures the balance of inflammation and the process of reactivation in the brain once the balance is disrupted. Furthermore, the emergence of brain inflammation and the factors that initiate it are subjects of inquiry. Lastly, alterations in various cerebral cytokines throughout the different stages of infection and their collaborative or antagonistic roles in infection progression warrant investigation. In our subsequent MR analysis, we discuss and propose the potential answers.

First, we revisited the question: What will be changed in body when chronic *T. gondii* infection occurred, and in what aspects do these changes differentiate the host from normal individuals. *T. gondii* chronic infection impacts the levels of neurotrophic factors and inflammatory mediators in different brain regions, leading to behavioral alterations in mice [33]. In conjunction with our research, in the common 41 inflammatory factors, *T. gondii* infection leads to a decrease in the expression levels of IP10, MCP1, and TRAIL. MCP1, also known as CCL2, which experiences a transient yet substantial increase during brain injury, plays a role in the chemotaxis of microglia and monocytes [34,35]. Brain inflammation constitutes a distinct form of cerebral damage. This appears to be independent of elevated MCP1 levels. Differences in MCP1 expression have been noted across various types of encephalitis [36–38]. The CCL2/CCR2 chemokine axis is a conserved mechanism of monocyte recruitment in mice and humans [39]. Examination of brain sections from mice and a limited set of samples from healthy human brains suggested that the majority of intracellular cysts are not

Table 3
Sensitivity analysis between Toxoplasma gondii infection and inflammatory biomarkers.

Category	Outcome	Method	nsnp	Q	P	Intercept	P
Chemokines	IP10	MR-Egger	20	17.53	0.49	0.03	0.14
Chemokines	IP10	Inverse variance weighted	20	19.85	0.40		
Chemokines	MCP1	MR-Egger	20	12.70	0.81	0.03	0.06
Chemokines	MCP1	Inverse variance weighted	20	16.90	0.60		
Others	TRAIL	MR-Egger	20	12.82	0.80	0.03	0.08
Others	TRAIL	Inverse variance weighted	20	16.37	0.63		

nsnp: Number of SNP.

associated with immune infiltration [40]. Nevertheless, in the same brain section, one can observe inflammatory areas harboring parasites or parasite remnants, along with activated microglia, macrophages, and T cells [41]. We observed that *T. gondii* infection did not lead to an increase in MCP1 expression; in fact, it suppressed its expression. This may be related to the database used in our MR analysis, where the infection status and timing of the population in the database are unknown. It is possible that during the acute phase of *T. gondii* infection, MCP1 may transiently increase or not increase at all. However, when entering the chronic infection phase, both the results of the MR analysis and our experiments on a mouse chronic *T. gondii* infection model indicate a decrease in MCP1 expression. This reveals that *T. gondii* infection may, by reducing MCP1 expression, hinder the proper elimination of the parasite by various immune cells, including microglial cells and macrophages. Once the host's immunity declines, the reactivation of *T. gondii* infection may become both easier and more intense.

The phenomenon of *T. gondii* secreting regulatory metabolites to modulate immune signal transduction in host cells has been increasingly recognized [42]. IP10 and TRAIL are two other inflammatory biomarkers that we identified. IP10 is a chemokine produced by cells that is typically released during viral infections and cellular stress. The negative regulatory effects of *T. gondii* may be similar to those of MCP1. TRAIL is associated with apoptosis (programmed cell death) and can induce apoptosis in certain types of cancer cells. During the inflammatory process, the activity of TRAIL might be regulated, thereby influencing both cellular apoptosis and immune responses. In the context of TE, TRAIL may be involved in the regulation of immune cells and immune activity at the site of infection. The extensive evolutionary history shared by *T. gondii* and mammalian hosts becomes apparent when the mechanisms employed by infected cells to identify and eliminate parasites are examined [43]. Autonomous immunity against *T. gondii* is complex. Based on our MR analysis, this might be related to its interaction with TRAIL. While the inflammatory response to protozoa is less explored than that to bacterial and viral pathogens, existing knowledge about *T. gondii* recognition indicates significant distinctions [44,45]. In contrast to cell death caused by other common inflammatory factors, pyroptotic host cell death has not been detected in murine or human cells [45–47]. The dynamic changes and inflammation balance in the host after *T. gondii* infection are highly complex. Alterations in different inflammatory factors may set the stage for the future reactivation of *T. gondii* infection in the body. Changes in inflammatory factors will affect the overall immune regulation of the body, such as immune cell surveillance, immune cell defense, and cell apoptosis, among others. In the future, by enhancing the expression of MCP-1, IP10, and TRAIL, it may be possible to avert more severe symptoms of *T. gondii* infection, such as preventing the occurrence of TE. This has significant implications for both the long-term treatment and diagnosis of *Toxoplasma* infections.

The highlight of this study is that we monitored the complete infection process of *T. gondii* in the mouse brain and provided a detailed pathological report. We also investigated the infection of *T. gondii* and the potentially altered inflammatory factors based on the largest GWAS meta-analysis. This has guiding significance for the future diagnosis of the severity of toxoplasmosis and prevention of further deterioration in *T. gondii* infection.

However, this study had several limitations. First, we limited our study cohort to individuals of European ancestry to mitigate potential biases stemming from population stratification, which may have resulted in reduced generalizability to individuals with different genetic backgrounds. Second, the constraints inherent in the MR analysis hinder the comprehensive exploration of the second and third assumptions, potentially introducing bias. For example, *T. gondii* often remains latent in the human body. Sudden changes may be associated with other diseases, and immune dysregulation caused by these diseases can lead to alterations in the levels of inflammatory factors. We aimed to address the limitations of the aforementioned MR analysis to some extent through experimental modifications. However, our experiments were limited to the validation of phenotypic aspects. Our findings offer valuable insights into the mechanisms underlying toxoplasmic encephalitis and suggest directions for the prevention and treatment of *T. gondii* infections. However, further mechanistic studies are required to fully elucidate these mechanisms.

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Ethics statement

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Anhui Medical University. And our ethical approval number is LISC20200873.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Yong Yao: Writing – original draft, Visualization, Validation, Investigation. **Taiyu Shi:** Methodology, Investigation, Formal analysis, Data curation. **Panyin Shu:** Resources, Methodology. **Yixin Zhang:** Software, Resources. **Hao Gu:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition.

Declaration of Competing interest

There is no declaration of Interest Statement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24228.

References

[1] S. Ji-Long, Y. Li, [Prevalence and Fundamental researches of prevention and treatment of toxoplasmosis in China: an overview], Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 31 (2019) 71–76, https://doi.org/10.16250/j.32.1374.2019012.

[2] J.G. Montoya, O. Liesenfeld Toxoplasmosis, Lancet 363 (2004) 1965–1976, https://doi.org/10.1016/s0140-6736(04)16412-x.

[3] S.A. Elmore, J.L. Jones, P.A. Conrad, S. Patton, D.S. Lindsay, J.P. Dubey Toxoplasma Gondii, Epidemiology, feline clinical aspects, and prevention, Trends Parasitol. 26 (2010) 190–196, https://doi.org/10.1016/j.pt.2010.01.009.

[4] I. Bechmann, I. Galea, V.H. Perry, What is the blood-brain barrier (not)? Trends Immunol. 28 (2007) 5–11, https://doi.org/10.1016/j.it.2006.11.007.

[5] E.A. Vizcarra, A. Ulu, T.A. Landrith, X. Qiu, A. Godzik, E.H. Wilson, Group 1 metabotropic glutamate receptor expression defines a T cell memory population during chronic Toxoplasma infection that enhances ifn-gamma and perforin production in the cns, Brain Behav. Immun. 114 (2023) 131–143, https://doi.org/10.1016/j.bbi.2023.08.015.

[6] G. Constantin, Chemokine signaling and integrin activation in lymphocyte migration into the inflamed brain, J. Neuroimmunol. 198 (2008) 20–26, https://doi.org/10.1016/j.jneuroim.2008.04.023.

[7] C.A. Hunter, J.S. Remington Immunopathogenesis of Toxoplasmic Encephalitis, J. Infect. Dis. 170 (1994) 1057–1067, https://doi.org/10.1093/infdis/170.5.1057.

[8] R. Gazzinelli, Y. Xu, S. Hieny, A. Cheever, A. Sher, Simultaneous depletion of Cd4+ and Cd8+ T lymphocytes is required to reactivate chronic infection with Toxoplasma gondii, J. Immunol. 149 (1992) 175–180.

[9] Y. Suzuki, F.K. Conley, J.S. Remington, Treatment of toxoplasmic encephalitis in mice with recombinant gamma interferon, Infect. Immun. 58 (1990) 3050–3055, https://doi.org/10.1128/iai.58.9.3050-3055.1990.

[10] Y. Suzuki, M.A. Orellana, R.D. Schreiber, J.S. Remington Interferon-Gamma, The major mediator of resistance against Toxoplasma gondii, Science 240 (1988) 516–518, https://doi.org/10.1126/science.3128869.

[11] L.L. Johnson A, Protective role for endogenous tumor necrosis factor in Toxoplasma gondii infection, Infect. Immun. 60 (1992) 1979–1983, https://doi.org/10.1128/iai.60.5.1979-1983.1992.

[12] T. Robinson, R.M. Martin, J. Yarmolinsky, Mendelian randomisation analysis of circulating adipokines and C-reactive protein on breast cancer risk, Int. J. Cancer 147 (2020) 1597–1603, https://doi.org/10.1002/ijc.32947.

[13] M. Nazarzadeh, A.C. Pinho-Gomes, Z. Bidel, A. Dehghan, D. Canoy, A. Hassaine, et al., Plasma lipids and risk of aortic valve stenosis: a mendelian randomization study, Eur. Heart J. 41 (2020) 3913–3920, https://doi.org/10.1093/eurheartj/ehaa070.

[14] S. van Oort, J.W.J. Beulens, A.J. van Ballegooijen, M.L. Handoko, S.C. Larsson, Modifiable lifestyle factors and heart failure: a mendelian randomization study, Am. Heart J. 227 (2020) 64–73, https://doi.org/10.1016/j.ahj.2020.06.007.

[15] X. Li, S. Peng, B. Guan, S. Chen, G. Zhou, Y. Wei, et al., Genetically determined inflammatory biomarkers and the risk of heart failure: a mendelian randomization study, Front Cardiovasc Med 8 (2021) 734400, https://doi.org/10.3389/fcvm.2021.734400.

[16] Z.W. Chen, J.M. Gao, X.X. Huo, L. Wang, L. Yu, F. Halm-Lai, et al., Genotyping of Toxoplasma gondii isolates from cats in different geographic regions of China, Vet. Parasitol. 183 (2011) 166–170, https://doi.org/10.1016/j.vetpar.2011.06.013.

[17] A. Biswas, T. French, H.P. Düsedau, N. Mueller, M. Riek-Burchardt, A. Dudeck, et al., Behavior of neutrophil granulocytes during Toxoplasma gondii infection in the central nervous system, Front. Cell. Infect. Microbiol. 7 (2017) 259, https://doi.org/10.3389/fcimb.2017.00259.

[18] S. Burgess, R.A. Scott, N.J. Timpson, G. Davey Smith, S.G. Thompson, Using published data in mendelian randomization: a blueprint for efficient identification of causal risk factors, Eur. J. Epidemiol. 30 (2015) 543–552, https://doi.org/10.1007/s10654-015-0011-z.

[19] A.V. Ahola-Olli, P. Würtz, A.S. Havulinna, K. Aalto, N. Pitkänen, T. Lehtimäki, et al., Genome-wide association study identifies 27 loci influencing concentrations of circulating cytokines and growth factors, Am. J. Hum. Genet. 100 (2017) 40–50, https://doi.org/10.1016/j.ajhg.2016.11.007.

[20] A. Auton, L.D. Brooks, R.M. Durbin, E.P. Garrison, H.M. Kang, J.O. Korbel, et al., A global reference for human genetic variation, Nature 526 (2015) 68–74, https://doi.org/10.1038/nature15393.

[21] B.L. Pierce, H. Ahsan, T. J. Vanderweele power and instrument strength requirements for mendelian randomization studies using multiple genetic variants, Int. J. Epidemiol. 40 (2011) 740–752, https://doi.org/10.1093/ije/dyq151.

[22] M.J. Brion, K. Shakhbazov, P.M. Visscher, Calculating statistical power in mendelian randomization studies, Int. J. Epidemiol. 42 (2013) 1497–1501, https://doi.org/10.1093/ije/dyt179.

[23] S. Burgess, A. Butterworth, S. G. Thompson mendelian randomization analysis with multiple genetic variants using summarized data, Genet. Epidemiol. 37 (2013) 658–665, https://doi.org/10.1002/gepi.21758.

[24] J. Bowden, G. Davey Smith, P.C. Haycock, S. Burgess, Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator, Genet. Epidemiol. 40 (2016) 304–314, https://doi.org/10.1002/gepi.21965.

[25] J. Bowden, G. Davey Smith, S. Burgess, Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression, Int. J. Epidemiol. 44 (2015) 512–525, https://doi.org/10.1093/ije/dyv080.

[26] M. Verbanck, C.Y. Chen, B. Neale, R. Do, Detection of widespread horizontal pleiotropy in causal relationships inferred from mendelian randomization between complex traits and diseases, Nat. Genet. 50 (2018) 693–698, https://doi.org/10.1038/s41588-018-0099-7.

[27] W.A. Cañón-Franco, N. López-Orozco, J.E. Gómez-Marín, J.P. Dubey, An overview of seventy years of research (1944-2014) on toxoplasmosis in Colombia, South America, Parasit Vectors 7 (2014) 427, https://doi.org/10.1186/1756-3305-7-427.

[28] J.P. Dubey, E.G. Lago, S.M. Gennari, C. Su, J.L. Jones Toxoplasmosis, Humans and animals in Brazil: high prevalence, high burden of disease, and Epidemiology, Parasitology 139 (2012) 1375–1424, https://doi.org/10.1017/s0031182012000765.

[29] N. Guigue, L. Léon, S. Hamane, M. Gits-Muselli, Y. Le Strat, A. Alanio, et al., Continuous decline of Toxoplasma gondii seroprevalence in hospital: a 1997-2014 longitudinal study in Paris, France, Front. Microbiol. 9 (2018) 2369, https://doi.org/10.3389/fmicb.2018.02369.

[30] J.P. Dubey, D.S. Lindsay, C.A. Speer, Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts, Clin. Microbiol. Rev. 11 (1998) 267–299, https://doi.org/10.1128/cmr.11.2.267.

[31] C.R. Sturge, F. Yarovinsky, Complex immune cell interplay in the gamma interferon response during Toxoplasma gondii infection, Infect. Immun. 82 (2014) 3090–3097, https://doi.org/10.1128/iai.01722-14.

[32] B.J. Luft, J.S. Remington, Toxoplasmic encephalitis in aids, Clin. Infect. Dis. 15 (1992) 211–222, https://doi.org/10.1093/clinids/15.2.211.

[33] R.M.M. Brito, M.C.M. da Silva, F. Vieira-Santos, C. de Almeida Lopes, J.L.N. Souza, A.L. Bastilho, et al., Chronic infection by atypical Toxoplasma gondii strain induces disturbance in microglia population and altered behaviour in mice, Brain Behav Immun Health 30 (2023) 100652, https://doi.org/10.1016/j.bbih.2023.100652.
[34] J.J. Sheehan, C. Zhou, I. Gravanis, A.D. Rogove, Y.P. Wu, D.F. Bogenhagen, et al., Proteolytic activation of monocyte chemoattractant protein-1 by plasmin underlies excitotoxic neurodegeneration in mice, J. Neurosci. 27 (2007) 1738–1745, https://doi.org/10.1523/jneurosci.4987-06.2007.
[35] Y. Yao, S.E. Tsirka, The C terminus of mouse monocyte chemoattractant protein 1 (Mcp1) mediates Mcp1 dimerization while blocking its chemotactic potency, J. Biol. Chem. 285 (2010) 31509–31516, https://doi.org/10.1074/jbc.M110.124891.
[36] M. Guasp, G. Muñoz-Sánchez, E. Martínez-Hernández, D. Santana, Á. Carbayo, L. Naranjo, et al., Csf biomarkers in covid-19 associated encephalopathy and encephalitis predict long-term outcome, Front. Immunol. 13 (2022) 866153, https://doi.org/10.3389/fimmu.2022.866153.
[37] J. Kalita, R. Srivastava, M.K. Mishra, A. Basu, U.K. Misra, Cytokines and chemokines in viral encephalitis: a clinicoradiological correlation, Neurosci. Lett. 473 (2010) 48–51, https://doi.org/10.1016/j.neulet.2010.02.017.
[38] Y. Yao, J. Gu, M. Li, G. Li, L. Zhao, J. Ai, Mri changes and expressions of neuron-specific enolase and monocyte chemoattractant protein-1 in cerebrospinal fluid in patients with severe herpes simplex virus encephalitis, Cell. Mol. Biol. 68 (2022) 78–82, https://doi.org/10.14715/cmb/2022.68.11.13.
[39] A. Safronova, A. Araujo, E.T. Camanzo, T.J. Moon, M.R. Elliott, D.P. Beiting, et al., Alarmin S100a11 initiates a chemokine response to the human pathogen Toxoplasma gondii, Nat. Immunol. 20 (2019) 64–72, https://doi.org/10.1038/s41590-018-0250-8.
[40] L. Pusch, B. Romeike, M. Deckert, C. Mawrin, Persistent Toxoplasma bradyzoite cysts in the brain: incidental finding in an immunocompetent patient without evidence of a toxoplasmosis, Clin. Neuropathol. 28 (2009) 210–212, https://doi.org/10.5414/npp28210.
[41] O.A. Mendez, A.A. Koshy Toxoplasma Gondii, Entry, association, and physiological influence on the central nervous system, PLoS Pathog. 13 (2017) e1006351, https://doi.org/10.1371/journal.ppat.1006351.
[42] X.Y. Zhao, S.E. Ewald, The molecular biology and immune control of chronic Toxoplasma gondii infection, J. Clin. Invest. 130 (2020) 3370–3380, https://doi.org/10.1172/jci136226.
[43] U.B. Müller, J.C. Howard, The impact of Toxoplasma gondii on the mammalian genome, Curr. Opin. Microbiol. 32 (2016) 19–25, https://doi.org/10.1016/j.mib.2016.04.009.
[44] P. Broz, V.M. Dixit Inflammasomes, Mechanism of assembly, regulation and signalling, Nat. Rev. Immunol. 16 (2016) 407–420, https://doi.org/10.1038/nri.2016.58.
[45] K.V. Swanson, M. Deng, J.P. Ting, The Nlrp3 inflammasome: molecular activation and regulation to therapeutics, Nat. Rev. Immunol. 19 (2019) 477–489, https://doi.org/10.1038/s41577-019-0165-0.
[46] S.E. Ewald, J. Chavarria-Smith, J.C. Boothroyd, Nlrp1 is an inflammasome sensor for Toxoplasma gondii, Infect. Immun. 82 (2014) 460–468, https://doi.org/10.1128/iai.01170-13.
[47] L. Gov, C.A. Schneider, T.S. Lima, W. Pandori, M.B. Lodoen, Nlrp3 and potassium efflux drive rapid il-1β release from primary human monocytes during Toxoplasma gondii infection, J. Immunol. 199 (2017) 2855–2864, https://doi.org/10.4049/jimmunol.1700245.