Association between toxoplasmosis and bipolar disorder: a systematic review and meta-analysis

Running head: Toxoplasmosis and bipolar disorder

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Abstract

Background
The relationship between toxoplasma gondii (T. gondii) infection and bipolar disorder (BD) is poorly understood. This review explores this relationship by estimating the strength of the association between the two conditions using data from published studies.

Methods
Following PRISMA guidelines, we performed a review and meta-analysis of published articles obtained from a systematic search of PubMed, PsycINFO, EMBASE and the Cochrane library up to January 10th, 2021. We included observational studies that compared seroprevalence of IgG class antibodies against T. gondii in patients with a diagnosis of BD with healthy controls. We excluded studies that included <10 participants in each study arm and patients with a serious concomitant medical illness. Discrepancies between the two independent researchers were resolved by consulting a third experienced researcher. Summary data were extracted from published reports. Analysis was conducted using both fixed-effects and random-effects models. The study is registered with PROSPERO number CRD42021237809.

Findings
The search yielded 23 independent studies with a total of 12690 participants (4021 with BD and 8669 controls). Persons with BD had a greater odd of seropositivity with toxoplasmosis than controls, both in the fixed-effects model (OR=1.34 [95%CI: 1.19 to 1.51] and the random-effects model (OR=1.69 [95%CI: 1.21 to 2.36]. No publication bias was detected but reported results showed a high heterogeneity (I2=84% [95%CI:77% to 89%]).

Interpretation
The findings support the relationship between toxoplasmosis infection and BD and suggests a need for studies designed to explore possible causal relationship. Such studies may also improve our understanding of the pathophysiology of BD and open other avenues for its treatment.

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Research in context

Evidence before this study

Recent systematic reviews and meta-analysis have examined the relationship between *T. gondii* infection and various psychiatric disorders. The results of these studies suggest that toxoplasmosis is an associated factor for schizophrenia, addiction, but not for major depression.

T. gondii relationship with bipolar disorder (BD) is still relatively understudied. Three previous meta-analyses were performed and only 2 highlighted a statistically significant association while the third shows a positive association that does not reach statistical significance. Since data was small and the observed association is weak, the question remains still open. If this association will be confirmed it could represent an important target for public health purpose in geographical area where the infection is endemic.

A double search was performed 1) Bipolar Disorder AND toxoplasmosis, 2) Bipolar Disorder AND Toxoplasma on the following databases: PubMed/Medline; EMBASE; PsycINFO and the Cochrane library. No time restriction was applied; the cut-off date was January 10, 2021.

Added value of this study

This meta-analysis, including 23 studies and 12690 participants, represents a major update and extension of previous studies and provides the best currently available evidence about the relationship between toxoplasmosis infection and BD. Patients with BD have an increased chance of being found seropositive with toxoplasmosis than controls, irrespectively of major characteristics of the studies such as age, gender ratio, overall sample size, or methodological quality.

Implications of all the available evidence

Our findings of a higher frequency of Toxoplasma gondii infection in patients diagnosed with BD than in healthy controls highlight a risk that cannot be overlooked, especially in countries where toxoplasmosis is endemic and widespread. A more accurate assessment of patients with BD for potential parasitosis at their first contact is needed. This is particularly important since toxoplasmosis can have a chronic course and lead to severe consequences, such as encephalitis in immunocompromised individuals, vision loss because of posterior uveitis, and pregnancy loss. A deeper investigation of the potential neurobiological effects of the Toxoplasma gondii infection on the brain may provide some new understanding of the etiology of BD and may open new avenues of treatment.

Introduction

T. gondii is an intestinal coccidium that parasitizes cats and has a wide range of intermediate hosts. It has been found worldwide, and nearly one-third of humans have been exposed to the parasite, with prevalence rates that vary from 8% to 90% between countries (Aguirre et al., 2019]. Central and Southern Europe, Latin America and tropical African countries are the areas most affected (Robert-Gangneux and Dardé, 2012). Most common forms of the infection in humans are asymptomatic but, in some conditions, including immunocompromised patients and congenitally infected fetuses and newborns, the infection may cause severe diseases (Weiss et Dubey, 2009). In immunocompetent individuals, due to specific brain tissue tropism where the parasite, forming cysts, can survives in latent intracellular stages. According to some hypotheses T. Gondii could play various roles in the etiopathogenesis of different mental disorders and neuropsychiatric symptoms (Dalimi and Abdoli, 2012). The manipulation hypothesis states that a parasite may be able to alter the behavior of a host for its own selective benefit by enhancing its transmission rate (Webster, 2001). Studies on rats and mice have demonstrated that T. gondii causes a decrease in neophobic (the innate fear of novelty) and predator vigilance behavioral traits, turning rodents' innate aversion into a 'suicidal' fatal feline attraction (Webster, 2007). Humans may become infected with the parasite accidentally, and any behavioral alterations induced may be termed as parasitic 'constraint' (Moore et al., 1990). Several mechanisms have been hypothesized through which infection might influence host behavior, which range from inflammation in the brain to changes in hormones or neurotransmitters (Johnson S. K. and Johnson P., 2021). These biological alterations may lead to behavioral changes and to uncover psychiatric symptoms. Recent systematic review and meta-analysis have examined the relationship between *T. gondii* infection and various psychiatric disorders. The results of these studies suggest that toxoplasmosis is an associated factor for schizophrenia (OR 1.81; $P < 0.00001$) (Sutterland et al., 2015), epilepsy (OR 2.25; $P = 0.005$) (Ngoungou et al. 2015), addiction (OR 1.91; $P< 0.00001$) (Johnson S. K. and Johnson P., 2021)., OCD (OR 1.96; 95% CI: 1.32–2.90) (Nayeri et al., 2019) but not for major depression (OR 1.21; $P= 0.28$) (Johnson S. K. and Johnson P., 2021).; (OR 1.36; 95% IC: 0.58-3.22) (Wang et al. 2014).

Bipolar Disorder (BD) is a complex and heterogeneous mental illness marked by shifts in a person mood, energy and behavior. It is characterized by severe features including an early age of onset, a chronic outcome and an important suicidal risk (Oswald et al., 2007). Lifetime prevalence for BD is estimated at 1.02% (95% CI, 0.81%- 1.29%) with rates that vary

significantly across studies and geographical regions (Moreira et al., 2017). Considering a dimensional composition of the bipolar spectrum that comprises less marked manifestations, estimated lifetime prevalence rates range from 2.8 to 6.5% (Bauer and Pfennig, 2015). A current paradigm for the etiopathogenesis of BD postulates the emergence of the illness as a result of an interaction between genetic and epigenetic factors (Kalcev et al. 2021) with environmental influences (Rybakowski, 2021). In contrast to its well-documented relationship with schizophrenia and other psychiatric disorders, T. gondii relationship with BD is still relatively understudied. Three previous meta-analyses were performed on the topic and only 2 demonstrated a statistically significant association (De Barros et al., 2017; Sutterland et al., 2015), while the third shows a positive association that does not reach statistical significance (Snijders et al., 2019). Since data was small and the observed association is weak, the question remains still open. If this association will be confirmed it could represent an important target for public health purpose in areas where infection is endemic. The objective of this study is to evaluate whether Toxoplasma gondii infection is more prevalent in BDcompared with healthy controls by performing a systematic review and meta-analysis of published studies. We will explore the heterogeneity of the studies by looking at potential moderators, biases and limits of the reported findings and, using the reported findings to highlight possible future research on the topic.

Methods

Search strategy and selection criteria

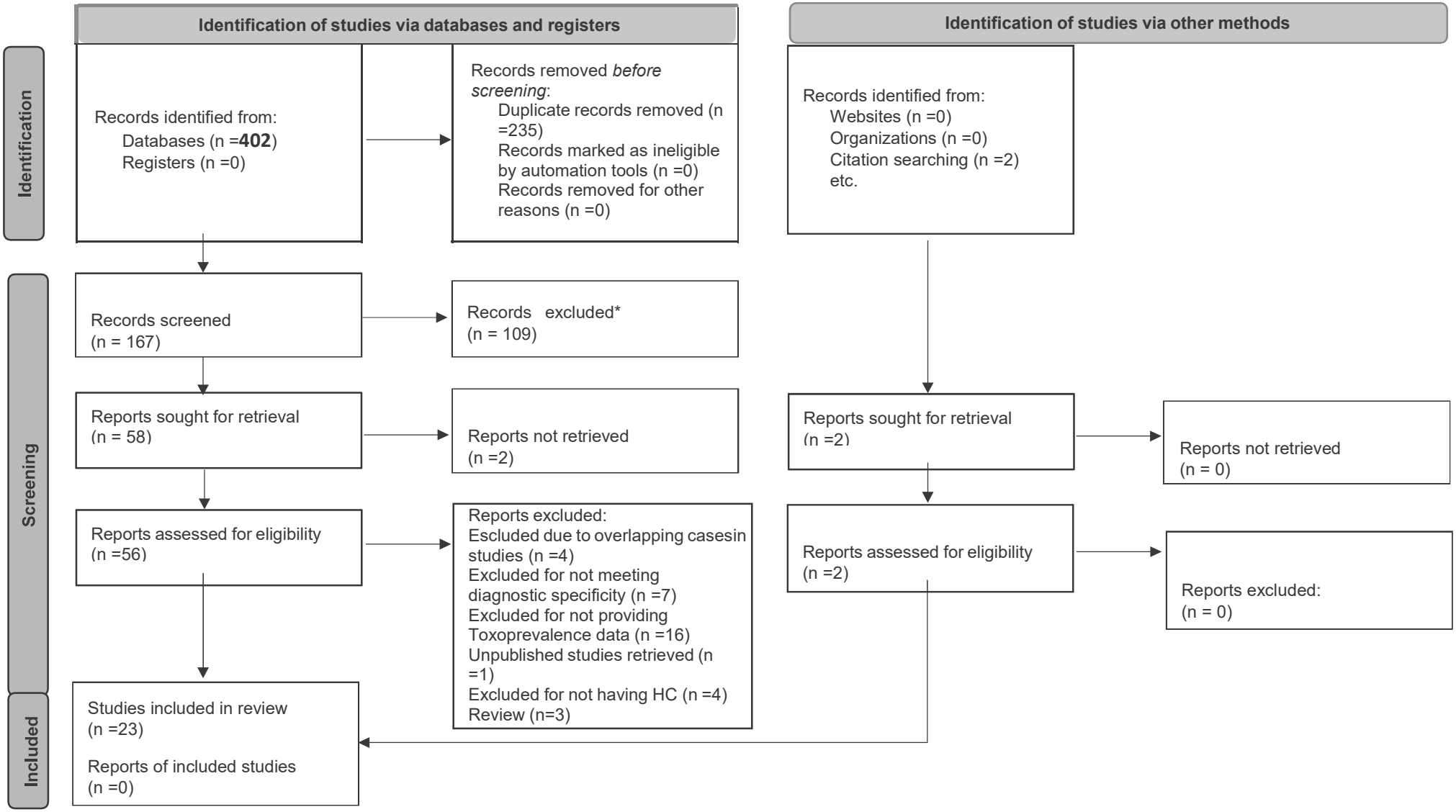
The systematic review is guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009) and is registered on Prospero with the number CRD42021237809-31/03/2021. A double search was performed 1) Bipolar Disorder AND toxoplasmosis, 2) Bipolar Disorder AND Toxoplasma on the following databases: PubMed/Medline (https://pubmed.ncbi.nlm.nih.gov/), EMBASE (https://www.embase.com/), PsycINFO (https://www.apa.org/pubs/databases/psycinfo), and the Cochrane library (https://www.cochranelibrary.com/).

Search cut-off date was 10 January 2021. Pre-specified inclusion criteria were: (1) original research, published in a peer-reviewed journal; (2) published in English, (3) human observational comparative studies, comparing (4) patients with a current diagnosis of BD (defined using DSM-V, ICD-10 or other specified criteria) with (5) healthy controls, (6) >10 participants in each study arm; (7) measuring the seroprevalence of IgG class antibodies against HSV-1, HSV-2, EBV, CMV or T. gondii according to validated serologic procedures; and (8) no time restrictions.

We included studies published in a peer-reviewed journal. Grey literature were excluded. Indeed, there is evidence that selection bias is higher in unpublished literature than in published literature (Egger et al., 2003; Ferguson and Brannick, 2012).

Duplicates across databases were excluded, as were articles repeating previously reported results of a trial or based on overlapping samples. Titles and abstracts were inspected (TIAB) to exclude unrelated articles (i.e., studies on animals) (Figure 1: PRISMA Flow chart). Included articles were then carefully read to decide whether they matched the inclusion criteria. Discrepancies between the two blind researchers (GC and AP) were resolved consulting a third experienced researcher (MGC). The references of the retrieved articles and of the extracted reviews on the topic were also scanned to identify potentially missed studies. At the end of this procedure, 23 independent studies were included in the systematic analysis and the subsequent meta-analysis (Figure 1: PRISMA Flow chart). From the included studies, the two researchers who conducted the search extracted the following variables: authors and year of publication of the study; location of the study; criteria and instrument for BD diagnosis; criteria for diagnosis of toxoplasmosis; sample size; sample mean age; sample gender ratio; number of cases positive for toxoplasmosis in the sample. Quality assessment was performed according to the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (nhlbi.nih.gov/health-topics/study-quality-assessment-tools). Discrepancies in extraction of data were resolved by discussion within the research team.

Fig. 1



* Articles unrelated to the research topic excluded blind by GC and AP. *From:* Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Data analysis

The analysis was carried out in R version 4.0.2 (R Core Development Team, 2015) with the 'meta' package (Balduzzi et al. 2019) and the 'metafor'package (Viechtbauer, 2010). The outcome of the meta-analysis was the proportion of cases that were found positive for toxoplasmosis according to the method that was used in the study. All proportions were estimated with the variance-stabilizing Freeman and Tukey double arcsine transformation (Murray et al., 1950), since there is evidence that it outperforms other proposed methods (e.g., logit transformation) of estimating prevalence, especially when the proportion of cases is expected to be small (Barendregt et al., 2013).

Comparison by diagnosis was carried out by odds ratio (OR), which was calculated with the Mantel-Haenszel method with continuity correction of 0.5 in studies with zero cell frequencies. The inverse variance method was used for pooling (Fleiss, 1993). The tau-squared ($\tau 2$) statistics was used to calculate between studies variance and variance of the effect size parameters across the population. The $\tau 2$ reflects the variance of the true effect sizes: the lower, the better. Calculation was done with the Empirical Bayes estimator (Veroniki et al., 2016); its 95% CI was calculated by using the Q-Profile method (Viechtbauer, 2010) with Knapp and Hartung correction (Knapp and Hartung, 2003). Both fixed-and random-effects summary estimates were reported, along with a corresponding 95% confidence interval (CI) for each outcome in forest plots.Forest plots are based on package 'meta' with an adaptation courtesy of Guido Schwarzer.

Heterogeneity was assessed with Cochran's Q and I^2 statistics (Huedo-Medina et al., 2006). The higher the I^2, the greater the impact of the variance in true effects. Values of I^2 between 30% and 60% were considered indicative of moderate heterogeneity; values above 75% were considered indicative of substantial heterogeneity (Higgins et al., 2019). The funnel plot, the Egger's test (Egger et al. 1997), and the Begg's test (Begg and Mazumdar, 1994) were used as a proxy index of bias in publication. The radial plot was used to assess model adequacy (Galbraith, 1994), and effect size sampling variance. For each study, the observation of a large standardized residual (above 2, as a rule of thumb) suggests that the study does not fit the assumed model (i.e., it may be an outlier). We used meta-regression techniques to evaluate the impact of the following clinical variables: gender ratio, mean age of the sample, overall sample size, and thequality of the study.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis,

data interpretation, or writing of the report.

Results

Overall, 23 studies were found (Tab.1) with details about the proportion of cases with toxoplasmosis in samples of patients diagnosed with bipolar disorder and a comparison with healthy controls. These studies were: 4 from US (Frye et al., 2019; Parks et al., 2018;; Tanaka et al., 2017; Yolken et al., 2017), 7 from Europe (France: 3 (Hamdani et al., 2015; Hamdani et al., 2018); Germany: 3 (Gerber et al., 2012; Hinze-Selch, et al., 2010; Oliveira et al., 2017; Stich et al. 2015); The Netherlands: 1 (Snijders et al., 2019)), 4 from Asia (China: 3 (Chen et al., 2019; Xiao et al., 2010; Xu et al., 2020)); India: 1 (Sundaresh et al., 2018)), 5 from Middles East or Arabic countries (Iran: 4 (Abdollahian et al., 2017; Dalimiasl et al., 2016 Khademvatan et al., 2014; Kheirandish et al., 2016); Saudi Arabia: 1 (Afifi et al., 2018)), 2 from Africa (Egypt: 1 (Hussein et al., 2020); Ethiopia: 1 (Tedla et al., 2011)), 1 from Mexico (Alvarado-Esquivel et al., 2019). Clinical diagnosis was carried out according to the DSM criteria in 15 studies (DSM-IV in 14 studies (Afifi et al., 2018; Chen et al., 2019; Frye et al., 2019; Gerber et al., 2012; Hamdani et al., 2015; Hamdani et al., 2018; Khademvatan et al., 2013; Oliveira et al., 2017; Snijders et al., 2019; Stich et al., 2015; Sundaresch et al., 2018; Tanaka et al., 2017; Xu et al., 2020; Yolken et al., 2017), DSM-5 in 1 study (Hussein et al., 2020). In the remaining studies, the ICD-10 criteria were used in 3 studies (Alvarado-Esquivel et al., 2019; Hinze-Selch et al., 2010; Tedla et al., 2011), and 5 studies applied clinical criteria without detailing the procedure for the diagnosis (Abdollahian et al., 2017; Dalimiasl et al., 2016; Kheirandish et al., 2016; Parks et al., 2018; Xiao et al., 2010). Overall, patients with bipolar disorder included in the meta-analysis were 4021 (range per study: 30 to 1207; mean per sample: 175±254); healthy controls were 8669 (range: 20 to 2634; mean per sample: 377±602). The mean age of the combined sample was 40.5 (±5.1; range: 32.6 to 49.4), the average proportion of males in the samples was 49% (±10%; range: 28% to 77%). There were 984 patients with bipolar disorder who were found positive for toxoplasmosis (range: 3% to 95%; mean per sample: 37%±29%; median: 30%); in healthy controls, 1178 were found positive for toxoplasmosis (range: 2% to 87%; mean per sample: 28%±23%; median: 21%). Studies that were done in Europe (48%±34%) or the Middle East or Arabic countries (43%±23%) had higher positivity for toxoplasmosis in patients than studies done in Asia (21%±13%) or US (14%±6%), although without reaching the threshold for statistical significance: $F[3;16] = 2.19$, $p=0.13$.

The effect was more evident in controls: Europe (38%±20%), the Middle East/Arabic countries (37%±18%), Asia (7%±4%), US (11%±6%): $F[3;16] = 5.29$, $p=0.011$. No other characteristic of the studies was associated with the positivity of toxoplasmosis in the samples.

The probability of being found positive for toxoplasmosis was higher in patients diagnosed with bipolar disorder than in healthy controls (Figure 2) in both the fixed-effects model (OR=1.34 [95%CI: 1.19 to 1.52]; z=4.85; p<0.0001) and the random-effects model (OR=1.69 [1.21 to 2.37]; z=3.22; p=0.0039). Heterogeneity was substantial: $\tau 2$=0.449; Q=133.7; df=22; p<0.0001; I^2=84% (77% to 89%). No publication bias was detected by the Egger's test (t=1.95; df=21; p=0.065) or the Begg's test (z=0.32; p=0.75). The funnel plot was reasonably symmetric (Figure A2a/A2b in supplementary material).

However, the radial plot indicated that three studies scored outside two standard deviations from the estimated mean in the sample, with the studies of Chen et al., 2019, Afifi et al.,2018, and Frye et al., 2019 being four standard deviations outside the mean estimation in the sample (Figure A3 in supplementary material). When repeated by excluding these three outliers, patients with bipolar disorder still had a higher chance of being found positive for toxoplasmosis than controls (Figure A4). There was a modest decrease of the heterogeneity after the exclusion of the three influential points (I^2=59% [32% to 75%]), and a more important decrease of $\tau 2$ (0.041), indicating a better estimate of the true effect (Figure A4).

There was no impact of age (beta=-0.05; s.e.=0.04; t=-1.42; df=17; p=0.17; amount of heterogeneity accounted for: 7%), gender ratio (F[1;17] = 1.29, p =0.27; amount of heterogeneity accounted for: 2%), or overall sample size (beta=-0.001; s.e.=0.001; t=-1.87; df=21; p=0.08; amount of heterogeneity accounted for: 13%), neither the quality of the studies (fair vs. poor) (Tab. A1) had any impact on the OR estimates (F[1;21] = 0.04, p =0.85; amount of heterogeneity accounted for: 0%).

We also explored in a series of sensitivity analyses the role of the geographical location of the studies, of the design (cross-sectional vs. case-control), and of the type of assay used to ascertain the positivity for toxoplasmosis. We compared studies that were done in Europe (n=7), the US (n=4), Asia (n=4), and the Middle East or Arabic countries (n=5). No effect of the geographical location of the studies was found on the OR, but a modest effect on the heterogeneity (F[3;16] = 2.33, p=0.11; amount of heterogeneity accounted for: 22%). No effect was found on the OR between the 10 studies with a cross-sectional design and the 13 studies that adopted a case-control design (F[1;21] = 2.77, p=0.11; amount of heterogeneity accounted for: 10%).

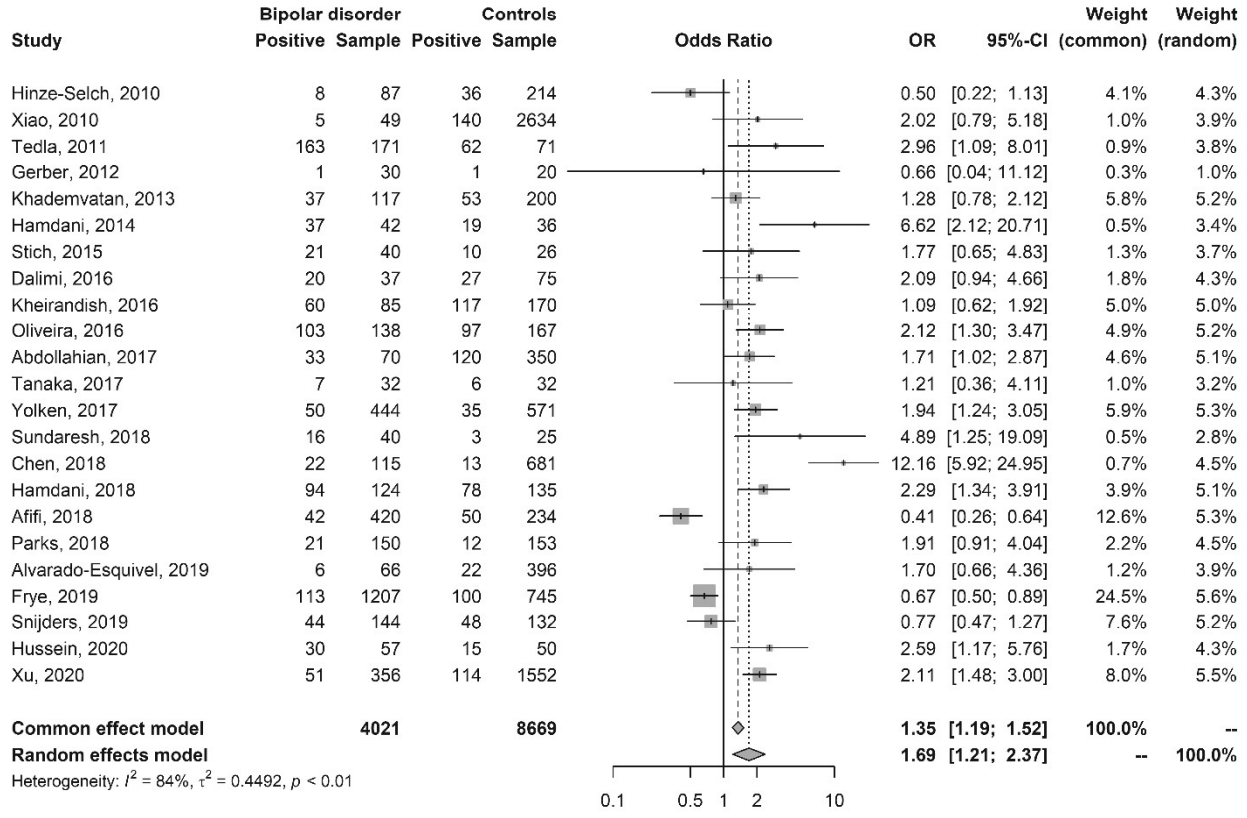
We also found no effect on the OR between the 9 studies that used Elisa to test for toxoplasmosis and the 12 studies that used an enzyme immunoassay (F[1;19] = 2.62, p=0.12; amount of heterogeneity accounted for: 10%).

Table 1 **Characteristics of the included studies**

Author, year	Country	Design	Sample	Age	Male %	Diagnosis	Toxo test	RR; 95%-CI
Hinze-Selch, 2010	Germany	Cross-sectional	BD 87, HC 214	46.3	Na	ICD-10	Spot Immunofluorescence	0.55; [0.26; 1.13]
Xiao, 2010	China	Cross-sectional	BD 49, HC 2634	Na	Na	Na	ELISA	1.92; [0.82; 4.47]
Tedla, 2011	Ethiopia	Case-control	BD 199, HC 80	Na	52.8%	ICD-10	IgG serology (unspecified)	1.09; [0.99; 1.20]
Gerber, 2012	Germany	Cross-sectional	BD 30, HC 20	42.6	40%	DSM-IV	Enzyme immunoassay	0.67; [0.04; 10.05]
Khademvatan, 2013	Iran	Cross-sectional	BD 117, HC 200	33.9	59%	DSM-IV	ELISA	1.19; [0.84; 1.70]
Hamddani, 2014	France	Cross-sectional	BD 42; HC 36	44.8	53%	DSM-IV	Enzyme immunoassay	1.67; [1.20; 2.32]
Stich, 2015	Germany	Case-control	BD 40; HC 26	48.3	37,5%	DSM-IV	Enzyme immunoassay	1.36; [0.77; 2.41]
Dalimiasl, 2016	Iran	Case-control	BD 37; HC 75	Na	42%	Na	ELISA	1.50; [0.98; 2.29]
Kheirandish, 2016	Iran	Case-control	BD 85, HC 170	37.2	57.1%	Na	ELISA	1.03; [0.86; 1.22]
Oliveira, 2016	France	Case-control	BD 138; HC 167	43.6	48.6%	DSM-IV	Enzyme immunoassay	1.28; [1.09; 1.51]
Abdollahian, 2017	Iran	Case-control	BD 70; HC 350	38	Na	DSM-IV	ELISA	1.38; [1.03; 1.83]
Tanaka, 2017	USA	Cross-sectional	BD 32; HC 32	38.72	14%	DSM-IV	Enzyme immunoassay	1.17; [0.44; 3.09]
Yolken, 2017	USA	Case-control	BD 444; HC 571	37.5	28.4%	DSM-IV	Enzyme immunoassay	1.84; [1.21; 2.78]
Sundaresh, 2018	India	Case-control	BD 150; HC 153	32.95	48%	DSM-IV	Enzyme immunoassay	3.33; [1.08; 10.29]
Chen, 2018	China	Case-control	BD 115; HC 681	38.83	Na	DSM-IV	Enzyme immunoassay	10.02; [5.20; 19.32]
Hamdani, 2018	France	Cross-sectional	BD 124; HC 135	44.2	47.2%	DSM-IV	Enzyme immunoassay	1.31; [1.10; 1.56]
Afifi, 2018	Saudi Arabia	Cross-sectional	BD 40; HC 25	32.6	47.5%	DSM-5	ELISA	0.47; [0.32; 0.68]
Parks, 2018	USA	Cross-sectional	BD 420; HC 234	43.2	48,1%	Na	Enzyme immunoassay	1.79; [0.91; 3.50]
Alvarado-Esquivel, 2019	Mexico	Case-control	BD 66; HC 396	40.05	50%	ICD-10	Enzyme immunoassay	1.64; [0.69; 3.88]
Frye, 2019	USA	Case-control	BD 1207; HC 745	43.2	38,5%	DSM-IV	Enzyme immunoassay	0.70; [0.54; 0.90]
Snijders, 2019	Holland	Cross-sectional	BD 760; HC 132	49.41	44.1%	DSM-IV	ELISA	0.84; [0.60; 1.17]
Hussein, 2020	Egypt	Case-control	BD 57; HC 50	34.65	77.2%	DSM-IV	ELISA	1.75; [1.07; 2.86]
Xu, 2020	China	Case-control	BD 356; HC 1552	Na	Na	DSM-IV	ELISA	1.95; [1.43; 2.66]

BD: Bipolar disorder; HC: healthy control; Na: not available; ICD-10: International Classification of Diseases; DSM-IV/5: Diagnostic and Statistical Manual of Mental Disorders; ELISA: Enzyme-linked immunosorbent assay

Fig. 2


Bipolar disorder
Controls
Study
Positive Sample Positive Sample
Odds Ratio
OR 95%-CI
Weight (common)
Weight (random)
Hinze-Selch, 2010 8 87 36 214 0.50 [0.22; 1.13] 4.1% 4.3%
Xiao, 2010 5 49 140 2634 2.02 [0.79; 5.18] 1.0% 3.9%
Tedla, 2011 163 171 62 71 2.96 [1.09; 8.01] 0.9% 3.8%
Gerber, 2012 1 30 1 20 0.66 [0.04; 11.12] 0.3% 1.0%
Khademvatan, 2013 37 117 53 200 1.28 [0.78; 2.12] 5.8% 5.2%
Hamdani, 2014 37 42 19 36 6.62 [2.12; 20.71] 0.5% 3.4%
Stich, 2015 21 40 10 26 1.77 [0.65; 4.83] 1.3% 3.7%
Dalimi, 2016 20 37 27 75 2.09 [0.94; 4.66] 1.8% 4.3%
Kheirandish, 2016 60 85 117 170 1.09 [0.62; 1.92] 5.0% 5.0%
Oliveira, 2016 103 138 97 167 2.12 [1.30; 3.47] 4.9% 5.2%
Abdollahian, 2017 33 70 120 350 1.71 [1.02; 2.87] 4.6% 5.1%
Tanaka, 2017 7 32 6 32 1.21 [0.36; 4.11] 1.0% 3.2%
Yolken, 2017 50 444 35 571 1.94 [1.24; 3.05] 5.9% 5.3%
Sundaresh, 2018 16 40 3 25 4.89 [1.25; 19.09] 0.5% 2.8%
Chen, 2018 22 115 13 681 12.16 [5.92; 24.95] 0.7% 4.5%
Hamdani, 2018 94 124 78 135 2.29 [1.34; 3.91] 3.9% 5.1%
Afifi, 2018 42 420 50 234 0.41 [0.26; 0.64] 12.6% 5.3%
Parks, 2018 21 150 12 153 1.91 [0.91; 4.04] 2.2% 4.5%
Alvarado-Esquivel, 2019 6 66 22 396 1.70 [0.66; 4.36] 1.2% 3.9%
Frye, 2019 113 1207 100 745 0.67 [0.50; 0.89] 24.5% 5.6%
Snijders, 2019 44 144 48 132 0.77 [0.47; 1.27] 7.6% 5.2%
Hussein, 2020 30 57 15 50 2.59 [1.17; 5.76] 1.7% 4.3%
Xu, 2020 51 356 114 1552 2.11 [1.48; 3.00] 8.0% 5.5%
Common effect model 4021 8669 1.35 [1.19; 1.52] 100.0% --
Random effects model 1.69 [1.21; 2.37] -- 100.0%
Heterogeneity: I^2 = 84%, τ^2 = 0.4492, p < 0.01
0.1
0.5
1
2
10

Discussion

We found in a meta-analysis including 4021 patients diagnosed with BD and 8669 healthy controls, extracted from 23 independent samples (Tab.1), that the probability of being found positive for toxoplasmosis was higher in patients diagnosed with BD than in healthy controls. No study characteristics impacts on the difference between patients with BD and controls.

This is the first meta-analysis that includes a large number of studies specifically addressing the relationship between bipolar disorder and toxoplasmosis. Our results corroborate previous studies of De Barros et al., 2017 (k=8; OR 1.26; 95% CI 1.08-1.47; p=0.004), Sutterland et al., 2015 (k=11; OR 1.52; 95% CI 1.06-2.18; p=0.002) and Snijders et al., 2019 (k=11; OR 1.40; 95% CI 0.95-1.90; p=0.009) that started to investigate this association.

Several mechanisms have been investigated as potential causal link between chronic toxoplasmosis infection and occurrence of neuropsychiatric illnesses (Chaudhury and Ramana, 2019), typically focused on one of three domains: generalized inflammation, alteration in endocrine signaling, and changes in neurotransmitter pathways (Johnson S. K. and Johnson P., 2021). Even though the cysts are distributed throughout the brain and selective tropism of the parasite toward a particular functional system has not been observed, however some brain regions are consistently more infected than others, including the hippocampus and the amygdala (McConkey et al., 2013; Berenreiterová et al., 2011). Alteration of the structure and function of corticolimbic circuits, which are involved in the modulation of impulsivity and aggression, could be responsible for behavioral changes observed in infected animals and humans (Coccaro et al., 2011; Coccaro et al., 2016). Genomic sequencing of T.gondii DNA has shown two genes encoding tyrosine hydroxylase which metabolizes phenylalanine and tyrosine with an overproduction of dopamine in tissue cysts of Toxoplasma (Zhang et al., 2020). This might explain the association between chronic toxoplasmosis and positive symptoms in schizophrenia and bipolar disorder. Another study noted increased extracellular glutamate and downregulation of glutamate transporter GLT-1 in astrocytes during toxoplasma chronic infection. Dysregulation of glutamate homeostasis can cause neuro excitotoxicity responsible for network connectivity alterations shown in EEG recording (David et al., 2016). Chronic T. gondii infection is characterized by increased levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-12 (IL- 12), and IL-1β (Dogruman-Al et al., 2011; Meira et al., 2014). It seems that there exist specific patterns of increased levels of cytokines in different phases of bipolar disorders: the pro-inflammatory cytokines including IL-2, IL-6, and IL-8 and interferon (IFN)-γ are increased during mania, whereas only IL-6 is increased during depressive episodes (Brietzke et al., 2009). These data suggest that mostly manic phase of bipolar disorder is

associated with a persistent and chronic pro-inflammatory state (Kim Y. K. et al., 2007). Another possible mediator could be the kynurenic acid (KYNA), an astrocytic harmful metabolite of tryptophan activated in T. gondii infection that alter dopaminergic and glutamatergic neurotransmission, which were found elevated in patients with schizophrenia (Schwarcz and Hunter, 2007). A recent meta-analysis suggests that a dysregulation of the kynurenine pathway occurs in bipolar disorder. Individuals with a manic episode showed a greater reduction in tryptophan levels, whereas kynurenic acid levels were more reduced among subjects in a depressive phase (Bartoli et al., 2021). The vesicles produced by toxoplasma in neurological locations have also been found to contain large amounts of thymosin beta4 (Pope and Lässer, 2013), a small peptide involved in neural maturation, angiogenesis, cell migration as well as in estrogen and progesterone derived hormones regulation and sleep disturbance (Carta et al., 2021). Blood levels of this peptide have been found to be altered in both depressive and bipolar disorders (Kim H. et al., 2021). The main strength of this meta-analysis is the inclusion of a larger set of studies than in previous investigations on the topic and the use of state-of-the-art statistical procedures in the conduction of the analyses. Moreover, no publication bias was detected in the global sample of studies included inthe meta-analysis, suggesting some confidence in the retrieved results. However, some caveats have to be taken into account. Some of the included studies (Gerber et al., 2012; Tanaka et al.,2017) have small sample size with very few subjects found positive for toxoplasmosis which implies little statistical power. Results were associated to a high heterogeneity (I^2=83%), which was not explained by the characteristics of the sample (the composition of the sample by age and gender) neither by the quality of the studies. None of the included studies quantified the extent of the infection or any possible neurological damage of toxoplasmosis with neuroimaging and their correlation with psychiatric disorders and symptoms. None of the included studies analyzed the psychotropic drugs as a possible confounding, even though the in-vitro antiprotozoal activity is well established for some drugs such as valproic acid, zuclopenthixol and haloperidol (Jones-Brando et al., 2003; Fond et al., 2014). Furthermore, these studies considered the prevalence of toxoplasmosis using categorial diagnosis such as those based on ICD or DSM criteria, with none exploring symptom and behavioral dimensions that could possibly lead to a better characterization of the psychopathological problems related to toxoplasmosis. The retrospective and cross-sectional nature of the included studies prevents any assessment of the direction of causality, so it also cannot be totally excluded the possibility that the co-occurrence of toxoplasmosis infection and bipolar disorder (or other mental health disorders) reflects from sharingsimilar sociodemographic risk factors (e.g. to live in deprived areas (Kroon et al., 2013; Bigna et al., 2020) or that suffering

from a mental illness could lead to lifestyles (eating habits; hygiene) that increase the risk of becoming infected (reverse causation) (Thebault et al., 2021).

Despite the limitations, this meta-analysis indicates a higher chance of Toxoplasma gondii infectionin patients diagnosed with BD than in healthy controls. This indicates the need for accurate assessment of patients with BD for potential parasitosis at their first contact. This is particularly important since toxoplasmosis can have a chronic course and lead to severe consequences, such as encephalitis in immunocompromised individuals, vision loss because of posterior uveitis, and pregnancy loss.

Longitudinal studies have the potential to help unravel the possibility that lifestyles andhealth behaviors of patients with BD are the basis of their higher risk to have toxoplasmosis infection or that the BD results from a disruption in neural circuits caused by the Toxoplasma gondii infection. Also, a deeper investigation of the potential neurobiological effects of the Toxoplasma gondii infection on the brain may provide some new understanding of the etiology ofBD and, as well, may open new avenues of treatment. A better investigation of this link in longitudinal studies will be worth exploring since it may clarify the pathophysiology of BD and open avenues for its treatment.

Contributions: All authors contributed to the study conception and design.
GC: Management and coordination responsibility for the research activity planning and execution.AP: Development design of methodology; creation of models.
DG: Preparation, presentation of the published work from the original research group.
OG: Oversight and leadership responsibility for the research activity planning and execution,including mentorship external to the core team.
MGC: Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.
All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. GC and AP have accessed and verified the data.
All authors read and approved the final manuscript.

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Data sharing: The data that support the findings of this study are available on request from thecorresponding author GC.

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References

- Abdollahian, E., Shafiei, R., Mokhber, N., Kalantar, K., & Fata, A. (2017). Seroepidemiological Study of *Toxoplasma gondii* Infection among Psychiatric Patients in Mashhad, Northeast of Iran. *Iranian journal of parasitology*, *12*(1), 117–122.
- Afifi, M. A., Jiman-Fatani, A. A., Al-Rabia, M. W., Al-Hussainy, N. H., El Saadany, S., & Mayah, W. (2018). More Than an Association: Latent Toxoplasmosis Might Provoke a Local Oxidative Stress That Triggers the Development of Bipolar Disorder. *Journal of microscopy and ultrastructure*, *6*(3), 139–144. https://doi.org/10.4103/JMAU.JMAU_22_18
- Aguirre, A. A., Longcore, T., Barbieri, M., Dabritz, H., Hill, D., Klein, P. N., Lepczyk, C., Lilly, E. L., McLeod, R., Milcarsky, J., Murphy, C. E., Su, C., VanWormer, E., Yolken, R., & Sizemore, G. C. (2019). The One Health Approach to Toxoplasmosis: Epidemiology, Control, and Prevention Strategies. *EcoHealth*, *16*(2), 378–390. https://doi.org/10.1007/s10393-019-01405-7
- Alvarado-Esquivel, C., Estrada-Martínez, S., & Pérez-Alamos, A. R. (2019). A Case-Control Seroprevalence Study on the Association Between *Toxoplasma gondii* Infection and Bipolar Disorder. *Frontiers in psychiatry*, *10*, 766. https://doi.org/10.3389/fpsyt.2019.00766
- Balduzzi, S., Rücker, G., & Schwarzer, G. (2019). How to perform a meta-analysis with R: a practical tutorial. *Evidence-based mental health*, *22*(4), 153–160. https://doi.org/10.1136/ebmental-2019-300117
- Barendregt, J. J., Doi, S. A., Lee, Y. Y., Norman, R. E., & Vos, T. (2013). Meta-analysis of prevalence. *Journal of epidemiology and community health*, *67*(11), 974–978. https://doi.org/10.1136/jech-2013-203104
- Bartoli, F., Misiak, B., Callovini, T., Cavaleri, D., Cioni, R. M., Crocamo, C., Savitz, J. B., & Carrà, G. (2021). The kynurenine pathway in bipolar disorder: a meta-analysis on the peripheral blood levels of tryptophan and related metabolites. *Molecular psychiatry*, *26*(7), 3419–3429. https://doi.org/10.1038/s41380-020-00913-1
- Bauer, M., & Pfennig, A. (2005). Epidemiology of bipolar disorders. *Epilepsia*, *46 Suppl 4*, 8–13. https://doi.org/10.1111/j.1528-1167.2005.463003.x
- Begg, C. B., & Mazumdar, M. (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics*, *50*(4), 1088–1101.
- Berenreiterová, M., Flegr, J., Kuběna, A. A., & Němec, P. (2011). The distribution of Toxoplasma gondii cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis. *PloS one*, *6*(12), e28925. https://doi.org/10.1371/journal.pone.0028925
- Bigna, J. J., Tochie, J. N., Tounouga, D. N., Bekolo, A. O., Ymele, N. S., Youda, E. L., Sime, P. S., & Nansseu, J. R. (2020). Global, regional, and country seroprevalence of Toxoplasma gondii in pregnant women: a systematic review, modelling and meta-analysis. *Scientific reports*, *10*(1), 12102. https://doi.org/10.1038/s41598-020-69078-9
- Brietzke, E., Stertz, L., Fernandes, B. S., Kauer-Sant'anna, M., Mascarenhas, M., Escosteguy Vargas, A., Chies, J. A., & Kapczinski, F. (2009). Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. *Journal of affective disorders*, *116*(3), 214–217. https://doi.org/10.1016/j.jad.2008.12.001
- Carta, M. G., Fanni, D., Orrù, G., & Faa, G. (2021). Commentary: Sleep Disturbance in Bipolar Disorder: Neuroglia and Circadian Rhythms. *Frontiers in psychiatry*, *12*, 730360. https://doi.org/10.3389/fpsyt.2021.730360

- Chaudhury, A., & Ramana, B. V. (2019). Schizophrenia and bipolar disorders: The *Toxoplasma* connection. *Tropical parasitology*, *9*(2), 71–76. https://doi.org/10.4103/tp.TP_28_19
- Chen, X., Chen, B., Hou, X., Zheng, C., Yang, X., Ke, J., Hu, X., & Tan, F. (2019). Association between Toxoplasma gondii infection and psychiatric disorders in Zhejiang, Southeastern China. *Acta tropica*, *192*, 82–86. https://doi.org/10.1016/j.actatropica.2019.02.001
- Coccaro, E. F., Sripada, C. S., Yanowitch, R. N., & Phan, K. L. (2011). Corticolimbic function in impulsive aggressive behavior. *Biological psychiatry*, *69*(12), 1153–1159. https://doi.org/10.1016/j.biopsych.2011.02.032
- Coccaro, E. F., Lee, R., Groer, M. W., Can, A., Coussons-Read, M., & Postolache, T. T. (2016). Toxoplasma gondii infection: relationship with aggression in psychiatric subjects. *The Journal of clinical psychiatry*, *77*(3), 334–341. https://doi.org/10.4088/JCP.14m09621
- Dalimi, A., & Abdoli, A. (2012). Latent toxoplasmosis and human. *Iranian journal of parasitology*, *7*(1), 1–17.
- Dalimiasl, A., Mosayebi, M., Ghorbanzadeh, B., Ghasemi, M., & Hajihossein, R. (2016). Association between anti-Toxoplasma gondii antibodies and schizophrenia and psychotic bipolar in patients hospitalized in the psychiatric ward. *Australasian Medical Journal, 9*, 313-318
- David, C. N., Frias, E. S., Szu, J. I., Vieira, P. A., Hubbard, J. A., Lovelace, J., Michael, M., Worth, D., McGovern, K. E., Ethell, I. M., Stanley, B. G., Korzus, E., Fiacco, T. A., Binder, D. K., & Wilson, E. H. (2016). GLT-1-Dependent Disruption of CNS Glutamate Homeostasis and Neuronal Function by the Protozoan Parasite Toxoplasma gondii. *PLoS pathogens*, *12*(6), e1005643. https://doi.org/10.1371/journal.ppat.1005643
- De Barros, J., Barbosa, I. G., Salem, H., Rocha, N. P., Kummer, A., Okusaga, O. O., Soares, J. C., & Teixeira, A. L. (2017). Is there any association between Toxoplasma gondii infection and bipolar disorder? A systematic review and meta-analysis. *Journal of affective disorders*, *209*, 59–65. https://doi.org/10.1016/j.jad.2016.11.016
- Dogruman-Al, F., Fidan, I., Celebi, B., Yesilyurt, E., Erdal, B., Babur, C., & Kustimur, S. (2011). Cytokine profile in murine toxoplasmosis. *Asian Pacific journal of tropical medicine*, *4*(1), 16–19. https://doi.org/10.1016/S1995-7645(11)60024-2
- Egger, M., Davey Smith, G., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical research ed.)*, *315*(7109), 629–634. https://doi.org/10.1136/bmj.315.7109.629
- Egger, M., Juni, P., Bartlett, C., Holenstein, F., & Sterne, J. (2003). How important are comprehensive literature searches and the assessment of trial quality in systematic reviews? Empirical study. *Health technology assessment (Winchester, England)*, *7*(1), 1–76.
- Ferguson, C. J., & Brannick, M. T. (2012). Publication bias in psychological science: prevalence, methods for identifying and controlling, and implications for the use of meta-analyses. *Psychological methods*, *17*(1), 120–128. https://doi.org/10.1037/a0024445
- Fleiss J. L. (1993). The statistical basis of meta-analysis. *Statistical methods in medical research*, *2*(2), 121–145. https://doi.org/10.1177/096228029300200202
- Fond, G., Macgregor, A., Tamouza, R., Hamdani, N., Meary, A., Leboyer, M., & Dubremetz, J. F. (2014). Comparative analysis of anti-toxoplasmic activity of antipsychotic drugs and valproate. *European archives of psychiatry and clinical neuroscience*, *264*(2), 179–183. https://doi.org/10.1007/s00406-013-0413-4

- Frye, M. A., Coombes, B. J., McElroy, S. L., Jones-Brando, L., Bond, D. J., Veldic, M., Romo-Nava, F., Bobo, W. V., Singh, B., Colby, C., Skime, M. K., Biernacka, J. M., & Yolken, R. (2019). Association of Cytomegalovirus and Toxoplasma gondii Antibody Titers With Bipolar Disorder. *JAMA psychiatry*, *76*(12), 1285–1293. https://doi.org/10.1001/jamapsychiatry.2019.2499
- Galbraith R. F. (1994) Some Applications of Radial Plots, Journal of the American Statistical Association, 89:428, 1232-1242, DOI: 10.1080/01621459.1994.10476864
- Gerber, S. I., Krienke, U. J., Biedermann, N. C., Grunze, H., Yolken, R. H., Dittmann, S., & Langosch, J. M. (2012). Impaired functioning in euthymic patients with bipolar disorder--HSV-1 as a predictor. *Progress in neuro-psychopharmacology & biological psychiatry*, *36*(1), 110–116. https://doi.org/10.1016/j.pnpbp.2011.09.003
- Hamdani, N., Daban-Huard, C., Lajnef, M., Gadel, R., Le Corvoisier, P., Delavest, M., Carde, S., Lépine, J. P., Jamain, S., Houenou, J., Galeh, B., Richard, J. R., Aoki, M., Charron, D., Krishnamoorthy, R., Yolken, R., Dickerson, F., Tamouza, R., & Leboyer, M. (2015). Cognitive deterioration among bipolar disorder patients infected by Toxoplasma gondii is correlated to interleukin 6 levels. *Journal of affective disorders*, *179*, 161–166. https://doi.org/10.1016/j.jad.2015.03.038
- Hamdani, N., Bengoufa, D., Godin, O., Doukhan, R., Le Guen, E., Daban-Huard, C., Bennabi, M., Delavest, M., Lépine, J. P., Boukouaci, W., Laouamri, H., Houenou, J., Jamain, S., Richard, J. R., Lecorvosier, P., Yolken, R., Rajagopal, K., Leboyer, M., & Tamouza, R. (2018). Immunoglobulin sub-class distribution in bipolar disorder and schizophrenia: potential relationship with latent Toxoplasma Gondii infection. *BMC psychiatry*, *18*(1), 239. https://doi.org/10.1186/s12888-018-1821-9
- Higgins JPT, Thomas J, Chandler J, et al. Cochrane Handbook for Systematic Reviews of Interventions 2nd Edition. Chichester (UK): John Wiley & Sons, 2019.
- Hinze-Selch, D., Däubener, W., Erdag, S., & Wilms, S. (2010). The diagnosis of a personality disorder increases the likelihood for seropositivity to Toxoplasma gondii in psychiatric patients. *Folia parasitologica*, *57*(2), 129–135. https://doi.org/10.14411/fp.2010.016
- Huedo-Medina, T. B., Sánchez-Meca, J., Marín-Martínez, F., & Botella, J. (2006). Assessing heterogeneity in meta-analysis: Q statistic or I2 index? *Psychological methods*, *11*(2), 193–206. https://doi.org/10.1037/1082-989X.11.2.193
- Hussein, E., Khalifa, H., Ramadan, G. K., Hassaan, S. H., Shaaban, I., & Farrag, H. (2020). Seroprevalence of Toxoplasma gondii among patients with schizophrenia and bipolar disorder in Upper Egypt: a comparative study with a control group. *Annals of parasitology*, *66*(2), 183–192. https://doi.org/10.17420/ap6602.253
- Johnson, S. K., & Johnson, P. (2021). Toxoplasmosis: Recent Advances in Understanding the Link Between Infection and Host Behavior. *Annual review of animal biosciences*, *9*, 249–264. https://doi.org/10.1146/annurev-animal-081720-111125
- Jones-Brando, L., Torrey, E. F., & Yolken, R. (2003). Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of Toxoplasma gondii. *Schizophrenia research*, *62*(3), 237–244. https://doi.org/10.1016/s0920-9964(02)00357-2
- Kalcev, G., Preti, A., Scano, A., Orrù, G., & Carta, M. G. (2021). Insight into susceptibility genes associated with bipolar disorder: a systematic review. *European review for medical and pharmacological sciences*, *25*(18), 5701–5724. https://doi.org/10.26355/eurrev_202109_26789

- Khademvatan, S., Saki, J., Khajeddin, N., Izadi-Mazidi, M., Beladi, R., Shafiee, B., & Salehi, Z. (2014). Toxoplasma gondii Exposure and the Risk of Schizophrenia. *Jundishapur journal of microbiology*, *7*(11), e12776. https://doi.org/10.5812/jjm.12776
- Kheirandish F, Nazari H, Mahmoudvand H, Yaseri Y, Tarahi MJ, Fallahi S, et al. Possible Link Between Toxoplasma gondii Infection and Mood Disorders in Lorestan Province, WesternIran. *Archives of ClinicalhInfectioushDiseases* 2016; 11. e36602. doi: 10.5812/archcid.36602.
- Kim, H., Rhee, S. J., Lee, H., Han, D., Lee, T. Y., Kim, M., Kim, E. Y., Kwon, J. S., Shin, H., Kim, H., Ahn, Y. M., & Ha, K. (2021). Identification of altered protein expression in major depressive disorder and bipolar disorder patients using liquid chromatography-tandem mass spectrometry. *Psychiatry research*, *299*, 113850. https://doi.org/10.1016/j.psychres.2021.113850
- Kim, Y. K., Jung, H. G., Myint, A. M., Kim, H., & Park, S. H. (2007). Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. *Journal of affective disorders*, *104*(1-3), 91–95. https://doi.org/10.1016/j.jad.2007.02.018
- Knapp, G., & Hartung, J. (2003). Improved tests for a random effects meta-regression with a single covariate. *Statistics in medicine*, *22*(17), 2693–2710. https://doi.org/10.1002/sim.1482
- Kroon, J. S., Wohlfarth, T. D., Dieleman, J., Sutterland, A. L., Storosum, J. G., Denys, D., de Haan, L., & Sturkenboom, M. C. (2013). Incidence rates and risk factors of bipolar disorder in the general population: a population-based cohort study. *Bipolar disorders*, *15*(3), 306–313. https://doi.org/10.1111/bdi.12058
- McConkey, G. A., Martin, H. L., Bristow, G. C., & Webster, J. P. (2013). Toxoplasma gondii infection and behaviour - location, location, location? *The Journal of experimental biology*, *216*(Pt 1), 113–119. https://doi.org/10.1242/jeb.074153
- Meira, C. S., Pereira-Chioccola, V. L., Vidal, J. E., de Mattos, C. C., Motoie, G., Costa-Silva, T. A., Gava, R., Frederico, F. B., de Mattos, L. C., & Toxoplasma Groups (2014). Cerebral and ocular toxoplasmosis related with IFN-γ, TNF-α, and IL-10 levels. *Frontiers in microbiology*, *5*, 492. https://doi.org/10.3389/fmicb.2014.00492
- Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS medicine*, *6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097
- Moore, J., Gotelli, N.J., Barnard, C.J., & Behnke, J.M. (1990). A phylogenetic perspective on the evolution of altered host behaviours: a critical look at the manipulation hypothesis.
- Moreira, A., Van Meter, A., Genzlinger, J., & Youngstrom, E. A. (2017). Review and Meta-Analysis of Epidemiologic Studies of Adult Bipolar Disorder. *The Journal of clinical psychiatry*, *78*(9), e1259–e1269. https://doi.org/10.4088/JCP.16r11165
- Murray F. Freeman, John W. Tukey "Transformations Related to the Angular and the Square Root," The Annals of Mathematical Statistics, Ann. Math. Statist. 21(4), 607-611, (December 1950)
- National Heart, Lung, and Blood Institute; National Institutes of Health; U.S. Department of Health and Human Services. Study Quality Assessment Tools 2013. https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools. (Accessed 16 december 2021)
- Nayeri Chegeni, T., Sarvi, S., Amouei, A., Moosazadeh, M., Hosseininejad, Z., A Aghayan, S., & Daryani, A. (2019). Relationship between toxoplasmosis and obsessive compulsive disorder: A systematic review and meta-analysis. *PLoS neglected tropical diseases*, *13*(4), e0007306. https://doi.org/10.1371/journal.pntd.0007306

- Ngoungou, E. B., Bhalla, D., Nzoghe, A., Dardé, M. L., & Preux, P. M. (2015). Toxoplasmosis and epilepsy--systematic review and meta analysis. *PLoS neglected tropical diseases*, *9*(2), e0003525. https://doi.org/10.1371/journal.pntd.0003525
- Oliveira, J., Oliveira-Maia, A. J., Tamouza, R., Brown, A. S., & Leboyer, M. (2017). Infectious and immunogenetic factors in bipolar disorder. *Acta psychiatrica Scandinavica*, *136*(4), 409–423. https://doi.org/10.1111/acps.12791
- Oswald, P., Souery, D., Kasper, S., Lecrubier, Y., Montgomery, S., Wyckaert, S., Zohar, J., & Mendlewicz, J. (2007). Current issues in bipolar disorder: a critical review. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, *17*(11), 687–695. https://doi.org/10.1016/j.euroneuro.2007.03.006
- Parks S, Avramopoulos D, Mulle J, McGrath J, Wang R, Goes FS, et al. HLA typing using genome wide data reveals susceptibility types for infections in a psychiatric disease enrichedsample. *Brain, Behavior, and Immunity* 2018; 70: 203–13.
- Pope, S. M., & Lässer, C. (2013). Toxoplasma gondii infection of fibroblasts causes the production of exosome-like vesicles containing a unique array of mRNA and miRNA transcripts compared to serum starvation. *Journal of extracellular vesicles*, *2*, 10.3402/jev.v2i0.22484. https://doi.org/10.3402/jev.v2i0.22484
- R Core Development Team. R: a language and environment for statistical computing, 3.2.1. https://www.r-project.org/2015. (accessed 15 April 2021)
- Robert-Gangneux, F., & Dardé, M. L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical microbiology reviews*, *25*(2), 264–296. https://doi.org/10.1128/CMR.05013-11
- Rybakowski J. (2021). Etiopathogenesis of bipolar affective disorder - the state of the art for 2021. Etiopatogeneza choroby afektywnej dwubiegunowej – stan wiedzy na rok 2021. *Psychiatria polska*, *55*(3), 481–496. https://doi.org/10.12740/PP/132961
- Schwarcz, R., & Hunter, C. A. (2007). Toxoplasma gondii and schizophrenia: linkage through astrocyte-derived kynurenic acid? *Schizophrenia bulletin*, *33*(3), 652–653. https://doi.org/10.1093/schbul/sbm030
- Snijders, G., van Mierlo, H. C., Boks, M. P., Begemann, M., Sutterland, A. L., Litjens, M., Ophoff, R. A., Kahn, R. S., & de Witte, L. D. (2019). The association between antibodies to neurotropic pathogens and bipolar disorder: A study in the Dutch Bipolar (DB) Cohort and meta-analysis. *Translational psychiatry*, *9*(1), 311. https://doi.org/10.1038/s41398-019-0636-x
- Stich, O., Andres, T. A., Gross, C. M., Gerber, S. I., Rauer, S., & Langosch, J. M. (2015). An observational study of inflammation in the central nervous system in patients with bipolar disorder. *Bipolar disorders*, *17*(3), 291–302. https://doi.org/10.1111/bdi.12244
- Sundaresh, A., Wu, C. L., Chinnadurai, R. K., Rajkumar, R. P., Mariaselvam, C. M., LeMaoult, J., Krishnamoorthy, R., Leboyer, M., Negi, V. S., & Tamouza, R. (2018). The HLA-G Genetic Contribution to Bipolar Disorder: A Trans-Ethnic Replication. *Immunological investigations*, *47*(6), 593–604. https://doi.org/10.1080/08820139.2018.1469649
- Sutterland, A. L., Fond, G., Kuin, A., Koeter, M. W., Lutter, R., van Gool, T., Yolken, R., Szoke, A., Leboyer, M., & de Haan, L. (2015). Beyond the association. Toxoplasma gondii in schizophrenia, bipolar disorder, and addiction: systematic review and meta-analysis. *Acta psychiatrica Scandinavica*, *132*(3), 161–179. https://doi.org/10.1111/acps.12423

- Tanaka, T., Matsuda, T., Hayes, L. N., Yang, S., Rodriguez, K., Severance, E. G., Yolken, R. H., Sawa, A., & Eaton, W. W. (2017). Infection and inflammation in schizophrenia and bipolar disorder. *Neuroscience research*, *115*, 59–63. https://doi.org/10.1016/j.neures.2016.11.002
- Tedla, Y., Shibre, T., Ali, O., Tadele, G., Woldeamanuel, Y., Asrat, D., Aseffa, A., Mihret, W., Abebe, M., Alem, A., Medhin, G., & Habte, A. (2011). Serum antibodies to Toxoplasma gondii and Herpesvidae family viruses in individuals with schizophrenia and bipolar disorder: a case-control study. *Ethiopian medical journal*, *49*(3), 211–220.
- Thebault, A, Kooh P, Cadavez V, Gonzales-Barron U, Villena I, Risk factors for sporadic toxoplasmosis: A systematic review and meta-analysis*, Microbial Risk Analysis* 2021; 17 100133. DOI: 10.1016/j.mran.2020.100133
- Veroniki, A. A., Jackson, D., Viechtbauer, W., Bender, R., Bowden, J., Knapp, G., Kuss, O., Higgins, J. P., Langan, D., & Salanti, G. (2016). Methods to estimate the between-study variance and its uncertainty in meta-analysis. *Research synthesis methods*, *7*(1), 55–79. https://doi.org/10.1002/jrsm.1164
- Viechtbauer, W. (2010). Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software*, *36*(3), 1–48. https://doi.org/10.18637/jss.v036.i03
- Wang, X., Zhang, L., Lei, Y., Liu, X., Zhou, X., Liu, Y., Wang, M., Yang, L., Zhang, L., Fan, S., & Xie, P. (2014). Meta-analysis of infectious agents and depression. *Scientific reports*, *4*, 4530. https://doi.org/10.1038/srep04530
- Webster J. P. (2001). Rats, cats, people and parasites: the impact of latent toxoplasmosis on behaviour. *Microbes and infection*, *3*(12), 1037–1045. https://doi.org/10.1016/s1286-4579(01)01459-9
- Webster J. P. (2007). The effect of Toxoplasma gondii on animal behavior: playing cat and mouse. *Schizophrenia bulletin*, *33*(3), 752–756. https://doi.org/10.1093/schbul/sbl073
- Weiss, L. M., & Dubey, J. P. (2009). Toxoplasmosis: A history of clinical observations. *International journal for parasitology*, *39*(8), 895–901. https://doi.org/10.1016/j.ijpara.2009.02.004
- Xiao, Y., Yin, J., Jiang, N., Xiang, M., Hao, L., Lu, H., Sang, H., Liu, X., Xu, H., Ankarklev, J., Lindh, J., & Chen, Q. (2010). Seroepidemiology of human Toxoplasma gondii infection in China. *BMC infectious diseases*, *10*, 4. https://doi.org/10.1186/1471-2334-10-4
- Xu, F., Ma, X., Zhu, Y., Sutterland, A., Cheng, R., Miao, S., Chen, J., Qiu, L., & Zhou, Y. (2020). Effects of Toxoplasma gondii infection and schizophrenia comorbidity on serum lipid profile: A population retrospective study from Eastern China. *Microbial pathogenesis*, *149*, 104587. https://doi.org/10.1016/j.micpath.2020.10458
- Yolken, R., Torrey, E. F., & Dickerson, F. (2017). Evidence of increased exposure to Toxoplasma gondii in individuals with recent onset psychosis but not with established schizophrenia. *PLoS neglected tropical diseases*, *11*(11), e0006040. https://doi.org/10.1371/journal.pntd.0006040
- Zhang, Z., Li, Y., Li, H., Song, X., Ma, Z., Lu, H., Liu, S., Zhao, Y., Tan, M., Wang, S., & Li, X. (2020). Identification of *Toxoplasma Gondii* Tyrosine Hydroxylase (TH) Activity and Molecular Immunoprotection against Toxoplasmosis. *Vaccines*, *8*(2), 158. https://doi.org/10.3390/vaccines8020158

Supplementary material

Table A1

Quality Assessment Tool for observational cohort and cross-sectional studies (National Heart, Lung, and Blood Institute, 2013)																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	Hinze-Selch 2010	Xiao 2010	Tedla 2011	Gerber 2012	Khademvatan 2013	Hamdani 2014	Stich 2015	Dalimiasl 2016	Kheirandish 2016	Oliveira 2016	Abdollahian 2017	Tanaka 2017	Yolken 2017	Sundaresh 2018	Chen 2018	Hamdani 2018	Afifi 2018	Parks 2018	Alvarado-Esquivel 2019	Frye 2019	Snjiders 2019	Hussein 2020	Xu 2020
1.	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
2.	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	X	V	V	V	V	V	V
3.	NR	NR	NR	NR	NR	NR	V	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
4.	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
5.	X	X	X	V	X	X	V	X	X	V	X	V	X	X	X	V	X	V	X	X	V	X	V
6.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	V	X	X	X
7.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
8.	V	X	X	X	X	X	V	X	X	X	X	V	X	X	X	V	X	X	X	V	V	X	X
9.	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
10.	X	V	X	X	X	X	V	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
11.	V	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	V	X	V	V	V	V	V
12.	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	X	NR	NR	NR	NR	NR	V	NR	NR	NR	NR	NR
13.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14.	X	X	X	V	X	X	X	X	X	V	X	X	V	X	X	V	X	X	X	V	V	X	X
Mean	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**poor**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**	**poor**	**fair**	**fair**	**fair**	**fair**	**fair**	**fair**

QUALITY RATING: 0-4 poor/5-10 fair/11-14 good. V=Yes; X=NO; NR=Not reported; NA= Not Applicable

1. Was the research question or objective in this paper clearly stated?
2. Was the study population clearly specified and defined?
3. Was the participation rate of eligible persons at least 50%?
4. Were all the subjects selected or recruited from the same or similar populations?
5. Was a sample size justification, power description, or variance and effect estimates provided?
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if existed?
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure?
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
10. Was the exposure(s) assessed more than once over time?
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented? consistently across all study participants?
12. Were the outcome assessors blinded to the exposure status of participants?
13. Was loss to follow-up after baseline 20% or less?
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship? between exposure(s) and outcome(s)?

Fig. A2a

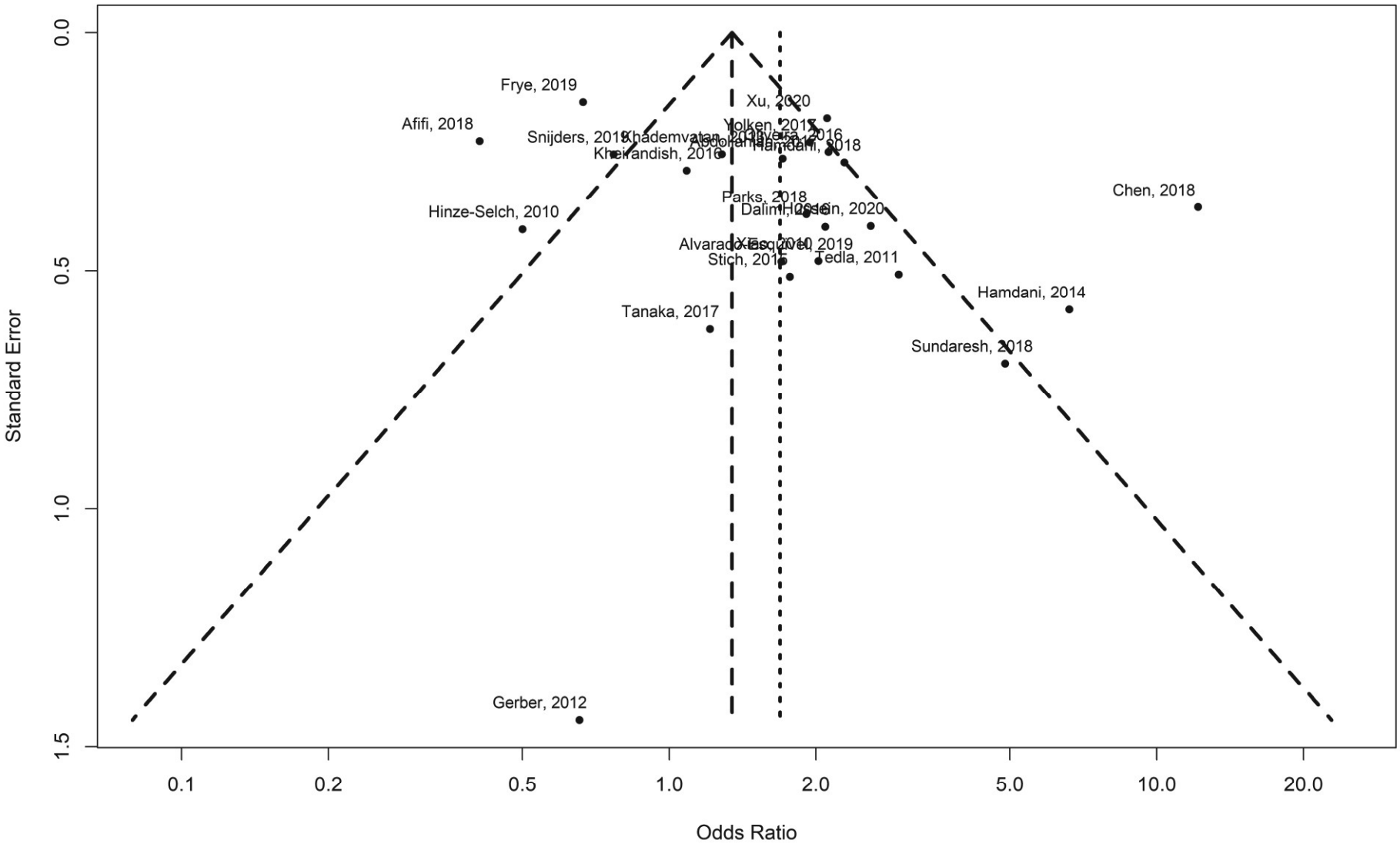

Standard Error
0.0
0.5
1.0
1.5
Frye, 2019
Afifi, 2018
Snijders, 2019
Khademvatan, 2016
Khorandish, 2016
Xu, 2020
Volken, 2015
Hamdani, 2018
Parks, 2018
Chen, 2018
Hinze-Selch, 2010
Dalimi Hosein, 2020
Alvarado-Esquivel, 2019
Stich, 2015
Tedla, 2011
Hamdani, 2014
Tanaka, 2017
Sundaresh, 2018
Gerber, 2012
0.1
0.2
0.5
1.0
2.0
5.0
10.0
20.0
Odds Ratio

Fig. A2b

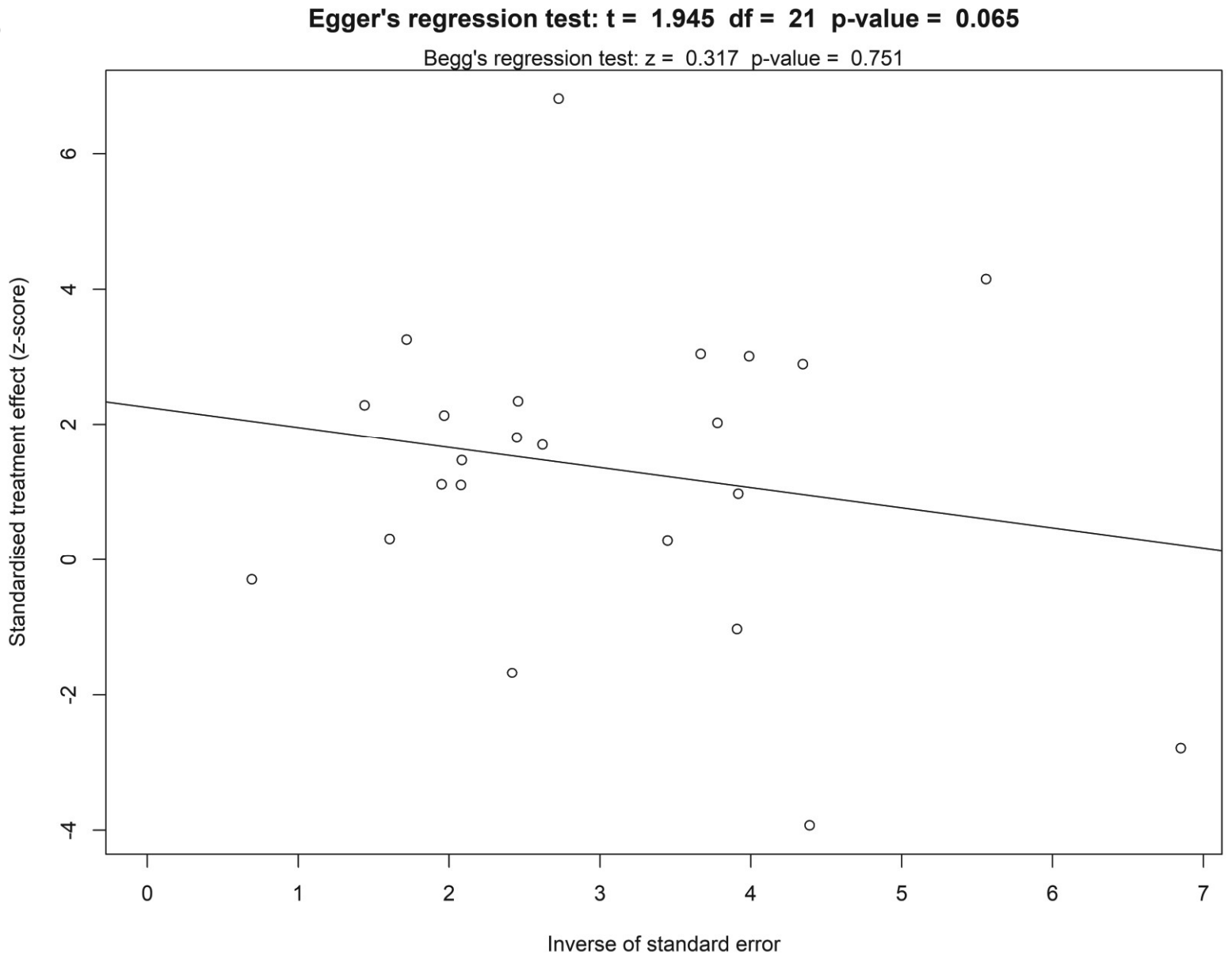

Egger's regression test: t = 1.945 df = 21 p-value = 0.065
Begg's regression test: z = 0.317 p-value = 0.751
Standardised treatment effect (z-score)
Inverse of standard error
6
4
2
0
-2
-4
0
1
2
3
4
5
6
7

Fig A3

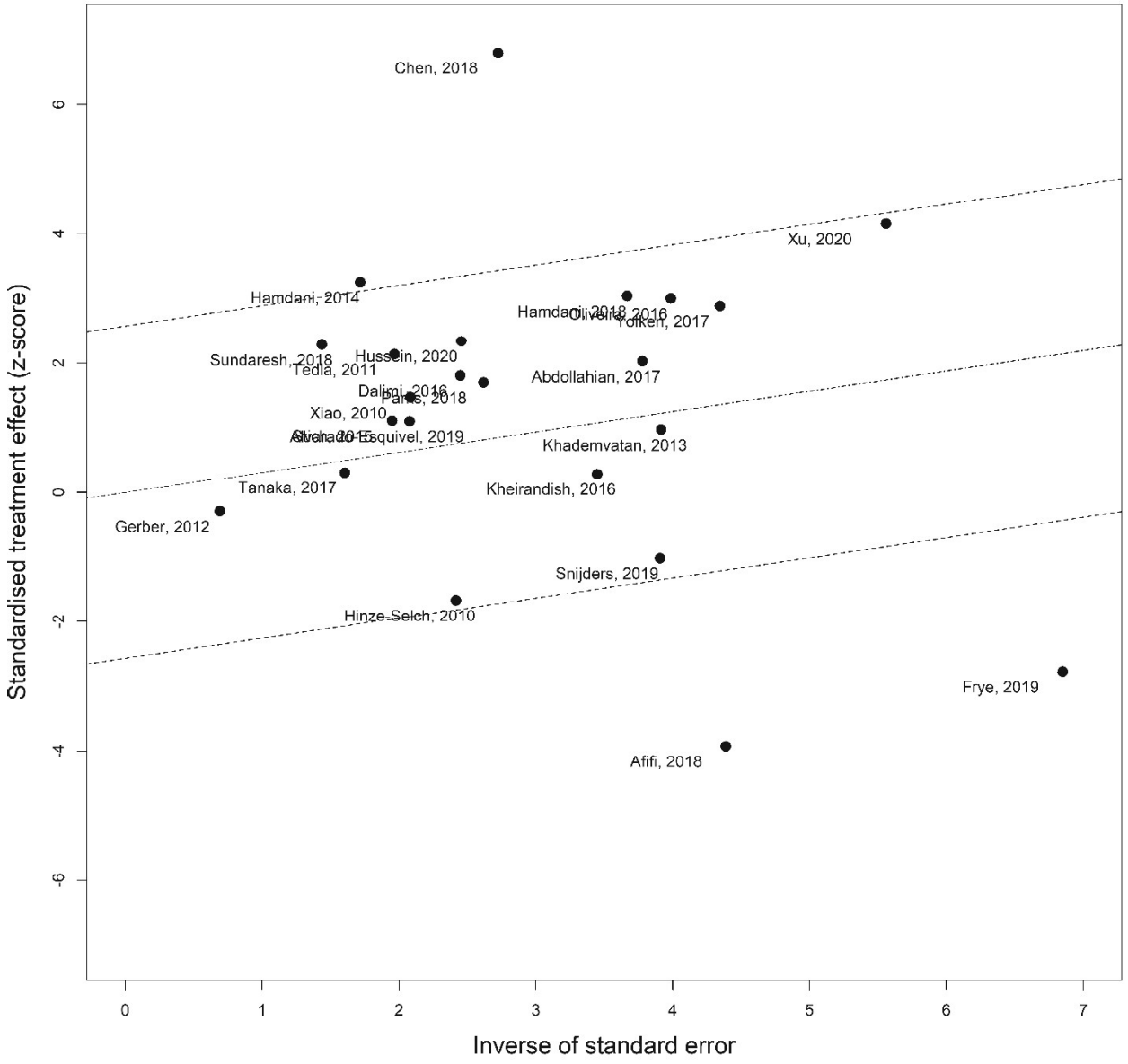
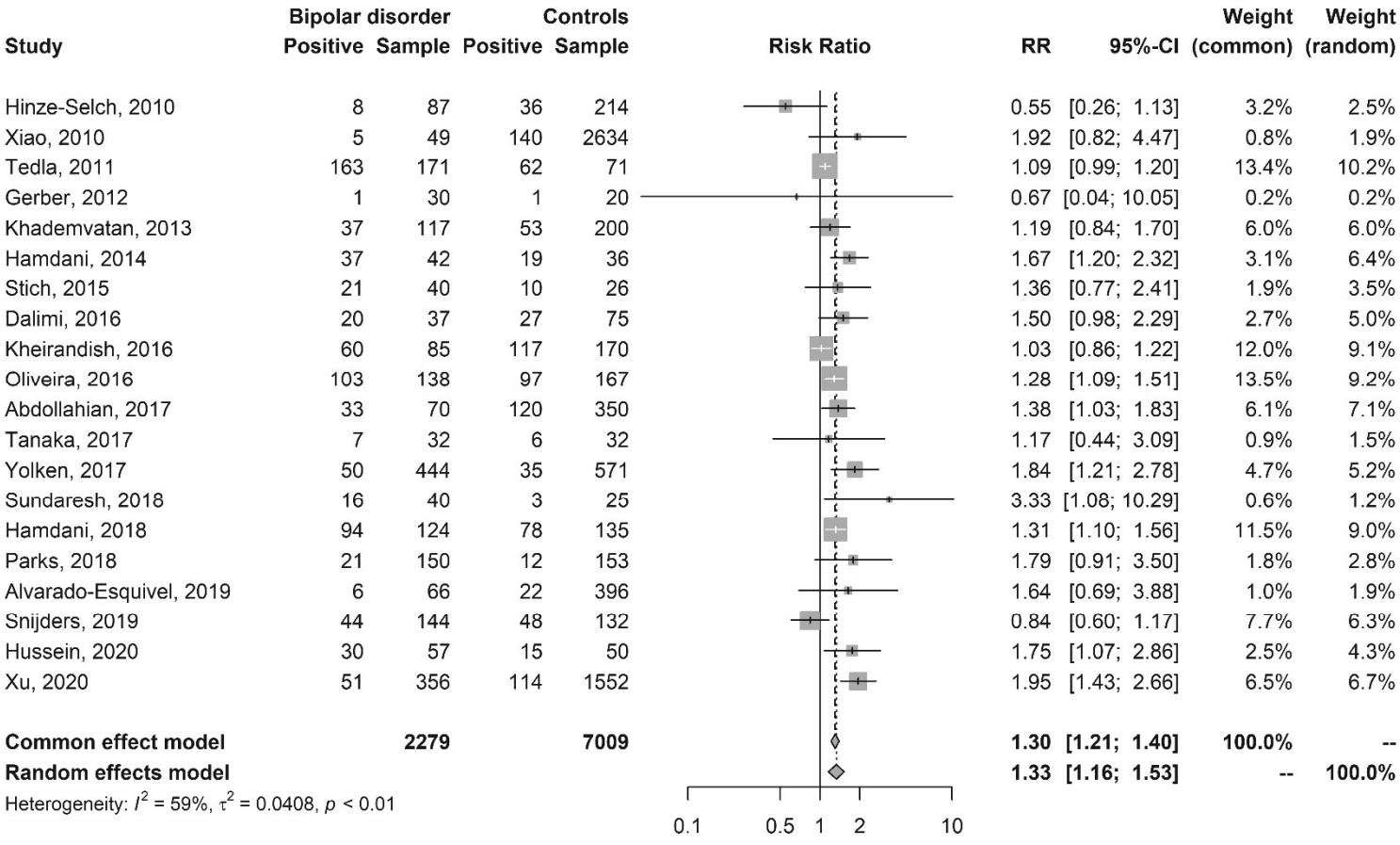

Chen, 2018
Xu, 2020
Hamdani, 2014
Hamdani, 2018
Oliveira, 2016
Tolken, 2017
Sundaresh, 2018
Hussein, 2020
Tedla, 2011
Abdollahian, 2017
Dalimi, 2016
Parks, 2018
Xiao, 2010
Khademvatan, 2013
Tanaka, 2017
Kheirandish, 2016
Gerber, 2012
Snijders, 2019
Hinze-Selch, 2010
Frye, 2019
Afifi, 2018
Standardised treatment effect (z-score)
Inverse of standard error
6
4
2
0
-2
-4
-6
0
1
2
3
4
5
6
7

Fig A4


Bipolar disorder
Controls
Study
Positive Sample Positive Sample
Risk Ratio
RR 95%-CI
Weight (common)
Weight (random)
Hinze-Selch, 2010 8 87 36 214 0.55 [0.26; 1.13] 3.2% 2.5%
Xiao, 2010 5 49 140 2634 1.92 [0.82; 4.47] 0.8% 1.9%
Tedla, 2011 163 171 62 71 1.09 [0.99; 1.20] 13.4% 10.2%
Gerber, 2012 1 30 1 20 0.67 [0.04; 10.05] 0.2% 0.2%
Khademvatan, 2013 37 117 53 200 1.19 [0.84; 1.70] 6.0% 6.0%
Hamdani, 2014 37 42 19 36 1.67 [1.20; 2.32] 3.1% 6.4%
Stich, 2015 21 40 10 26 1.36 [0.77; 2.41] 1.9% 3.5%
Dalimi, 2016 20 37 27 75 1.50 [0.98; 2.29] 2.7% 5.0%
Kheirandish, 2016 60 85 117 170 1.03 [0.86; 1.22] 12.0% 9.1%
Oliveira, 2016 103 138 97 167 1.28 [1.09; 1.51] 13.5% 9.2%
Abdollahian, 2017 33 70 120 350 1.38 [1.03; 1.83] 6.1% 7.1%
Tanaka, 2017 7 32 6 32 1.17 [0.44; 3.09] 0.9% 1.5%
Yolken, 2017 50 444 35 571 1.84 [1.21; 2.78] 4.7% 5.2%
Sundaresh, 2018 16 40 3 25 3.33 [1.08; 10.29] 0.6% 1.2%
Hamdani, 2018 94 124 78 135 1.31 [1.10; 1.56] 11.5% 9.0%
Parks, 2018 21 150 12 153 1.79 [0.91; 3.50] 1.8% 2.8%
Alvarado-Esquivel, 2019 6 66 22 396 1.64 [0.69; 3.88] 1.0% 1.9%
Snijders, 2019 44 144 48 132 0.84 [0.60; 1.17] 7.7% 6.3%
Hussein, 2020 30 57 15 50 1.75 [1.07; 2.86] 2.5% 4.3%
Xu, 2020 51 356 114 1552 1.95 [1.43; 2.66] 6.5% 6.7%
Common effect model 2279 7009 1.30 [1.21; 1.40] 100.0% --
Random effects model 1.33 [1.16; 1.53] -- 100.0%
Heterogeneity: I^2 = 59%, τ^2 = 0.0408, p < 0.01
0.1
0.5
1
2
10