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Review

Homocysteine as a peripheral biomarker in bipolar disorder: A meta-analysis

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ABSTRACT

Background: Bipolar disorder (BD) is a psychiatric disorder with an uncertain aetiology. Recently, special attention has been given to homocysteine (Hcy), as it has been suggested that alterations in 1-carbon metabolism might be implicated in diverse psychiatric disorders. However, there is uncertainty regarding possible alterations in peripheral Hcy levels in BD.
Methods: This study comprises a meta-analysis comparing serum and plasma Hcy levels in persons with BD and healthy controls. We conducted a systematic search for all eligible English and non-English peer-reviewed articles.
Results: Nine cross-sectional studies were included in the meta-analyses, providing data on 1547 participants. Random-effects meta-analysis showed that serum and plasma levels of Hcy were increased in subjects with BD in either mania or euthymia when compared to healthy controls, with a large effect size in the mania group ($g = 0.98$, 95% CI: 0.8–1.17, $P < 0.001$, $n = 495$) and a small effect in the euthymia group ($g = 0.3$, 95% CI: 0.11–0.48, $P = 0.002$, $n = 1052$).
Conclusions: Our meta-analysis provides evidence that Hcy levels are elevated in persons with BD during mania and euthymia. Peripheral Hcy could be considered as a potential biomarker in BD, both of trait (since it is increased in euthymia), and also of state (since its increase is more accentuated in mania). Longitudinal studies are needed to clarify the relationship between bipolar disorder and Hcy, as well as the usefulness of peripheral Hcy as both a trait and state biomarker in BD.

1. Introduction

In order to better understand the physiopathology underlying bipolar disorder (BD), increasing research efforts have attempted to identify potential biomarkers in the peripheral blood of those with BD and also to advance the elusive field of precision psychiatry [1–4]. Neurotrophins such as BDNF [5–9] and inflammatory markers such as C-reactive protein [10–12] have

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consistently been proposed as promising peripheral biomarkers in BD. More recently, special attention is being given to homocysteine (Hcy) [13], as it has been suggested that alterations in 1-carbon metabolism might be implicated in psychiatric disorders, including BD [14–17].

In the 1-carbon metabolism, a carbon unit from serine or glycine is transferred to tetrahydrofolate (THF) to form methylene-THF. Homocysteine is a non-protein and nonessential amino acid sulfur with a central role in 1-carbon metabolism, and its regulation depends on multiple enzymes, with methylenetetrahydrofolate reductase (MTHFR) the most extensively investigated. Folate and vitamin B12 are also key elements of Hcy metabolism, since they act as enzymatic cofactors [18,19]. Accordingly, either a reduced enzymatic activity of MTHFR or a nutritional deficiency in folate or vitamin B12 may lead to hyperhomocysteinemia [19–21]. This may promote neurotoxic and vasculotoxic effects by several proposed mechanisms, including but not limited to mitochondrial dysfunction [22], oxidative stress induction [14,23,24], inflammation [25], neuroapoptosis [26,27], direct vascular damage [25,28], aberrant DNA methylation [29,30] and impaired DNA synthesis [31]. These pathways overlap considerably with those pathways documented as drivers of the process of neuroprogression evident in BD [32]. Equally, this is concordant with the notion of shared pathways to comorbidity of both psychiatric and common medical disorders [18,33].

Hyperhomocysteinemia is a known risk factor for cardiovascular diseases [34] and Alzheimer's dementia [35,36]. Additionally, several studies have described an association between higher levels of Hcy and depression [37–41], autism [42] and schizophrenia [17,29,43]. In addition, a recent meta-analysis by Numata et al. provided evidence that increased Hcy levels is causally related with an increased risk of developing schizophrenia using a Mendelian Randomization analysis [16]. In BD, higher levels of peripheral Hcy have been associated with worse cognitive performance [44,45].

Contrary to schizophrenia, where better evidence exists, data on peripheral levels of Hcy in BD are more limited, with discrepant findings across studies conducted to date; some studies show increased levels of Hcy in persons with BD when compared to healthy controls [46], while others find no evidence for this association [47]. The aim of this study was therefore to verify if alterations in peripheral Hcy levels are present in BD in the different mood states compared to healthy controls. To this end, we performed a meta-analysis of all available cross-sectional studies that measured peripheral Hcy levels in BD compared to healthy subjects in order to evaluate the potential of Hcy as a biomarker in BD. The null hypothesis was that there would be no difference between individuals with BD compared to healthy controls.

2. Methods

We performed a meta-analysis comparing peripheral levels of Hcy in subjects with BD across different mood states versus healthy controls. We adhered to the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) statement and guidelines from the Cochrane Collaboration [48]. The literature search, decisions on inclusion, data extraction and quality control were all independently performed by two of the authors (E.S., B.S.F.). Disagreements were resolved through consensus. An a priori defined but unpublished protocol was followed.

2.1. Search strategy

We conducted a systematic search for all potentially eligible peer-reviewed articles using PubMed and SCOPUS in January 2016. We included studies published in English, Dutch, French, German, Italian, Portuguese and Spanish, with no year or country restrictions [48]. The Boolean terms used for the electronic database search were: (homocysteine or MTHFR or methylenetetrahydrofolate reductase or folate or folic acid or B12) and (bipolar or mania or psychosis). We manually searched bibliographies of the identified articles in order to identify further relevant references that might have been missed in the initial search. Study selection eligibility and exclusion criteria were pre-specified.

2.2. Study selection

We included studies meeting the following inclusion criteria:

- adult subjects with diagnosis of BD, as defined by Diagnostic and Statistical Manual of Mental Disorders (DSM), regardless of current mood state;
- pairwise comparison with a control group of healthy volunteers
- studies that assessed homocysteine levels *in vivo*.

Exclusion criteria were:

- duplicate reports;
- studies conducted in subjects aged less than 18 years;
- reviews or meta-analyses articles;
- lack of a control group of healthy volunteers;
- genetic studies not measuring Hcy levels;
- studies that included samples with mixed psychiatric diagnoses unless data for BD were reported separately or were obtained after contacting the authors;
- studies not specifying the mood state of the subjects at the time Hcy was assessed;
- studies which did not report data on subjects with BD separately according to mood state;
- animal studies.

We used the Newcastle–Ottawa Scale (NOS) for case-control studies [49] as recommended by the Cochrane Collaboration [50] to assess the quality of the eligible studies. Overall, quality score was defined as the frequency of criteria that were met by the particular study. The NOS scale contains eight items for assessing the quality of case-control studies, categorised into the three domains of selection, comparability, and exposure. A series of response options is provided for each item. A star system was used to enable semi-quantitative assessment of study quality, such that the highest quality studies are awarded a maximum of one star for each item, with the exception of the comparability domain, which allows the assignment of two stars. As such, the Newcastle–Ottawa scale ranges between zero and nine stars [49]. Item "non-response rate" from Exposure in the case-control scale was not applicable; therefore, a maximum of eight stars was considered. The quality score of the included was four and five. All studies were included in the posterior analyses.

2.3. Data extraction

Two independent reviewers extracted data [*n*, mean and standard deviation (SD)] using a predesigned form [48]. Whenever necessary, authors of included studies were electronically contacted to provide data in at least two separate occasions. Whenever data on the same participants were provided by different articles, only the most comprehensive data set was included. When necessary, means and SD were calculated from available graphs using procedures described in details elsewhere [51].

All variables were extracted separately for each mood state (euthymia, mania or depression). We extracted the following data: sample size, age, sex, length of illness in years, body mass index

(BMI), smoking status, mean score of the Hamilton Depression Rating Scale (HDRS) [52] and the Young Mania Rating Scale (YMRS) [53], medications in use, study population, type of assay, and sampling source (plasma or serum).

2.4. Publication bias

Studies with negative (i.e., non-significant) results are less likely to be published than studies with positive (i.e., statistically significant) results. To account for potential publication bias, an acknowledged problem in the field of peripheral biomarkers in psychiatric disorders including BD and major depressive disorder [54,55], we inspected funnel plot graphs, which are scatter plots of the effect size (ES) against a measure of study sample size, and the Egger's regression test [56]. In addition, the Orwin's fail-safe N test (file drawer statistic) was used to quantify the number of possible negative omitted studies that would be required to render statistically significant results non-significant (i.e., $P > 0.05$) [57].

2.5. Statistical analysis

Our primary aim was to quantify differences in peripheral Hcy levels in persons with BD compared to healthy volunteers. We employed Comprehensive Meta-Analysis Software (CMA) version 2.0 in all analyses. Since eligible studies included different assay methods, we used standardized mean differences to estimate differences in peripheral Hcy levels in individuals with BD compared to healthy controls. We used Hedges' adjusted *g*, as it provides a relatively unbiased ES estimate adjusted for sample size. We also calculated the 95% confidence interval (95% CI) of the ES. An ES of 0.2 was considered as a small ES, i.e., it indicates a small difference in Hcy levels between subjects with BD and controls, while an ES of 0.5 was considered as a moderate effect and an ES $\geq$ 0.8 as a large effect [58]. The direction of the ES values was negative if subjects with BD showed decreased Hcy levels when compared to healthy controls, and positive if they showed increased Hcy levels.

Heterogeneity across studies was assessed using the Cochran Q test, a weighted sum of the squares of the deviations of individual study ES estimates from the summary ES estimate, and a *P* value of < 0.10 was considered significant (i.e., indicating heterogeneity). Inconsistency across studies was also evaluated using the I^2 metric, which describes the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error. An $I^2 > 50\%$ is considered indicative of substantial heterogeneity [48].

Both measures of heterogeneity (i.e., the Cochran Q test and the I^2 metric) have a limited power to detect heterogeneity unless very large data sets are available. Thus, we pooled ES estimates of individual studies using random-effects modelling, which allows population-level inferences and is more stringent than fixed-effects models. Random-effects models assume that the true effect size differs across studies and incorporates a between-study variance into the calculations [48,59].

Unrestricted maximum likelihood random-effects meta-regressions of ES were performed in euthymic subjects, with mean age and percentage of female sex as moderators, to determine whether these covariates influenced the ES. Meta-regression analyses with data from fewer than five datasets may provide spurious findings [48]. Therefore, we explored potential sources of heterogeneity with this tool only when independent data from at least five studies were available. Studies were weighted such that the studies with the most precise parameters, quantified by the sample size and 95% CI, had more influence in the regression analyses.

The meta-analyses consisted of three steps. Firstly, we performed the meta-analyses according to mood state. As only a single study assessing Hcy levels in BD subjects with a depressive episode was identified [60], we limited our analyses to mania and euthymia. Secondly, sensitivity analyses were conducted to ascertain whether the results of our analyses were strongly influenced by any single study or studies sharing some characteristics. The overall significance was recomputed after each study or group of studies with a common feature were deleted from the analysis. Thirdly, we performed meta-regression analyses in order to investigate possible moderators of Hcy levels. The level of significance was set at $P < 0.05$.

3. Results

3.1. Selection and inclusion of studies

In total, 2597 studies were identified through the systematic electronic search. Of those, we excluded 2540 on the basis of title and abstract. We went through 57 full-text articles of which: two were excluded because they were duplicates of included studies with overlapping samples [61,62]; eighteen studies were excluded because they lacked a control group [38,46,63–78]; twelve were excluded because they were not performed in subjects with a diagnosis of BD [41,79–89]; twelve were excluded because they did not measure Hcy levels [90–101]; and two were excluded because they presented mixed data on BD and other psychiatric disorders [102,103]. The authors of a study that analysed together the total mean Hcy level for subjects with BD in different mood states were contacted to request mean Hcy levels separately according to mood states. Since no response was provided, this article was also excluded [104]. In total, we identified 10 studies fulfilling our inclusion criteria, of which two studies measured Hcy levels in mania (227 persons with BD and 268 healthy controls) [105,106], eight in euthymia (436 persons with BD and 616 healthy controls) [44,106–112] and one in depression [60] (Fig. 1). As there was only one article assessing Hcy levels in persons with BD in depression, which found no differences in Hcy levels between persons with BD and the control group [60], a meta-analysis could not be conducted. All studies included in the analyses were cross-sectional and they provided data on 1547 participants; of these, 663 comprised subjects with BD and 884 were healthy controls. One study [106] provided more than one pairwise comparison.

3.2. Study characteristics

The characteristics of the included studies are summarized in Table 1. The studies were published from 2004 to 2013. They included participants of either sex with a mean age between 33.6 and 49.8. The diagnosis of bipolar disorder was made according to the DSM-IV. Severity of affective symptoms was assessed with the YMRS for manic symptoms and the HDRS for depressive symptoms. Only one study provided data on YMRS in the mania group [106] and the median score was 27.5 (19–30.7). The euthymia group was defined as scoring less than 6 on the YMRS and less than 8 on the HDRS across studies.

In six of the nine studies, the control group was matched by sex and age [90,106–108,111,112]. All subjects with BD were on psychopharmacological treatment. Seven studies provided details on the medications used [105,107–112], the most common being mood stabilizers, antidepressants, and first and second generation antipsychotics. All studies excluded subjects with either long-term somatic conditions or substance use disorders.

3.3. Hcy levels are increased in BD compared to healthy controls

We included two studies in the meta-analysis of Hcy levels in subjects with BD in manic state compared with healthy controls [105,106] and eight studies in the meta-analysis that compared subjects with BD in euthymic state compared with healthy

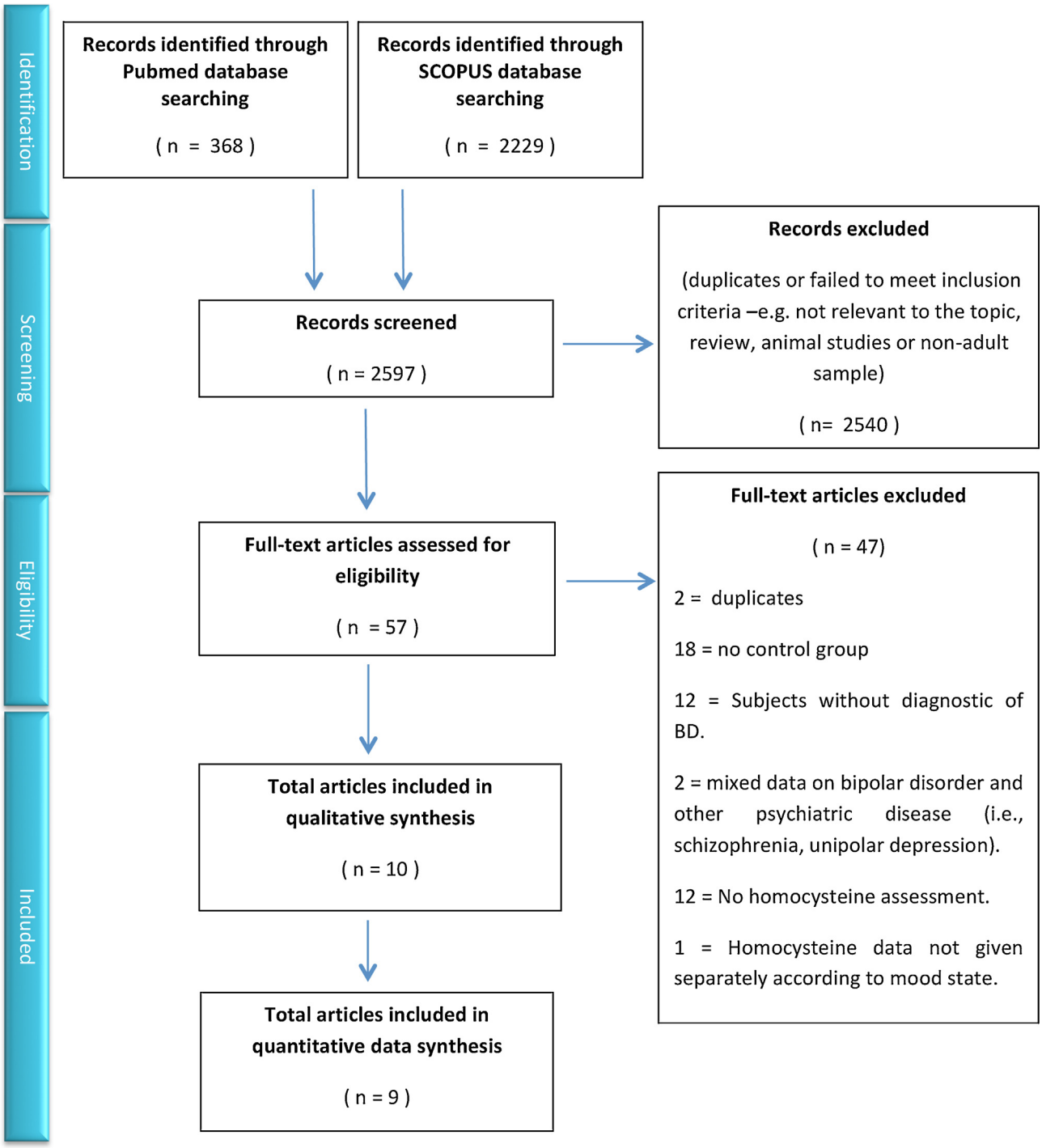

Fig. 1. Flow diagram of the systematic review, showing the study inclusion and exclusion process. Some studies included subjects in more than one mood state, being included in more than one analysis.

controls [44,106–112]. Random-effects meta-analysis showed that serum and plasma levels of Hcy were increased in subjects with BD either in mania or in euthymia when compared to healthy controls, with a large effect in the mania group ($g = 0.98$, 95% CI: 0.80–1.17, $P < 0.001$, 2 between-group comparisons, $n = 495$) and a small effect in the euthymia group ($g = 0.30$, 95% CI: 0.11–0.48, $P = 0.002$, 8 between-group comparisons, $n = 1052$) (Fig. 2, Table 2).

3.4. Hcy levels in BD are not associated with age or sex

We performed meta-regression analyses to identify possible effect moderators. Since there were a small number of studies assessing Hcy in mania, we only performed the meta-regression analyses for the euthymia group. In univariable meta-regression models for euthymia, we found that neither age (slope = –0.01, 95% CI: –0.04 to 0.03, $P = 0.711$) nor sex (slope = 0.001, 95% CI: –0.02 to 0.02, $P = 0.943$) were related to the difference in Hcy levels in euthymia between those with BD and controls (Table 2).

3.5. Sensitivity analyses and publication bias

We found no heterogeneity between studies performed in persons with BD in manic state ($I^2 = 0\%$, $P = 0.39$). In contrast, heterogeneity was moderate between studies performed in

Table 1
Demographic characteristics of studies included in the meta-analysis of serum or plasma homocysteine (Hcy) levels in bipolar disorder.

Study	State and subjects characteristics	*n* (male/female)	Age[a]	YMRS[a] (SD)	HDRS[a] (SD)	Length of illness (years)[a]	Source and assay	Comorbidities and exclusion criteria	Medication
Osher et al., 2004	*Euthymia* Outpatients, euthymic for $\geq$1 month	41 (23/18)	49.8	NA	NA	NA	Serum, high-pressure liquid chromatography (HPLC) with fluorescence detection	Patients with diabetes, cardiovascular disease, renal disease, endocrinological disease, neurological disease or substance abuse were excluded	Lithium carbonate; Valproic acid; Carbamazepine, antipsychotics and antidepressants
	Control	305 (151/154)	49.7				Plasma, HPLC with fluorescence detection		
Osher et al., 2008	*Euthymia* Outpatients, euthymic for $\geq$1 month	57 (35/22)	39.4	<5	<7	13.54 (SD 10.3)	Serum, HPLC with fluorescence detection	Patients with any serious physical illness or substance abuse were excluded	Lithium carbonate; Valproic acid; Carbamazepine, typical or atypical antipsychotics and antidepressants
	Control	84 (41/43)	43.6				Plasma, HPLC with fluorescence detection		
Ozbeck et al., 2008	*Mania* Inpatients	197 (74/123)	40.5	NA	NA	NA	Serum, Fluorescence Polarization Immunoassay (FPIA)	Patients with neurological comorbidity, mental retardation, substance abuse, family or personal history of cardiovascular disease, endocrinological and metabolic disease were excluded	Typical and atypical antipsychotics or combination
	Control	238 (67/171)	41.3						
Dittman et al., 2008	*Euthymia* Outpatient in euthymia for $\geq$1 month on a stable treatment	74 (37/37)	42.5	1.01 (1.45)	1.36 (1.56)	16.7 (SD 10.6)	Plasma, HPLC with fluorescence detection	Patients with severe physical or neurological illnesses, present substance abuse, neurodegenerative disorders, mental retardation were excluded	Lithium carbonate; Valproic acid; lamotrigine; Oxcarbazepine; typical and atypical antipsychotic; antidepressant
	Control	42 (20/22)	43	0.10 (0.37)	0.19 (0.5)		Plasma, HPLC with fluorescence detection		
Bromberg et al., 2009	*Euthymia*	49 (29/20)	39	NA	NA	14.41 (SD 11.12)	Plasma, HPLC with fluorescence detection	Subjects with Alzheimer's disease, diabetes, cardiovascular disease or renal failure were excluded	Lithium carbonate; Valproic acid; other mood stabilizers
	Control	27 (17/10)	41.6				Plasma, HPLC with fluorescence detection		
Dias et al., 2009	*Euthymia* Outpatients in euthymia for $\geq$1 month	65 (24/41)	37.8	1 (1.72)	2.6 (2.36)	13.3 (SD 8.78)	Serum, HPLC with fluorescence detection	Patients with neurological disorders, head trauma, significant physical illnesses, substance abuse or ETC in the preceding 6 months were excluded	Patients under medication, treatment not detailed
	Control	49 (14/35)	33.6	0.1 (0.28)	0.7 (1.28)		Serum, HPLC with fluorescence detection		
Doğanavşargil-Baysal et al., 2013	*Euthymia* Outpatients	60 (NA)	NA	0.1 (0.54)	0.75 (1.14)	12.13 (SD 8.9)	Serum	Patients with neurological diseases, cardiovascular diseases, renal or hepatic diseases, diabetes mellitus, hypo/hyperthyroidism, non-psychotropic drug use that could affect Hcy levels, substance abuse, IQ $<$ 80 or ETC in the preceding 6 months were excluded	Lithium carbonate; Valproic acid; neuroleptics
	Control	20 (NA)	NA						

Table 1 (*Continued*)

Study	State and subjects characteristics	*n* (male/female)	Age[a]	YMRS[a] (SD)	HDRS[a] (SD)	Length of illness (years)[a]	Source and assay	Comorbidities and exclusion criteria	Medication
Vuksan-Cusa et al., 2013	*Euthymia* Inpatients, 1 day prior to their discharge from the hospital	60 (29/31)	44.4	≤5	≤7	10.95 (SD 9.47)	Serum, capillary gas chromatography-mass spectrometry	Patients with disorders of lipoprotein metabolism, substance abuse, eating disorders, neurological disorders, pregnancy and lactation, supplementation with B-vitamins, current treatment with fibrates/niacin/metformin/ anti-inflammatory/ immunosupressant drugs were excluded	Atypical antipsychotics, mood stabilizers
	Control	20 (11/9)	42.2						
Chiarani et al., 2013	*Mania* Inpatients	30 (15/15)	41.2	27.5 (19–30.7)	3.5 (1.7–5)	NA	Serum, Stable isotope dilution and electrospray tandem mass spectrometry	Patients with significant comorbid medical conditions were excluded	Patients under medication, treatment not detailed
	Euthymia	30 (15/15)	41.2	1 (0–4)	1 (0–2.5)	NA		Patients with significant comorbid medical conditions were excluded	Patients under medication, treatment not detailed
	Control	30 (15/15)							

YMRS: Young Mania Rating Scale; HDRS: Hamilton Depression Rating Scale; BMI: body mass index in kg/m^2; ETC: electroconvulsive therapy; NA: not available.
[a] Mean ± standard deviation, except for the study of Chiarani et al., which provides median and interquartile range.

euthymia (I^2 = 43.3%, P = 0.09). To examine potential sources of heterogeneity detected in the euthymia group, we conducted sensitivity analysis. We excluded studies one at a time, in order to define its individual influence on the estimated overall effect and, therefore, determine the robustness of the analyses, and we did not find any particular study being responsible for heterogeneity in the euthymia group.

In addition, we found no evidence of publication bias on the funnel plot. The trim-and-fill estimate did not suggest any missing studies and the Orwin's fail-safe N test suggested that 148 negative missing studies would be necessary to turn our statistically significant results non-significant [57].

4. Discussion

4.1. Summary of main findings

Our results indicate that higher Hcy levels are found in persons with BD in manic and euthymic states when compared to healthy controls. The meta-regression analyses performed in the euthymia group showed that these differences were not significantly moderated by age or sex. This is the first meta-analysis to assess peripheral Hcy as a putative biomarker in BD.

4.2. Implications

Although there is growing literature showing an association between increased Hcy levels and psychiatric disorders, the nature of this relationship remains unclear. The role of Hcy in the Central Nervous System (CNS) is complex, as it participates in a large number of processes. It has been proposed that an alteration in glutamatergic neurotransmission may be a main mechanism linking Hcy and psychiatric disorders, including BD [19,113]. Homocysteine and its related compounds may have a role as a partial excitatory agonist on the N-Methyl-D-aspartate (NMDA) subtype of glutamate receptors and also on modulatory sites. Thus, long-term activation of NMDA receptors due to hyperhomocysteinemia would result in an increased calcium ion influx, elevated intracellular second messenger calcium, and accumulation of reactive oxygen species and, ultimately, would lead to cell damage and induction of both apoptotic and necrotic cell death [19,26,114]. Higher Hcy levels induced by methylenetetrahydrofolate reductase deficiency alters glutamatergic and GABAergic levels and contributes to neurotoxicity in the CNS [115].

It has also been hypothesized that long exposure to excessive levels of Hcy in the CNS can activate inflammatory pathways involving oxidative stress and pro-inflammatory cytokines, producing neural toxicity and microvascular damage [20,47,116,117]. Moreover, the brain depends on the 1-carbon metabolism to obtain methyl groups, with homocysteine acting as a methyl donor through its transformation to S-adenosylmethionine (SAMs), which is the main methyl donor in the CNS [18,31]. Hence, dysregulation of the 1-carbon metabolism may promote aberrant DNA methylation, which has as well been proposed as a potential mechanism involved in the development of psychiatric illnesses, including BD [107,118–123]. Finally, the 1-carbon metabolism also participates in monoamine regulation, so alterations in Hcy metabolism could lead to inadequate monoamine production and, consequently to dysregulation of serotonin, norepinephrine and dopamine [31,47]. Hyperhomocysteinemia may interfere with cholinergic metabolism by reducing choline acetyltransferase activity in some neuronal populations potentially leading to cognitive effects [124]. Cognitive impairment is a key factor involved in psychosocial functioning [125], so the relevance of homocysteine in BD may go beyond its potential use as another

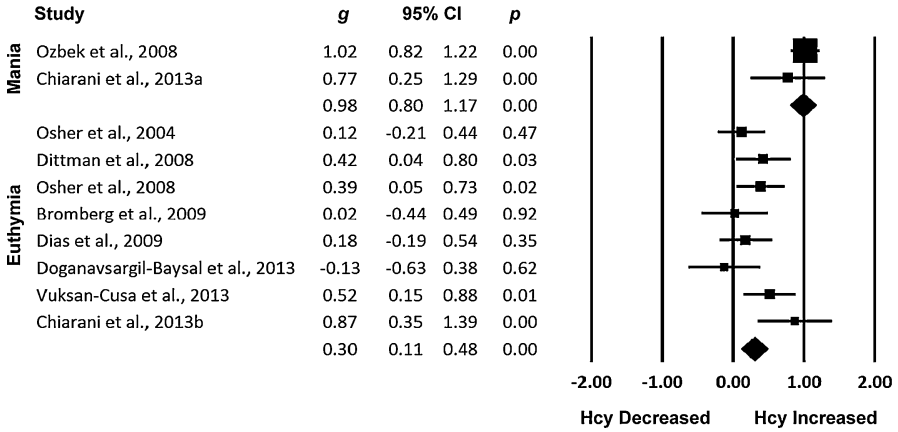

Fig. 2. Forest plot for random-effects meta-analysis on serum and plasma homocysteine levels in persons with bipolar disorder compared to healthy controls.

Table 2
Statistics on between-group meta-analysis regarding serum and plasma Hcy levels in bipolar disorder.

Group-wise	No. of pairwise	No. of subjects		Meta-analysis			Heterogeneity		
State		BD	HC	Hedges' *g*	95% CI	*P*	I^2	Q	*P*
Mania vs. HC	2	227	268	0.98	0.80–1.17	< 0.001	0.00	0.75	0.388
Euthymia vs. HC	8	436	616	0.30	0.11–0.48	0.002	43.32	12.35	0.09

Moderator	No. of pairwise	No. of subjects		Meta-regression			Meta-regression		
		BD	HC	Slope	95% CI	*P*	Intercept	*Z*	*P*
Age – *euthymia*	7	376	596	–0.01	–0.04 to 0.03	0.104	0.62	0.79	0.427
% Female – *euthymia*	7	376	596	0.01	–0.02 to 0.02	0.943	0.3	0.61	0.544

BD: bipolar disorder; HC: healthy controls.

peripheral biomarker but also as a tentative biomarker of prognosis pertaining neuroprogression. That would certainly need further longitudinal studies. The association between homocysteine levels and cognitive problems suggests that pharmacological interventions capable of decreasing Hcy levels could be beneficial in BD and, perhaps conversely, interventions that proved effective in functional and cognitive decline might decrease hyperhomocysteinemia [126,127]. Folate and vitamin B12 are key elements of Hcy metabolism, since they act as enzymatic cofactors that decrease Hcy production, with folate and vitamin B12 being cofactors for the methylation of Hcy into methionine and tetrahydrofolate (THF), which is the immediate acceptor of 1-carbon units for the synthesis of thymidine, purines (RNA and DNA), and methionine [18,19]. However, the benefit of prescribing folate to patients with depression without folate deficiency is controversial. One clinical trial found that folate supplementation using folic acid, the most commonly employed form of pharmacological supplementation, did not decrease the occurrence of depression in young people with increased risk of mood disorders, although it delayed the occurrence of the first episode of depression [128], and a clinical trial with 475 persons with an acute unipolar major depressive episode without vitamin deficiencies showed no benefit with folate [129]. A recent meta-analysis confirmed no short-term benefit of folate or B12 supplementation for depression, although there was a suggestion of a possible long-term benefit to prevent incidence or relapse [130].

However, this lack of effect might be due to the fact that the blood-brain barrier (BBB) is relatively impermeable to folic acid and folate, therefore the effect of supplementation with folic acid might only appear after several months, with a slow and cumulative effect of folate [131]; it has been hypothesised that probably methylfolate, the biologically active form of folate that can freely cross the BBB and cell membranes and that is also the predominant form of folate acquired through diet, would be a better choice as a pharmacological treatment. In addition, folic acid, the synthetic form of folate employed in abovementioned studies, needs to undergo enzymatic reduction by the enzyme dihydrofolate reductase (DHFR) in order to become biologically active within the cell [132]. Also, one clinical trial suggested that folic acid could significantly reduce the effect of lamotrigine in bipolar depression [133]. Consequently, methylfolate may be associated with a reduced interaction with drugs that inhibit dihydrofolate reductase, such as is the case with lamotrigine, than folic acid [132].

It is possible that the direction of the association between BD and Hcy is reversed, meaning that BD leads to increased Hcy levels, and not the opposite. It is also possible that confounders associated with both BD and Hcy play a role in the findings of increased Hcy levels in BD. Most likely, the reality is a combination of these three scenarios. It is also worth noting that low levels of physical activity are associated with elevated Hcy levels [134], and persons with BD are known to be more sedentary than healthy controls [135]. Exercise interventions, particularly resistance exercise, can reduce Hcy levels [136], and future research may wish to consider if exercise can reduce Hcy levels, cardiovascular risk and improve other health parameters in people with BD [137].

Although older age has been linked to higher levels of Hcy, for instance, due to dietary factors [21,114,138], our meta-regression analyses found no relationship between age and Hcy levels in persons with BD in euthymia. This might suggest that higher levels of Hcy may be present since the beginning of the illness at a younger age in BD, independently of environmental factors. This is in line with the results of Misiak et al., who found that Hcy plasma levels were significantly higher in first episode schizophrenia patients than in healthy controls [139]. Likewise, higher levels of

Hcy have been described in males compared to females with BD [75], which suggests a potentially protective role of estrogens amongst many other biological and social factors [140]. Nevertheless, sex did not emerge as a significant moderator in our meta-regression analyses.

Finally, the new field of research focused on the microbiome-gut-brain axis in psychiatry may be of relevance [141]. Gut microbiota synthesise B-group Vitamins [142] and maladaptive metabolism of these vitamins may be a function of microbiota dysbiosis, which in turn may be both cause and consequence of mood disorders [143]. As this field develops, more insights into the possible mechanistic pathways between microbes and the brain will possibly emerge; given the modifiable nature of the gut microbiota, this may yield new targets for intervention [144].

5. Strengths and limitations

Our study has some strengths and limitations that deserve discussion. Firstly, we included a relatively small number of studies with a cross-sectional design, which does not allow inferring of causality, besides having other potential limitations such as confounding and selection bias. However, we found no evidence of heterogeneity across studies performed in persons with BD in mania and moderate heterogeneity between studies performed in euthymia. A strength of this meta-analysis is that we found no evidence of publication bias, a common issue with biomarkers in psychiatric disorders such as BD [55] and major depressive disorder [54], as the funnel plot was symmetric. In addition, we estimated that 148 missing studies with null results would be necessary to turn our significant results into negative ones. In addition, due to the small number of available studies we were not able to analyse the relationship between depressive symptoms and Hcy as we only identify one article assessing this issue. Similarly, the limited number of identified studies for mania prevented us from conducting meta-regressions analysing possible associations between sex, age and Hcy levels in this subgroup. Another point is that Hcy levels are known to be influenced by several factors, including physical activity, numerous systemic illnesses, diet (especially vitamin B12 and folic acid levels), BMI, and smoking. As only a minority of studies provided data on these factors, we were not able to perform meta-regression analyses using them as moderators. Importantly, Hcy levels are also influenced by medications such as lamotrigine or valproate [20] and all the included patients were on pharmacological treatment. Fourthly, all studies determined Hcy levels in the periphery, but as it is known that Hcy metabolism pathways differ between the CNS and the periphery [145,146], it is not sure that peripheral Hcy levels are representative of central Hcy levels. Data on key genotype variants would also have been informative if available. It would similarly be valuable to have longitudinal data to determine if Hcy is a biomarker of trait or state in from a pathophysiological perspective, and if it also could be a biomarker with clinical value as a biomarker of response to treatment and/or prognosis, given that it will have adequate sensitivity and specificity [147,148].

6. Conclusions

Our meta-analysis provides evidence that Hcy levels are elevated in persons with BD in mania or euthymia independently of sex and age. These findings provide preliminary evidence that peripheral Hcy levels could be a potential biomarker in BD, both of trait (since it is increased in euthymia), and also of state (since its increase is more accentuated in mania). Larger longitudinal studies taking into account the confounding effect of the aforementioned variables and assessing patients in the different mood states are needed to clarify the relationship between bipolar disorder and Hcy as well as the clinical usefulness of peripheral Hcy as a biomarker for diagnosis, of response to treatment and/or prognosis.

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