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Review Article

Epigenetic correlates of the psychological interventions outcomes: A systematic review and meta-analysis

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ABSTRACT

Background: In the last years, there was a growing interest in exploring the potential epigenetic effects of psychotherapeutic approaches, but no clear directions have been identified. This article aimed to test and summarize the epigenetic correlates of the different psychological treatments' outcomes.

Methods: We considered only studies, published from inception to July 2021, that provided an epigenetic outcome assessed pre-and post- psychological treatment in either clinical or non-clinical populations. The article adhered to PRISMA, STROBE, Cochrane, and New Castle Ottawa bias scales, and it has been registered on PROSPERO.

Results: A systematic review of 14 studies and a meta-analysis on 2 observative prospective cohort studies were processed. The findings of the systematic review showed that, in the majority of the studies, subjects responding to therapy were associated with a specific decrease or increase in the methylation status, that in subjects not responding to therapy was associated in the opposite way. The meta-analysis showed a significant increase in the methylation status of the MAOA gene after a symptom-vocalization CBT only in the respondent participants with anxiety.

Limitations: The design of the majority of the studies included in the systematic review was observational, which is more prone to confounders and selection bias. There were few eligible studies for the meta-analyses, making the findings to be considered as preliminary.

Conclusions: The findings suggest dynamic changes in epigenetic mechanisms after a psychological treatment that are related to clinical outcomes with an inverse association between remitters and not-remitters.

1. Introduction

In recent years, the advent of epigenetics contributed to provide important evidence in support of the close relationship between biological heritage and the environment. The epigenetic mechanisms are influenced by environmental stimuli that can activate or silencing the gene expression. Recent studies have shown that different types of environmental exposures can modulate synaptic connectivity, stimulate neuroplasticity, and re-establish neural growth potential (Miller, 2017). The DNA methylation patterns seem to be dynamic (Wong et al., 2010) and responsive to environmental influences across the lifespan (Dekkers et al., 2016; Joehanes et al., 2016). Considering the psychotherapy as a form of "environmental regulation" (Yehuda 2013) that may alter neural connections (LeDoux, 2002) and the epigenetic status (Yehuda, 2013; Sweatt, 2009; Nestler, 2009), in the last years there was a growing interest in exploring the potential for psychotherapeutic approaches, not only to the reduction of the symptoms but also to the epigenetic processes.

Recent studies reported that cognitive behavioural psychotherapy (CBT) for depressive disorder (Kahl, 2016) and anxiety disorder (Roberts, 2014, Roberts et al., 2015; Ziegler, 2016; Schiele, 2018, 2019) lead to changes in the expression of genes associated with the symptomatology after the treatment. Moreover, it has been reported that CBT can lead to epigenetic regulation in subjects with post-traumatic stress disorder (PTSD), which in the pre - treatment presented methyl alterations in those genes responsible for the stress response (Yehuda, 2013;

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Morath, 2014). Previous findings showed that there were significant alterations of the brain-derived neurotrophic factor (BDNF) gene expression after a dialectical behavioural therapy (DBT) in participants with borderline personality disorder (BPD) (Perroud, 2013; Thomas, 2018). These findings seem to suggest an important role of psychotherapy on the modulation of gene expression also in people with personality disorders. A recent narrative review (Kumsta, 2019) discussed the results of six studies evaluating the effect of psychological interventions on the methylation status of different genes in subjects with different disorders. Interestingly, Kumsta (2019) reported that, rather than the pre-treatment DNA methylation status, the change in DNA methylation levels over the course of treatment was associated with the treatment outcome, where responders and non-responders diverged in the direction of change. Taken together these few previous findings sustain the notion, previously noted by Kandel (1998), that the changes in behaviours observed after psychotherapy could be brought about by intervention-associated alternations in gene expression. Considering that the gene expression status is controlled by the epigenetic mechanisms, those seem to be the prime candidates to explore how psychological interventions may lead to changes in neuroplasticity processes, extinguishing or forming memories and emotional meanings related to them. Previous research showed that the Mind Body Therapy (Cozzolino et al., 2017) and mindfulness interventions have also obtained promising outcomes, leading to methyl and histone changes and regulating the expression of biomarker genes associated with inflammation and immune function that were altered before treatment, in subjects with carcinoma (Muñoz et al., 2018) or with generic stress symptoms (Kaliman, 2014). Today, as highlighted by a recent review (Schiele et al., 2020), the studies are very heterogeneous in terms of samples, candidate genes, type of intervention, sampled tissue, and biochemical analyses of DNA methylation, and a systematic comparison of the studies on the epigenetic mechanisms after psychological intervention could be useful to identify whether the epigenetic changes could be specific correlates of the treatment, or whether therapy outcome could share common biological mechanisms.

The present work aimed to test and summarize the epigenetic correlates of the short-terms outcomes of the psychosocial interventions in participants with psychological disease, through a systematic review and meta-analyses. It was tested whether the variations in clinical symptoms after the psychological intervention were associated with changes in epigenetic outcomes.

2. Methods

A focused research question based on the PICO framework (Population, Intervention, Comparison, Outcome) and eligibility criteria were developed before the searches were performed. The methods used fulfilled the PRISMA guidelines (Page et al., 2021) and it has been registered on the International Prospective Register of Systematic Reviews (PROSPERO ID number CRD42020211866).

2.1. Eligibility criteria

The articles eligible for inclusion had to fulfill all selection criteria:

- *Population*: study participants were adults ($\geq$ _18 years), adolescent ($<$ 18 years) or children ($<$ 13 years) with any psychological disease,
- *Intervention*: dynamic psychotherapies, cognitive-behavioural therapies, psychosocial support intervention, mindfulness protocols, or dialectical-behavioural therapies,
- *Comparator*: the comparison was conducted between the pre- and the post- psychological intervention assessment,
- *Outcomes*: primary outcomes were the DNA methylation levels and basal DNA mechanisms. Only original articles that provided an epigenetic outcome assessed pre- and post- psychological intervention were included. The studies that considered the epigenetic outcome exclusively as a predictor of the psychological intervention outcomes were excluded.
- Secondary outcomes were anxiety and depression symptoms, PTSD symptomatology, stress levels, psychopathological symptoms, borderline symptoms, and impulsiveness. Patient-reported outcomes had to be assessed using validated measures.
- *Types of study included the following*: randomized controlled trials (RCT) observational prospective cohort studies (OPC) were eligible for the inclusion. The OPC study are longitudinal cohort studies that follow over time a group of similar individuals (cohorts) who differ with respect to certain condition of the study, to determine how these factors affect rates of a certain outcome. Studies had to be in English and involved human subjects. The publication date was considered from the inception to the 2021.

2.2. Data sources and study selection

Published articles concerning the effects of psychotherapy on epigenetic mechanisms were identified by searching in *PubMed, PsycINFO, PsycArticles*, and the *Cochrane Library* from inception to July 2021. Search terms included "epigenetic*", "psychotherapy", "genetic*", "expression gene", "post-traumatic stress disorder", "major depressive disorder". A manual search of the literature was also performed, and reference lists of the retrieved articles were examined for further studies not yet identified. The duplicates were excluded.

Two reviewers (DS and GRP) performed pilot calibration exercises on a random sample of 50 references. Specifically, each reviewer applied the inclusion and exclusion criteria to a common set of titles and abstracts. Each reviewer discussed the level of agreement, that was, whether the article was included or excluded. Regular meetings were set up to discuss discrepancies between the two reviewers and the process was repeated until the 90% agreement was reached.

The articles resulting from the search were divided among the reviewers. Two reviewers (GRP and DS) independently screened the set of references and apply the inclusion criteria firstly on the title and abstract, and then on the full text. Meetings took place to discuss discrepancies and a consensus was reached through discussion with the senior author (CL).

2.3. Data collection process

A calibration exercise with a random sample of 10 articles was performed. Two reviewers extracted relevant data from each article. Once an agreement level of 90% was met within each pair, we proceeded to data extract of the full set of articles.

A synoptic table was created with all the relevant data extracted from each study, including the following information: first author and year, Country, aim of the study, research design, presence of follow-up, sample size, age and gender of study population, kind and duration of psychological intervention, psychological condition of the participants and psychological measure, epigenetic measure, epigenetic outcome, findings on psychological variables, findings on epigenetic variables. Both significant and insignificant p-values have been included in the synoptic table.

2.4. Assessment of risk of bias and certainty of evidence

To assess the risk of bias for the RCT studies, the *Cochrane Bias Scale* (Higgins, 2011) scale was applied, which takes into account various domains: random sequence generation describing the method used to generate the sequence allocation file; the allocation concealment allowing to determine whether the assignments were planned before or after enrolment; the selective reporting which contains the description of methodological bias. Furthermore, the presence of blinding participants and personnel were assessed, and the blinding outcome assessment which allows observing whether the assessment took place blind to

what was measured. Finally, the last domain concerns the incomplete outcome data, which evaluates whether the outcome data are reported in a complete and detailed manner.

To assess the risk of bias for the cohort studies, the *New Castle Ottawa Scale* (Wells, 2000) was applied, which takes into account various domains: the representativeness of the cohort of the exposed; the selection of the cohort of not exposed if the participants are drawn from the same or a different population of the exposed; the assessment of exposure (objective data or self-report); demonstration that no outcomes have been added *in itinere*; presence of follow-up; detection of measures result (independent and blinded or non-independent and use of self-report data); the completeness of the follow-up for all enrolled subjects.

Moreover, the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Checklist (Von Elm et al., 2007; Von Elm et al., 2008) was used to describe the included studies following 11 domains: 1: article's title and abstract; 2–3: introduction; 4–12: methods; 13–17: results; 18–21: discussion; 22: further information. The assessment was conducted independently by authors CC and DS, and any disagreements were resolved by a third author (CL).

2.5. Data synthesis and analysis

For the systematic review of the literature, it was carried out a comparative table with the distribution of frequencies of the significant (increase or decrease in the methylation status) and not-significant changes of the epigenetic outcomes in the post- vs pre- psychological treatment *per* different kind of treatments and psychological condition. The qualitative analyses were performed considering the number of studies, the number of samples, and the number of trials. The term "study" referred to the original article, the term "sample" referred to each cohort of participants considered in the studies, and the term "trial" referred to each sample with a specific outcome. The samples considered were only the experimental ones. Moreover, when it was reported by the studies the differentiation between treatment responder and non-responder participants, the distribution of the samples with the specification of the responder and not-responder participants *per* different kind of treatments and psychological condition on the epigenetic outcome was reported. All the epigenetic outcomes were grouped into categories according to their functions. The psychological treatments were grouped into different categories according to the different treatment approaches.

A meta-analysis was planned in case of two or more homogeneous studies reporting similar epigenetic outcomes. The meta-analyses were performed with the Review Manager software (RevMan) version 5.4 (The Cochrane Collaboration, 2020). To perform the meta-analyses, the number of the participants, means, and standard deviation of the epigenetic outcomes at pre-and post-treatment of the experimental samples were inserted in RevMan. On the studies with the similar epigenetic outcome, the analyses were performed on the comparison between the pre- and post- psychological intervention assessment of the epigenetic outcome. When the studies provided the distinction between responders and non-responders to treatment, a second analysis was performed, including only the pre- and post-intervention epigenetic outcome data of the responders. The difference of means within a random effect model with a 95% confidence interval was processed. The random effect model was chosen because it assumes heterogeneity between studies exists, and it has the effect of giving more weight to the smaller studies than the fixed-effect model (Copas and Shi, 2000). Heterogeneity was quantified with the I^2 metrics, which is independent of the number of studies in the meta-analysis (Higgins et al., 2003) and it takes values between 0 and 100%, with higher values denoting a greater degree of heterogeneity (I^2=0–25%, no heterogeneity; I^2= 25–50%, moderate heterogeneity; I^2 = 50–75%, large heterogeneity, and I^2 = 75–100%, extreme heterogeneity). *P*-values < 0.05 were considered to indicate statistical significance.

3. Results

3.1. Search results

The literature search yielded 489 records. After a preliminary screening for title and abstract, 446 studies were excluded. Reading the full text of the remaining 43 studies, 17 were excluded for duplicate records and lack of pre- treatment assessment. From the remaining 26 studies, 12 were excluded because the studies did not specifically address epigenetic changes. Finally, 14 studies (1 RCT and 13 OPC) were included in the systematic review. From these 14 studies, because of the lack of useful data, only 2 OPC studies were included in the metanalysis. A flowchart of the study selection process is presented in Fig. 1.

3.2. Characteristics of the included studies in the systematic review

The included studies were published between 2013 (Perroud, 2013; Yehuda, 2013) and 2019 (Vinkers et al., 2019) and they provided an assessment pre- and post- of the CBT and Mindfulness interventions on the DNA methylation status of genes involved in immunoregulation and inflammation, in cellular functioning, in neural plasticity, and neurotransmission, and on basal DNA mechanisms (see Table 1).

The systematic review included 14 studies (1 RCT and 13 OPC), 5 of these (all OPC) provided a follow-up (3–12 mm). The total samples were 25 (15 experimental and 10 controls). The experimental sample of the RCT studies was 1; the experimental samples of the OPC studies were 14, the controls of the OPC studies were 9. The sample size varied widely among the studies, from a minimum of 16 persons (Yehuda, 2013) to a maximum of 116 individuals (Roberts, 2014) in the experimental samples. Total participants of the experimental samples were 741; the RCT study (Morath, 2014) included 38 participants (19 in experimental and 19 in control samples), the OPC studies included 722 participants of the experimental samples and 315 of the control samples.

Most of the investigations were conducted in Europe (n = 12), 7 studies in Germany (Morath, 2014; Kahl, 2016; Knoblich, 2017; Schiele, 2018; Thomas, 2018; Ziegler, 2019, 2016), 2 studies in the United Kingdom (Roberts et al., 2015, 2014), 1 study in Spain (Kaliman et al., 2014), 1 study in Switzerland (Perroud, 2013), 1 study in the Netherlands (Vinkers et al., 2019) while the other researches were carried out in the USA (n = 2, Bishop et al., 2018; (Yehuda, 2013). The RCT study (Morath, 2014) included 6 females and 32 males, and the OPC studies included 467 females and 255 males. The mean age of the RCT study was 28.7 ± 9.54 years, in the OPC studies the individuals' mean age ranged from 9.3 (Roberts et al., 2015) to 49.89 (Kaliman, 2014) years. Two studies (Roberts et al., 2015, 2014) with 2 experimental samples and 3 trials were conducted on pediatric age group with anxiety disorders.

The 14 studies provided 15 samples with 38 trials. The more representative epigenetic outcome was the methylation status of the genes (36/38 trials) and only in 2/38 trials were considered basal DNA mechanisms. The description of the epigenetic outcomes among the studies was reported in Table 2.

The psychological interventions were categorized in two treatment categories: Cognitive Behavioural Therapy (CBT) and Mindfulness treatments. The more representative psychological intervention was the CBT in 20 trials coming from 13 samples and 12 studies (11 OPC and 1 RCT) (Table 3). A summary of the distribution of the trials of the epigenetic changes post- vs pre- psychological intervention in each psychological disease was reported in Table 4.

3.4. Epigenetic outcome

3.4.1. Genes involved in immunoregulation and inflammation

All the studies employed blood samples for the analysis of the methylation status, except for Roberts and colleagues (2015) where the DNA was extracted from saliva samples.

PRISMAFlow chart of the selection of studies included in the systematic review and meta-analysis

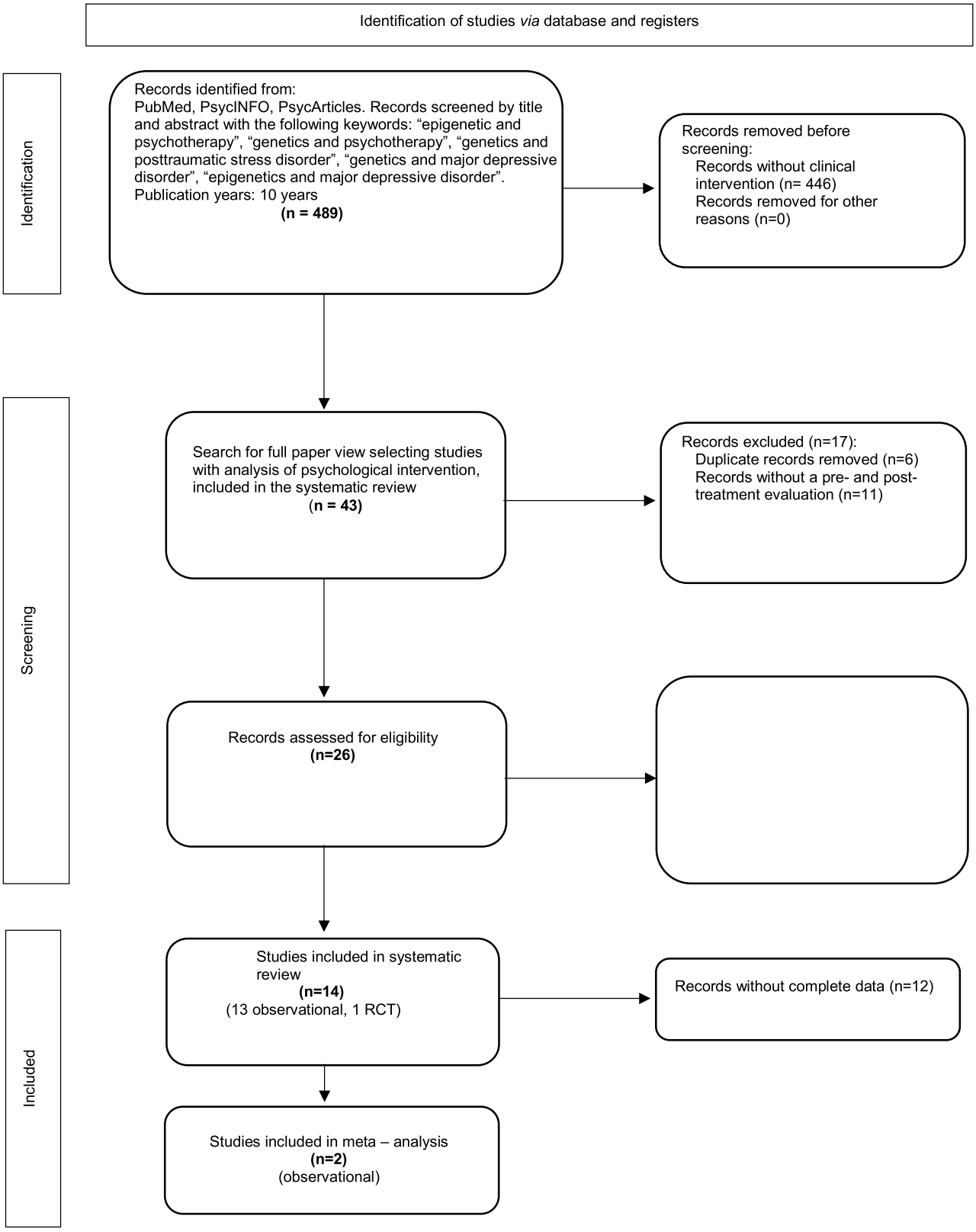

Fig. 1. PRISMA flowchart of the selection of studies included in the systematic review and meta-analysis.

Table 1
Characteristic of the included studies.

Author and year	Country	Study aim*	Study design	Follow-up points**	Sample size	Age (yrs)*mean (SD)*	Female gender:*n (%)*	Treatment	Psychological Measure	Epigenetic Measure	Epigenetic Outcome	Results (psychological variables)	Results (epigenetic variables)
1) Morath et al. (2014)	DE	To investigate the effects of psychotherapy on DNA damage and repair.	RCT	T0 – T1 – T2	38 (exp gr. 19; ctrl gr.=19)	Exp gr.= 28.7 (9.54); ctrl gr.= 30.1(8.21)	6(31.6)	NET (12 sessions) WLC	PTSD: CAPS	FADU essay	Basal DNA breakage	In Exp group there was a reduction in PTSD symptoms pre-therapy vs. 4-month post-test (p<.0001).	Reduction in DNA strand breakage in Exp group (pretherapy vs. post-test: p=.01) but not in the WLC group (pre-therapy vs. post-test values: p = .35)
2) Kahl (2016)	DE	To examine changes in DNA methylation of promoter regions of GLUT1 and GLUT4 in depressed patients after inpatient treatment vs healthy subjects	OPC	T0 – T1	T0=70 (exp gr. 52; ctrl gr. 18); T1= 55 (exp gr. 37; ctrl gr. 18)	Exp gr.= 41.8 (11.1); ctrl gr.= 43.2(13.1)	10(55.6)	CBT	Depression: MADRS and BDI-II	PCR	Methylation of GLUT1 and GLUT4 genes	Remitters T1: MADRS = 5,2 ± 2,9; BDI – 2 = 11,1 ± 10,3. Non remitters T1: MADRS =21,7 ± 7,9; BDI – 2 = 15,3 ± 11,8	GLUT1 methylation decreased in remitters and was similar to controls (p<.001)
3) Bishop et al. (2018)	USA	To investigate the methylation of SLC6A4 and FKBP5 genes before and after mindfulness in veterans with PTSD.	OPC	T0 – T1	22 (exp gr.)	Responders: 60.4(14.5); non-responders:58.2 (10.2)	4(18%)	Mindfulness	PTSD: CAPS; PCL Depression: PHQ-9	PCR	Methylation of SLC6A4 and FKBP5 genes		Responders had an increase in FKBP5 methylation and non-responders had a decrease in methylation from before to after treatment in this region. There was no main effect of time for methylation changing in the primary component of SLC6A4.
4) Kaliman (2014)	SP	To explore the impact of a day of intensive practice of mindfulness meditation in experienced subjects on the expression of circadian, chromatin modulatory and inflammatory genes in peripheral blood mononuclear cells vs control group.	OPC	T0 – T1	40 (exp gr. 19; ctrl gr. 21)	Exp gr.= 49.89 (11.18); ctrl gr.= 50.38(8.96)	Exp gr.= 11 (57.9); ctrl gr.= 12 (57.1)	Intensive practice of mindfulness meditation (1 day) Intentional activities (such as reading, watching documentaries, or playing computer games, walking)	Stress: Trier Social Stress Test	Ficoll-Paque-plus method; PCR	Expression of: circadian genes, chromatin modification genes, proinflammatory genes		The Exp group showed decreased expression of HDACs (chromatin modulators) (p ranges from <0.05 to <0.01), RIPK2 and COX2 (inflammation regulators) (p<.01 and p<.05) after intervention. Compared to controls, meditators showed decreased expression of proinflammatory genes.
5) Knoblich (2017)	DE	To investigate differential DNA methylation of APBA3, MCF2, and NINJ2 as potential epigenetic biomarker for treatment outcome in borderline personality disorder patients.	OPC	T0-T1	T0: 88 (exp gr. 44; ctrl gr. 44) T1: exp gr. 24	Exp gr. 30.8 (8.8); ctrl gr. 29.7(8.8)	Exp gr.=20 (83.3); ctrl gr.= 37 (84.0)	DBT (12 weeks)	Psychopathological symptoms: SCL-90R; Borderline symptoms: BSL23; Childhood trauma: CTQ	PCR	Methylation of APBA3 and MCF2 genes		No effects of DBT on the DNA methylation status of APBA3 and MCF2 Before and after therapy shows that therapy responders display significantly higher overall DNA methylation values for APBA3 before therapy than non-responders (p<.01)

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Table 1 (*continued*)

Author and year	Country	Study aim*	Study design	Follow-up points**	Sample size	Age (yrs)*mean (SD)*	Female gender:*n (%)*	Treatment	Psychological Measure	Epigenetic Measure	Epigenetic Outcome	Results (psychological variables)	Results (epigenetic variables)
6) Perroud, et al. (2013)	CH	To investigate change in BDNF methylation status in borderline personality disorder patients after a psychotherapeutic treatment.	OPC	T0-T1	167(exp gr. 115; ctrl gr. 52)	Exp gr. 30.4 (9.2); ctrl gr. 40.6(12.0)	Exp gr.=108 (93.9); ctrl gr.= 24 (46.1)	I-DBT (4 weeks with daily group and individual therapy)	Depression: BDI-II; Hopelessness: BHS; Impulsiveness: BIS-10; Borderline personality disorder symptoms: SCID-II BPD part; Childhood trauma: CTQ	PCR; High-resolution melt assay	Methylation of BDNF gene; Protein levels of BDNF in plasma	Reduction in BPDs of depression (20.37 ± 12,42), negativity (7.98 ± 5,04) and impulsivity (65.99 ± 17,31).	In Exp group, BDNF methylation increased after I-DBT (p=.0001). Non-responders accounted for the majority of the increase. A decrease in BDNF protein levels over time in BPD subjects which was inversely associated with treatment response (p=.008).
7) Roberts et al., 2015	UK	To examine change in DNA methylation of FKBP5 and NR3C1 genes during the course of CBT in a sample of children with anxiety disorders.	OPC	T0-T1	98	9.3(1.9)	66(48.6)	CBT	Anxiety: ADIS-IV-C/P	PCR	Methylation of FKBP5 and NR3C1 genes		Treatment response was not associated with FKBP5 and GR polymorphisms. Change in FKBP5 DNA methylation was associated with treatment response (p=.007)
8) Roberts (2014)	UK	To compare SERT DNA methylation change from pre- to post-treatment in children receiving CBT for an anxiety disorder.	OPC	T0-T1-T2	116	9.34	56(48.3)	CBT	Anxiety: ADIS-IV-C/P	PCR	Methylation of SERT gene	For primary and all anxiety diagnoses, remission rates were 48.3 and 29.3% at posttreatment.	Percentage DNA methylation at pre- and posttreatment was not significantly different in the whole group. From pre- to post- treatment responders increased in methylation at follow-up, whereas nonresponders showed a decrease in DNA methylation of SERT gene (p=.004)
9) Schiele (2018)	DE	To investigate DNA methylation of the MAOA gene in female patients with acrophobia during a CBT intervention.	OPC	T0-T1	56(exp gr.=28; ctrl gr.=28)	Exp gr. 44.9 (13.7); ctrl gr. 39.6(6.7)	56(100)	Psycho-educative information+20 min of excitatory rTMS before each of exposure sessions (2 sessions).	Acrophobia: AQ and ATHQ	PCR	Methylation of MAOA gene	After the therapeutic intervention, acrophobia decreased significantly (p=<0.001).	Following therapy, MAOA methylation increased significantly in the patient group for average methylation (p=.04). Differences were observed regarding CpG1 (p = .003) with lower baseline methylation predicting impaired treatment response.
10) Thomas (2018)	DE	To assess the blood and salivary BDNF methylation status in a sample of BPD patients after a DBT treatment.	OPC	T0-T1	T0 saliva: 82 (Exp gr. 41; ctrl gr. 41); T0 blood: 80 (Exp gr. 39; ctrl gr. 41); T1 saliva: 26Exp gr.;	T0 Exp gr.30.4 (8.6); T0 ctrl gr. 30.7 (9.3)	Exp gr.: 35 (85.4) Ctrl gr.: 35(85.4)	DBT (12 weeks)	Borderline personality disorder symptoms: BSL 23 Psychopathological symptoms: SCL-90R Childhood trauma: CTQ	PCR	BDNF IV promoter methylation levels in saliva and in blood	Following psychotherapeutic intervention, patients showed a significant reduction in general symptomatology (p≤.01)	BDNF methylation in saliva, but not in blood, of BPD patients significantly decreased after a 12-week psychotherapeutic intervention (p.=04)

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Table 1 (*continued*)

Author and year	Country	Study aim*	Study design	Follow-up points**	Sample size	Age (yrs)*mean (SD)*	Female gender:*n (%)*	Treatment	Psychological Measure	Epigenetic Measure	Epigenetic Outcome	Results (psychological variables)	Results (epigenetic variables)
11) Vinkers (2019)	NL	To examine genome-wide DNA methylation profiles before and after trauma focused psychotherapy in PTSD patients	OPC	T0-T1-T2	T1 blood:23 Exp gr 67(Exp gr. 44; ctrl gr. 23)	27.3(8.7)	8(9)	tf-CBT+EMDR (EMDR: 10 sessions; tf-CBT: 8 sessions)	PTSD: CAPS; Psychopathological symptoms: 90-item Mood and Anxiety Symptom Questionnaire	Blood-based genoma-wide DNA methylation levels	DNA methylation profiles	Responders showed decreased PTSD symptoms after the therapy (p <0.001)	ZFP57 methylation increased following trauma-related psychological PTSD treatment, while it decreases when PTSD develops over three time points before and after deployment
12) Yehuda (2013)	USA	To examine methylation of the NR3C1 and FKBP5genes before and after prolonged exposure psychotherapy in veterans with PTSD.	OPC	T0-T1-T2	16	*Responders* (N = 8): 41.2(17.8) *Non responders* (N = 8): 57.9(7.4)	2(12.5)	PE (12 weeks)	PTSD: CAPS, PSS-SR; Childhood trauma: CTQ; Resilience: DRRI	PCR	Methylation of the NR3C1 and FKBP5genes	Responders showed decreased PTSD symptoms after the therapy (p<.001).	NR3C1methylation was not significantly altered in either group at post-treatment or follow- up, but NR3C1methylation at T0 predicted treatment outcome (p=.029). FKBP5 methylation decreased in responders and increased in non-responders after PE and in T2 (p=.02), but FKBP5 methylation at T0 did not predict treatment response.
13) Ziegler (2019)	DE	To analyze changes on the DNA methylome level along with clinical effects of a CBT in patients with panic disorder.	OPC	T0-T1	T0: 116(Exp gr.=56; ctrl gr.=60) T1:47 Exp gr	Exp gr.34.0 (10.0); ctrl gr. 33.2(9.2)	Exp gr. 43 (75); ctrl gr. 47(77)	CBT (6 sessions)	Anxiety: HAM-A	Epigenome-wide association analyses	DNA methylome levels	NR	Increase in methylation after CBT of the IL1R1 gene in treatment responders to CBT (p<.00001).
14) Ziegler (2016)	DE	To investigate MAOA methylation changes as a potential epigenetic correlate of treatment response to a CBT in adult patients with panic disorder.	OPC	Discovery sample: T0-T1 Replication sample: T0-T1-T2	Discovery sample: 56(Exp gr.28; ctrl gr.28) Replication sample: T0:20, T1:16, T2:6	Discovery sample: Exp gr. 34.6 (8.5); ctrl gr. 34.9(9.0) Replication sample: 33.5 (11.1)	Discovery sample: 28(100) Replication sample: 20 (100)	Discovery sample: CBT (6 sessions) Replication sample: CBT (12 sessions)	Agoraphobia: MI	PCR	Methylation of MAOA gene	NR	Discovery sample: In the overall patient group MAOA methylation did not change significantly from T0 to T1. In responder patients, methylation increased after therapy (mean change ± s.e., 3.37 ± 2.17%), while in non-responders it decreased (mean change ± s.e., − 2.00 ± 1.28%; p=.001). Replication sample: No significant effects among T0, T1 and T2. Increasing in MAOA methylation correlated with agoraphobic

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Table 1 (*continued*)

Author and year	Country	Study aim*	Study design	Follow-up points**	Sample size	Age (yrs)*mean (SD)*	Female gender:*n (%)*	Treatment	Psychological Measure	Epigenetic Measure	Epigenetic Outcome	Results (psychological variables)	Results (epigenetic variables)
													symptom reduction after CBT (p=.02)

Note. ADIS-IV-C/P: Anxiety Disorders Interview Schedule for DSM-IV, Child and Parent Versions; APBA3: Amyloid Beta Precursor Protein Binding Family A Member 3; AQ: Acrophobia Questionnaire; ATHQ: Attitude Towards Heights Questionnaire; BDI-II: Beck Depression Inventory II; BDNF: Brain-derived neurotrophic factor; BHS: Beck Hopelessness Scale; BIS-10: Beck Impulsiveness Scale; BPD: borderline personality disorder; BSL23: Borderline Symptom List 23; CAPS: Clinician Administered PTSD Scale; CBT: Cognitive Behavioural Therapy; Ctrl: control group; COX2: cytochrome c oxidase subunit II; CpG: cytosine-phosphate-guanine; CTQ: Childhood Trauma Questionnaire; DBT: Dialectical Behavioural Therapy; DRRI: Deployment Risk and Resiliency Inventory; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; Exp: experimental group; FADU: Fluorimetric Detection of Alkaline DNA Unwinding method; FKBP5: FK506-binding protein; GLUT1: glucose transporter 1; GLUT4: glucose transporter 4; GR: glucocorticoid receptor; HAM -A: Hamilton Anxiety Rating Scale; HDAC: histone deacetylase gene; I-DBT: Intensive Dialectical Behavioural Therapy; IL1R1: Interleukin 1 Receptor Type 1; MADRS: Montgomery-Asberg Depression Rating Scale; MAOA: monoamine oxidase; MCF2: Protein Coding gene; MDD: major depressive disorder; MI: Mobility Inventory; NET: Narrative Exposure Therapy; NR: Not reported; NR3C1: Nuclear Receptor Subfamily 3 Group C Member 1; PCL: PTSD Checklist; PCR: Polymerase chain reaction method; PE: prolonged exposure therapy; PHQ-9: Patient Health Questionnaire 9; PSS-SR: PTSD Symptom Scale- Self Report Version; PTSD: post-traumatic stress disorder; RIPK2: receptor-interacting serine-threonine kinase 2; rTMS: Repetitive Transcranial Magnetic Stimulation; SCID-II: Screening Interview for Axis II Disorder; SCL90R: Symptom Checklist 90-Revised; SERT: serotonin transporter promoter region; tf-CBT: trauma-focused cognitive behavioural therapy; ZFP57: Zinc Finger Protein 57.
*Patient Reported Outcome in bolt; **Epigenetic data measurements in bolt.

In 4 studies (Bishop et al., 2018; Roberts et al., 2015; Yehuda, 2013; Ziegler, 2019), the trials were differentiated for the participants who responded to the treatment and those who did not. In the respondents to the CBT, there was a significant post- to pre- treatment increase in the methylation status of the IL1R1 in participants with anxiety (Ziegler, 2019), and a significant post- to pre- treatment decrease in the methylation status of FKBP5 in participants with PTSD (Yehuda, 2013) and anxiety (Roberts et al., 2015). Conversely, in the no-responders to the CBT there was a significant post- to pre- treatment decrease in the methylation status of the IL1R1 in participants with anxiety (Ziegler, 2019) and a significant post- to pre- treatment increase in the methylation status of FKBP5 in participants with PTSD (Yehuda, 2013) and anxiety (Roberts et al., 2015). Finally, there were not significant effects on the NR3C1 methylation status in participants with PTSD (Yehuda et al., 2013) and anxiety (Roberts et al., 2015) after the CBT.

In the respondents to the Mindfulness there was a significant post- to pre- treatment increase in the methylation status of FKBP5 in participants with PTSD (Bishop et al., 2018), while, conversely, in the no-responders there was a significant post- to pre- treatment decrease in the methylation status of FKBP5 (Bishop et al., 2018). Moreover, after Mindfulness treatment there was a significant post- to pre- treatment increase in the methylation status of the RIPK2 and COX2 genes in participants with stress (Kaliman, 2014) and no significant effects on the methylation status of CCR7, CXCR1, IL-6, and TNF-α genes on the same sample of participants (Kaliman, 2014).

3.4.2. Genes involved in gene regulation and cellular functioning

All the studies employed blood samples for the analysis of the methylation status. In 3 studies (Vinkers et al., 2019; Kahl, 2016; Ziegler, 2019), the trials were differentiated for the participants who responded to the treatment and those who did not. In the respondents to the CBT, there was a significant post- to pre- treatment increase in the methylation status of ZFP622, ZFP57, and SLC43A2 genes in participants with anxiety (Ziegler, 2019) in participants with PTSD (Vinkers et al., 2019), and a significant post- to pre- treatment decrease in the methylation status of GLUT1 in participants with depression (Kahl, 2016). Conversely, in the no-responders to the CBT there was a significant post- to pre- treatment increase in the methylation status of the GLUT1 in participants with depression (Kahl, 2016), and a significant post- to pre- treatment decrease in the methylation status of ZFP57 in participants with PTSD (Vinkers et al., 2019). Finally, there were not significant effects on the methylation status of APBA3, MCF2, and GLUT4 genes after CBT in participants with (Knoblich, 2017) and depression (Kahl, 2016).

After Mindfulness treatment there was a significant post- to pre-treatment increase in the methylation status of the HDAC2, HDAC3, and HDAC9 genes in participants with stress (Kaliman, 2014), and no significant effects on the methylation status of PER1, PER2, PER3, BMAL1, DBP, CRY1, and CRY2 on the same sample of participants (Kaliman, 2014).

3.4.3. Genes involved in neural plasticity

Perroud and colleagues (2013) analysed the DNA methylation status from blood samples, whereas Thomas and colleagues (2018) used both saliva and blood samples for the DNA analysis. In the study of Perroud and colleagues (2013) the trials were differentiated for the participants who responded to the treatment and those who did not. In the respondents to the CBT, there was a significant post- to pre- treatment decrease in the methylation status of the BDNF gene (Perroud, 2013; Thomas, 2018), while, conversely, in the no-responders there was a significant post- to pre- treatment increase in the methylation status of BDNF in participants with BPD (Perroud, 2013). Interestingly, after the CBT there were not significant effects in the trial from the blood sample of Thomas (2018) in participants with BPD.

There were no trials related to the effects of Mindfulness treatment.

Table 2
Distribution of the epigenetic outcomes among the studies.

		Studies	Samples	Trials
Genes involved in immunoregulation and inflammation (12/38 trials from 5 studies)	Receptor Interacting Serine/Threonine Kinase 2 - RIPK2	1/5 (Kaliman et al.2014)	1/12	1/12
	Cytochrome C Oxidase subunit II - COX2	1/5 (Kaliman et al.2014)	1/12	1/12
	C-C Motif Chemokine Receptor 7 - CCR7	1/5 (Kaliman et al.2014)	1/12	1/12
	C Motif Chemokine Receptor 1 - CXCR1	1/5 (Kaliman et al.2014)	1/12	1/12
	Interleukin-6 - IL-6	1/5 (Kaliman et al.2014)	1/12	1/12
	Tumor Necrosis Factor-alpha - TNF-α	1/5 (Kaliman et al.2014)	1/12	1/12
	Interleukin 1 receptor type 1 - IL1R1	1/5 (Ziegler et al., 2019)	1/12	1/12
	FK506 binding protein 51 - FKBP5	3/5 (Bishop et al., 2018; Roberts et al., 2015; Yehuda et al., 2013)	3/12	3/12
	Glucocorticoid receptor - NR3C1	2/5 (Roberts et al., 2015; Yehuda et al., 2013)	2/12	2/12
Genes involved in gene regulation and cellular functioning (17/38 trials from 5 studies)	Amyloid Beta Precursor Protein Binding Family A Member 3 - APBA3	1/5 (Knoblich et al., 2017)	1/17	1/17
	Cell Line Derived Transforming Sequence - MCF2	1/5 (Knoblich et al., 2017)	1/17	1/17
	Period Circadian Regulator 1 - PER1	1/5 (Kaliman et al.2014)	1/17	1/17
	Period Circadian Regulator 2 - PER2	1/5 (Kaliman et al.2014)	1/17	1/17
	Period Circadian Regulator 3 - PER3	1/5 (Kaliman et al.2014)	1/17	1/17
	Brain and Muscle ARNT-Like 1 - BMAL1	1/5 (Kaliman et al.2014)	1/17	1/17
	D Site-binding protein - DBP	1/5 (Kaliman et al.2014)	1/17	1/17
	Cryptochrome Circadian Regulator1 - CRY1	1/5 (Kaliman et al.2014)	1/17	1/17
	Cryptochrome Circadian Regulator2 - CRY2	1/5 (Kaliman et al.2014)	1/17	1/17
	Histone Deacetylase 2 - HDAC2	1/5 (Kaliman et al.2014)	1/17	1/17
	Histone Deacetylase 3 - HDAC3	1/5 (Kaliman et al.2014)	1/17	1/17
	Histone Deacetylase 9 - HDAC9	1/5 (Kaliman et al.2014)	1/17	1/17
	Glucose transporter 1 - GLUT1	1/5 (Kahl et al., 2016)	1/17	1/17
	Glucose transporter 4 - GLUT4	1/5 (Kahl et al., 2016)	1/17	1/17
	Zinc Finger Protein 622 - ZFP622	1/5 (Ziegler et al., 2019)	1/17	1/17
	Zinc Finger Protein 57 - ZFP57	1/5 (Vinkers et al., 2019)	1/17	1/17
	Solute Carrier Family 43 Member 2 - SLC43A2	1/5 (Ziegler et al., 2019)	1/17	1/17
Genes involved in neural plasticity (3/38 trials from 2 studies)	BDNF	2/2 (Perroud, et al., 2013; Thomas et al., 2018)	2/2	3/3
Genes involved in neurotransmission (4/38 trials from 4 studies)	MAO-A	2/4 (Schiele et al., 2018; Ziegler et al., 2016)	2/4	2/4
	SERT	2/4 (Bishop et al., 2018; Roberts et al., 2014)	2/4	2/4
Basal DNA mechanisms (2/38 trials from 1 study)	DNA strand brakeage	1/1 (Morath et al., 2014)	1/1	1/2
	DNA repair	1/1 (Morath et al., 2014)	1/1	1/2

3.4.4. Genes involved in neurotransmission

The studies employed blood samples for the analysis of the methylation status, except for Roberts and colleagues (2014) where the DNA was extracted from saliva samples. In the respondents to the CBT, there was a significant post- to pre- treatment increase in the methylation status of MAOA and SERT genes, while, conversely, in the no-responders there was a significant post- to pre- treatment decrease in the methylation status of the same genes in participants with anxiety (Roberts, 2014; Schiele, 2018; Ziegler et al., 2016).

The Mindfulness treatment did not produce significant effects in 1 trial (SERT) deriving from 1 study in participants with PTSD (Bishop et al., 2018).

3.4.5. Basal DNA mechanisms

The DNA analyses were conducted on blood samples. After the CBT there was a significant post- to pre- treatment reduction in DNA strand breakage and an increase in DNA repair mechanisms. There were no trials related to the effects of Mindfulness treatment.

3.5. Characteristics of the included studies in the meta-analysis

The meta-analysis included 2 OPC studies (Schiele, 2018; Ziegler, 2016) with 2 experimental samples for 2 trials. In the study of Ziegler and colleagues (2016) the trial was differentiated for the participants who responded (R) to the treatment and those who did not (NR). Total participants of the experimental samples were 56 (39 R, 17 NR) with anxiety disorders (panic disorder, agoraphobia, and acrophobia). The total number of participants of the control samples was 56. Both the studies included only female participants (n = 56 (100%)) in the experimental samples, and n = 56 (100%) in the control groups. The participants' mean age ranged from 34.6 (Ziegler, 2016) to 44.9 (Schiele, 2018) years. The study of Ziegler and colleagues provided 6 sessions of CBT protocol and the study of Schiele (2018) provided 2 sessions of CBT protocol in which each session was preceded by a psychoeducation and 20 min of Repetitive Transcranial Magnetic Stimulation. In both the studies, the epigenetic outcome was the pre- to post-treatment methylation status of the MAOA gene.

3.5.1. Results of meta-analyses

The forest plot in Fig. 2 reported the effects of the CBT on the post- vs pre- treatment MAOA methylation status in the samples of anxious patients. In this analysis were included data from both responders (R) and non-responders (NR) of the study of Ziegler (2016). The participants were 56 in the pre-treatment and 56 in the post-treatment. The CBT treatments did not produce significant effects on the post- vs pre-treatment MAOA methylation status (95% CI, Heterogeneity: Tau^2= 0.00, Chi^2= 37.20, df= 2 (p<.00001); I^2= 95%; Test for overall effect: Z = 0.48, p= .63).

The forest plot in Fig. 3 reported the effects of the CBT on the post- vs pre- treatment MAOA methylation status in the samples of anxious participants, excluding the NR participants. The participants were 39 in the pre-treatment and 39 in the post-treatment. The findings showed that the CBT treatment had a significant effect, increasing the methylation status of the MAOA gene after the treatment (95% CI, Heterogeneity: Tau^2= 0.00; Chi^2 = 0.33, df = 1 (p=.58); I^2 = 0%; Test for overall effect Z = 5.16, p<.0001).

3.6. Risk of bias within studies

The *Cochrane scale* showed that the study with RCT design (Morath, 2014) had low risks of bias (Table S1). For the bias risk of the OPC studies, the *New Castle Ottawa* bias scale reported that there was a

Table 3
Description of the psychological interventions and psychological outcomes among the included studies.

Cognitive Behavioural Therapies (CBT)(20 trials, 13 samples, 12 studies)	Studies	Duration	Psychological disease	Psychological Measure
Classical CBT protocol with focalization on symptomatic reduction	7/12	6 to 12 sessions	Depression (Kahl et al., 2016) Anxiety (Roberts et al., 2015;2014; Ziegler, et al., 2019) Acrophobia (Schiele et al., 2018) Agoraphobia (Ziegler, et al., 2019)	BDI-II; MADRS ADIS-IV-C/P; HAM-A AQ; ATHQ MI
Dialectical Behavioural Therapy (DBT)	3/12	4 to 12 weeks	Psychopathological symptoms (Knoblich et al., 2017; Thomas et al., 2018) Borderline personality disorder symptoms (Knoblich et al., 2017; Perroud et al., 2013; Thomas et al., 2018) Childhood trauma (Knoblich et al., 2017; Perroud et al., 2013; Thomas et al., 2018) Depression (Perroud et al., 2013) Hopelessness (Perroud et al., 2013) Impulsiveness (Perroud et al., 2013)	SCL90-R BSL23; SCID-II BPD part CTQ BDI-II BHS BIS-10
Prolonged Exposure Therapy (PE)	1/12	12 weeks	PTSD (Yehuda et al., 2013) Childhood trauma (Yehuda et al., 2013)	CAPS; PSS-SR CTQ
Narrative Exposure Therapy (NET)	1/12	12 sessions	PTSD (Morath et al., 2014)	CAPS
Mindfulness (18 trials, 2 samples, 2 studies)	2/2	one-day to 9 weeks	PTSD (Bishop et al., 2018) Depression (Bishop et al., 2018) Stress (Kaliman et al., 2014)	CAPS; PCL PHQ-9 TSST

Note. ADIS-IV-C/P: Anxiety Disorders Interview Schedule for DSM-IV, Child and Parent Versions; ATHQ: Attitude Towards Heights Questionnaire; BDI-II: Beck Depression Inventory II; BHS: Beck Hopelessness Scale; BIS-10: Beck Impulsiveness Scale; BSL23: Borderline Symptom List 23; CAPS: Clinician Administered PTSD Scale; CTQ: Childhood Trauma Questionnaire; HAM -A: Hamilton Anxiety Rating Scale; MADRS: Montgomery-Asberg Depression Rating Scale; MI: Mobility Inventory; PHQ-9: Patient Health Questionnaire 9; PSS-SR: PTSD Symptom Scale- Self Report Version; PTSD: post-traumatic stress disorder; SCL90-R: Symptom Checklist 90-Revised; TSST: Trier Social Stress Test.

low/moderate bias risk for the "representativeness of the exposed cohort" because the sample was not very representative. For the "selection of the non-exposed cohort", the bias risk was low, indicating that the controls had the same characteristics as the exposed population. The "ascertainment of exposure" had a moderate bias risk because the data were collected with medical records, biochemical parameters, and structured interviews, and with self-report measures. For the "demonstration that outcomes were not present at the start of study", the bias risk was low, demonstrating that outcomes were not added *in itinere*. For the "comparability of the cohort" the bias risk was low because the methodology was monitored over time for important prognostic factors. The "assessment of outcome" presented a moderate risk because were always used objective measures, self-report measures also if not blinded. The "adequacy of follow up of cohorts" presented a moderate-high risk because there were a lot of drops out (shown in Table S2).

The bias risk as referring to STROBE reported that 4/12 included studies lacked the item "6b = cohort study—for matched studies, give matching criteria and the number of exposed and unexposed, 12/12 included studies lacked the item "9 = describe any efforts to address potential sources of bias", 1/12 included studies lacked item "13b = give reasons for non-participation at each stage", all the studies lacked the item "13c = consider the use of a flow diagram", 7/12 included studies lacked the item "14c = cohort study—summarize follow-up time (e.g., average and total amount)" (shown in Table S3).

4. Discussion and future directions

This systematic review explored the effects of psychological interventions on the epigenetic mechanisms in participants with different psychological conditions and in healthy subjects. The main findings suggest dynamic changes in gene methylation status associated with clinical improvement after a psychological intervention, with an inverse association between remitters and non-remitters. The findings of the meta-analysis showed a significant effect of the symptom-focalization CBT on the methylation of the MAOA gene in participants with anxiety only for those participants who respond to the treatment. Specifically, only in respondent participants, the CBT seems to be associated with an increase in the methylation status of the MAOA gene (Ziegler, 2016; Schiele et al., 2018). The meta-analysis that included also the not responding participants showed no significant effects, suggesting that the observed methylation change occurred only in the respondent participants could be considered as a potential epigenetic correlate of the reduction of the anxiety symptomatology after the CBT treatment more than a direct correlate of the CBT outcome in anxious patients. MAO hypomethylation has been previously observed as an epigenetic risk pattern for patients with anxiety disorders ()(Domschke et al., 2012), since decreased methylation has been shown to activate MAOA expression, resulting in a decreased availability of monoamines in the synaptic cleft (Ziegler, 2016; (Checknita et al., 2015); Shumay et al., 2012). Considering this association between the MAOA hypomethylation and the risk for anxiety disorders, the findings of the meta-analysis suggested dynamic changes in MAOA methylation as a potential correlate of the anxiety symptoms after the CBT, tending to reverse the epigenetic risk of the MAOA hypomethylation (Ziegler et al., 2016). This effect of the CBT on the genes involved in neurotransmission was supported also by the findings of the systematic review that highlighted an increase in the methylation status of the MAOA and SERT genes in respondent participants with anxiety disorders (Roberts, 2014; Ziegler, 2016; Schiele, 2018).

Moreover, the systematic review highlighted that the CBT was the most investigated treatment (12/14 studies), and it seems to be associated with significant epigenetic effects mainly in anxiety disorders, while the Mindfulness protocol was considered in two studies, and it is associated with significant epigenetic effects in participants with stress and post-traumatic conditions. The detectable DNA methylation changes were associated with symptom improvement after psychological interventions. Specifically, after the relationship-centered CBT therapy, including Prolonged Exposure Therapy (Foa, 2007), Narrative Exposure Therapy (Neuner, 2002), and Dialectical Behavioural Therapy (Linehan, 1993), was detected a significant reduction in the methylation status of the gene involved in neuroplasticity (BDNF), of the genes involved in the immunoregulation in the respondent participants with borderline personality disorder (Perroud, 2013; Thomas, 2018), of the genes involved in immunoregulation and inflammation in PTSD (Yehuda, 2013; Morath, 2014) and anxiety disorders (Roberts et al., 2015), while was detected a significant increase in the methylation status of the same genes in the not-respondent participants. These above considered genes seem to be the biological correlates for traumatic

Table 4
Distribution of trials of the significant (s) and not-significant (ns) post- vs pre- treatment (Cognitive Behavioural Therapy (CBT) and Mindfulness) methylation status (increase (+) or decrease (-)) for considered epigenetic outcomes (genes involved in immunoregulation and inflammation, in cellular functioning, in neural plasticity and in neurotransmission, and basal DNA mechanisms) in each study (study ID in brackets) per each psychological condition. The post- vs pre- treatment methylation status differentiated by responders (R) and not-responders (NR) was reported when provided by the studies. All the included trials involved DNA analysis on blood samples except for those with * that refer to DNA analysis on saliva samples.

Psychological treatment(14 studies, 15 samples, 38 trials)		CBT(13 samples, 20 trials: 14 s, 6 ns)										Mindfulness(2 samples, 18 trials: 6 s, 12 ns)									
Psychological condition		PTSD(5 trials:4 s, 1 ns)		Anxiety(8 trials: 7 s, 1 ns)		Depression (2 trials: 1 s, 1 ns)		BPD(5 trials: 2 s, 3 ns)		Stress(0 trial)		PTSD(2 trials)		Anxiety(0 trial)		Depression(0 trial)		BPD(0 trial)		Stress(16 trials:5 s, 11 ns)	
Methylation status post- vs pre-treatment (38 trials:20 s, 18 ns)		s (2 +, 2 -)	ns (1)	s (7 +, 4 -)	ns (1)	s (1 +, 1 -)	ns (1)	s (1 +, 2 -)	ns (2)	s (0+,0-)	ns (0)	s (1+,1-)	ns (1)	s (0+,0-)	ns (0)	s (0+,0-)	ns (0)	s (0+,0-)	ns (0)	s (5 +, 0-)	ns (11)
Genes involved in immunoregulation and inflammation (12 trials)	RIPK2																			+ (4)	
	COX2																			+ (4)	
	CCR7																				/ (4)
	CXCR1																				/ (4)
	IL-6																				/ (4)
	TNF-α																				/ (4)
	IL1R1 (13): EWAS			+(13) (R) -(13) (NR)																	
	FKBP5 (12): exon 1 (7): 4 CGIs (3): 7CGIs	- (12) (R) +(12) (NR)		- (7)(R) * +(7) (NR)*								+(3) (R) -(3) (NR)									
	NR3C1 (12): exon 1F (7): 4 CGIs		/(12)		/(7) *																
Genes involved in gene regulation and cellular functioning (17 trials)	APBA3 (5): 2 CGIs								/(5)												
	MCF2 (5): 1 CGI								/(5)												
	PER1																				/ (4)
	PER2																				/ (4)
	PER3																				/ (4)
	BMAL1																				/ (4)
	DBP																				/ (4)
	CRY1																				/ (4)
	CRY2																				/ (4)
	HDAC2																			+ (4)	
	HDAC3																			+ (4)	
	HDAC9																			+ (4)	
	GLUT1 (2): 8 CGIs					- (2) (R) +(2) (NR)															

(*continued on next page*)

Table 4 (*continued*)

Psychological treatment(14 studies, 15 samples, 38 trials)		CBT(13 samples, 20 trials: 14 s, 6 ns)					Mindfulness(2 samples, 18 trials: 6 s, 12 ns)				
Psychological condition		PTSD(5 trials:4 s, 1 ns)	Anxiety(8 trials: 7 s, 1 ns)	Depression (2 trials: 1 s, 1 ns)	BPD(5 trials: 2 s, 3 ns)	Stress(0 trial)	PTSD(2 trials)	Anxiety(0 trial)	Depression(0 trial)	BPD(0 trial)	Stress(16 trials:5 s, 11 ns)
	GLUT4 (2): 24 CGIs			/(2) (R) /(2) (NR)							
	ZFP622 (13): EWAS		+(13) (R)								
	ZFP57 (11): GWAS	+(11) (R) -(11) (NR)									
	SLC43A2 (13): EWAS		+(13) (R)								
Genes involved in neural plasticity (3 trials)	BDNF (10): 4 CGIs (6): exons I and IV				-(6) (R) +(6) (NR) - (10) *	/(10)					
Genes involved in neurotransmission (4 trials)	MAO-A (9): 13 CGIs (14): 13 CGIs		+ (14) (R) - (14) (NR) + (9)								
	SERT (8): 6 CGIs (3): 42 CGIs		+(8)(R) * -(8) (NR)*					/(3) (R) /(3) (NR)			
Basal DNA mechanisms (total 2; 1+, 1-)											
	DNA strand brakeage	- (1)									
	DNA repair	+ (1)									

Note. APBA3: Amyloid Beta Precursor Protein Binding Family A Member 3; BDNF: brain-derived neurotrophic factor gene; BMAL1: Brain and Muscle ARNT-Like 1;BPD: borderline personality disorder; CBT: Cognitive Behavioural Therapy; CCR7: C—C Motif Chemokine Receptor 7; COX2: cytochrome c oxidase subunit II; CGI(s): CpG island(s); CRY1: Cryptochrome Circadian Regulator1; CRY2: Cryptochrome Circadian Regulator2; CXCR1: C-X-C Motif Chemokine Receptor 1; DBP: D Site-binding protein; EGR1: Early Growth Response 1; FKBP5: FK506-binding protein 51; GLUT1: glucose transporter 1; GLUT4: glucose transporter 4; HDAC2: histone deacetylase 2; HDAC3: histone deacetylase 3; HDAC9: histone deacetylase 9; IL-6: Interleukin-6; IL1R1: Interleukin 1 Receptor Type 1; MAOA: monoamine oxidase A; MCF2: Cell Line Derived Transforming Sequence; NR3C1: Nuclear Receptor Subfamily 3 Group C Member 1; PER1: Period Circadian Regulator 1; PER2: Period Circadian Regulator 2; PER3: Period Circadian Regulator 3; PTSD: post-traumatic stress disorder; R: responders to treatment; NR: not responders to treatment; RIPK2: receptor-interacting serine-threonine kinase 2; SERT: serotonin transporter; SLC43A2: Solute Carrier Family 43 Member 2; Stress: generic stress; TNF-α: Tumor Necrosis Factor-alpha; ZFP622: Zinc Finger Protein 622; ZFP57: Zinc Finger Protein 57.

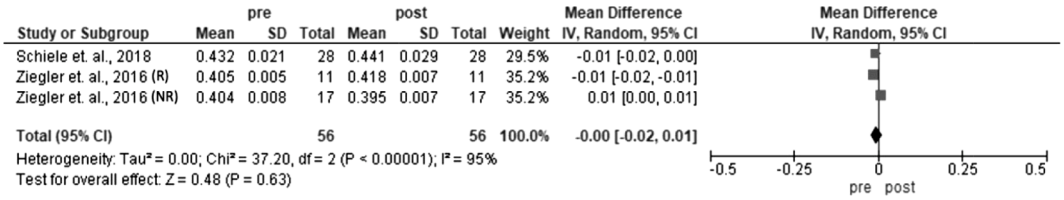

Fig. 2. Observational prospective cohort studies: effects of Cognitive Behavioural Therapy (CBT) on post- vs pre- treatment MAOA methylation status in samples of anxious patients. Both responders (R) and not-responders (NR) to treatment were included in the analysis (two studies and three trials).

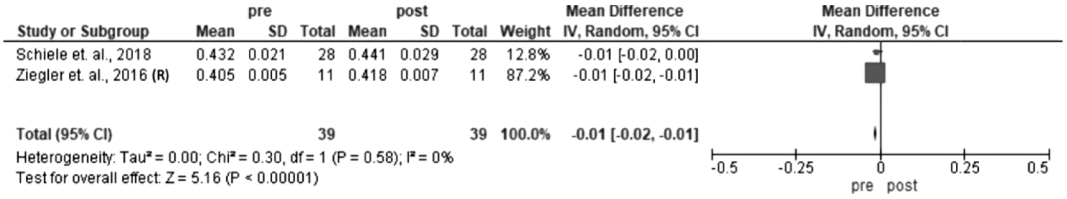

Fig. 3. Observational prospective cohort studies: effects of Cognitive Behavioural Therapy (CBT) on post- vs pre- treatment MAOA methylation status in samples of anxious patients. Only responders (R) to treatment were included in the analysis (two studies and two trials).

experiences and life adversity and this is consistent with the evidence that there is an altered methylation status of these genes in subjects with borderline personality disorder and PTSD (Hawn et al., 2019; Kundakovic et al., 2015).

It is interesting to note that, after the Dialectical Behavioural Therapy, the participants with borderline personality disorder showed a significantly decreased methylation status in two of the three considered trials (Perroud, 2013 and Thomas, 2018a) but not significant effects in the other one (Thomas, 2018b). In their study, Perroud and colleagues (2013) found a significant effect in peripheral blood, while Thomas and colleagues (2018) found a significant difference only in saliva, but not in blood. A possible explanation of this discrepancy could be attributable to the different methods employed for DNA methylation analysis (high resolution melt analysis in Perroud, 2013 and pyrosequencing in Thomas, 2018). Moreover, these contrasting findings may be ascribable to the fact that Perroud and colleagues found the effect only in respondent participants, while Thomas and colleagues did not split their sample in patients with and without significant alleviation of psychological symptoms after therapy. Nevertheless, these observations suggest that, considering the importance of tissue-specificity of DNA methylation in biomarker studies, BDNF methylation may be considered as a biological correlate for treatment success in patients with borderline personality disorder.

An additional significant finding was observed for symptom-centered CBT treatment that seems to be associated with a decrease in the methylation status of the GLUT1 gene, the major glucose transporter in the brain, only in the remitted participants with major depressive disorder.

The Mindfulness treatment showed promising results in hypo-regulating the expression of chromatin modulating genes (HDAC2, HDAC3, HDAC9 genes), and proinflammatory genes (COX 2 and RIPK2 genes) in stress conditions (Kaliman, 2014). These effects of psychological interventions in stress conditions were confirmed also by other studies (Wahbeh et al., 2009; Muñoz et al., 2018; Cozzolino, 2017) that were not included in the present study because they did not meet the inclusion criteria of the present systematic review. The Mind Body Therapy, which promotes resources to act on the immune response to stress (Wahbeh et al., 2009), resulted to have an effect in the regulation of the pro - inflammatory NF-kB and EGR1 genes (Muñoz et al., 2018), and in changes in the general methylation status (Cozzolino, 2017) in patients with stress condition. Moreover, differently from the studies of Roberts et al. (2015) and Yehuda (2013) that explored the association between CBT and methylation status of the FKBP5 in PTSD and anxiety, in the study of Bishop and colleagues (2018) after the Mindfulness treatment, the FKBP5 resulted hypermethylated in the respondent participants and hypomethylated in the non-respondent. This discrepancy among the studies could be attributable to the different regulator regions of the gene assessed (exon 1 promotor in Roberts et al. and Yehuda et al. vs intron 7 GRE in Bishop et al.), however, all the three studies observe changes in FKBP5 methylation status related to the symptom response after a psychological treatment.

Regarding the non-significant outcomes, it is particularly interesting to note that the expression of the NR3C1 gene (glucocorticoid receptor) did not seem to undergo any changes after CBT treatment neither in PTSD nor anxiety (Yehuda, 2013; Roberts et al., 2015). Considering that the NR3C1 gene has been repeatedly associated with early life adversity ((Turecki, 2016); Watkeys et al., 2018(Oberlander, 2008a)), it might be hypothesized that these early environmental influences on the expression of this gene are enough stable and long-lasting to account for the lack of epigenetic change after the psychotherapy (Yehuda, 2013).

In summary, the present work interestingly highlighted that the majority of the studies found different and opposite effects of the psychological treatment when splitting the samples into respondent and non-respondent participants. Subjects responding to CBT therapy were associated with a specific decrease or increase in the methylation status, that in subjects not responding to therapy was associated in the opposite way (Perroud, 2013; Yehuda, 2013; Roberts, 2014, Roberts et al., 2015; Ziegler, 2016; Kahl, 2016, 2019). Moreover, in PTSD and anxiety conditions, it was interesting to observe a not clear methylation response in the respondent participants for the genes involved in immune responses (greater methylation of IL1R1, and lower methylation of FKBP5), while, in the same psychological conditions, greater methylation of genes involved in cellular functioning (ZFP622, ZFP57, SLC43A2) and neurotransmission (MAOA and SERT) was found in respondent participants.

This evidence suggests that psychotherapy might be associated with significant and favourable epigenetic changes only when concomitant clinical improvement occurs. It is interesting to note that the outcome of therapy would seem to correspond both positively and negatively to an associated epigenetic response. This aspect could be also considered as a risk factor for subsequent adherence and prognosis to a treatment (Franco, 2017). These observations are highly intriguing, and they would suggest that the observed epigenetic changes after a psychological treatment could be related to the different meaning attribution processes that occur during therapeutic work. Moreover, this distinction between respondent and not-respondent participants in the epigenetic

response supports the emerging notion that the epigenetic changes could be the underlying neurobiological mechanisms of extinction-related psychotherapeutic interventions in different psychological disorders ((Stafford and Lattal, 2011); Kandel, 1998). It seems that epigenetic mechanisms are characterized by dynamic processes and are experience-dependent, thus that, if on the one hand adverse life experiences can lead to epigenetic vulnerability (Mitchel et al., 2016; Pellicano et al., 2020), on the other hand, we can think that positive life experiences, such as psychotherapy, can be reparative and protective (Cozzolino, 2017).

The present review study opens to new and suggestive implications in the field of mental health, pointing clinical practice to dialog and integration with the new epigenetic science. However, the younger field of epigenetics needs to consider the lesson learned from the genomic studies that highlighted the difficulty to replicate the findings of the candidate gene and candidate GxE studies (Border, 2019). The majority of studies collected in the present study are based on a candidate-gene selection in very small samples that may lead to a misinterpretation of the effects. Nevertheless, the emerging body of evidence on the epigenetic correlates of psychological treatment highlights promising future perspectives on the interaction between psychological and biological systems. The present study highlights an association between dynamic changes in epigenetic mechanisms and different psychological treatment that seems to be related to clinical improvement, with an inverse association between remitters and non-remitters.

These findings support the planning of future studies focused on epigenetic changes of genes involved mainly in inflammation response, in neurotransmission, and neuroplasticity, considering also other psychological treatments, as psychodynamics, and their interactions with pharmacological treatments that remains still not investigated. Finally, it remains to investigate the duration and stability of the changes in epigenetic mechanisms associated with the improvement of the symptoms after the psychological intervention.

4.1. Limitation

The difference between the large number of studies using CBT treatment compared only to the mindfulness intervention does not allow determining which therapeutic protocol could be more useful in bringing about epigenetic changes. The analysis of the action of the different types of treatment suffers greatly from the lack of studies using other approaches less oriented to the behavioural symptom but more focused on the analysis of the affective dynamics, the process, and the relationship, such as, for example, psychodynamic treatments.

The meta-analysis compared only two studies with the same epigenetic outcome and, despite the methodology was correctly set, the small number of subjects could indicate a possible methodological recruitment bias. In addition, since most of the studies of the systematic review are observational studies, blinded assessment is not ensured as in RCT studies, and this could lead to a risk of bias in the ascertainment of exposure and in the assessment of outcomes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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