



## Review article

## Stress, burnout and depression: A systematic review on DNA methylation mechanisms

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## ABSTRACT

Despite that burnout presents a serious burden for modern society, there are no diagnostic criteria. Additional difficulty is the differential diagnosis with depression. Consequently, there is a need to dispose of a burnout biomarker. Epigenetic studies suggest that DNA methylation is a possible mediator linking individual response to stress and psychopathology and could be considered as a potential biomarker of stress-related mental disorders. Thus, the aim of this review is to provide an overview of DNA methylation mechanisms in stress, burnout and depression. In addition to state-of-the-art overview, the goal of this review is to provide a scientific base for burnout biomarker research. We performed a systematic literature search and identified 25 pertinent articles. Among these, 15 focused on depression, 7 on chronic stress and only 3 on work stress/burnout. Three epigenome-wide studies were identified and the majority of studies used the candidate-gene approach, assessing 12 different genes. The glucocorticoid receptor gene (NR3C1) displayed different methylation patterns in chronic stress and depression. The serotonin transporter gene (SLC6A4) methylation was similarly affected in stress, depression and burnout. Work-related stress and depressive symptoms were associated with different methylation patterns of the brain derived neurotrophic factor gene (BDNF) in the same human sample. The tyrosine hydroxylase (TH) methylation was correlated with work stress in a single study. Additional, thoroughly designed longitudinal studies are necessary for revealing the cause-effect relationship of work stress, epigenetics and burnout, including its overlap with depression.

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## 1. Introduction

Psychosocial stress is a major contributor to morbidity, mortality, and health-care costs. While acute intermittent stress may be essential for successful adaptation to changing natural and social environments, chronic excessive stress carries a high risk of damaging consequences to the health [1]. Chronically persisting environmental and work-related stress can lead to severe psychosocial syndromes such as burnout and depression [2], which are among the top causes of disability and disease burden in modern society.

Burnout is a prolonged response to chronic occupational stress, and is defined as a combination of (emotional) exhaustion, cynicism (also termed as depersonalization), and lack of professional efficacy (or reduced personal accomplishment) [3]. So far, no binding diagnostic criteria exist for identifying cases of burnout [4]. It is not present in

the *Diagnostic and Statistical Manual of Mental Disorders*, currently in its fifth edition [5], and only appears as a factor influencing health status and contact with health services in the *International Classification of Diseases* [6]. In contrast, the *DSM-5* [5] provides the definition and classification of depression. Accordingly, depression covers a broad spectrum of disorders, which are multifactorial in origin including genetic, developmental, and environmental risk factors [7,8]. Whether burnout is a form of depression or a distinct phenomenon is still an object of controversy. Despite a considerable amount of research on both concepts, the distinction between burnout and depression remains unclear [9]. Furthermore, in spite of a rapid increase in research dedicated to physical and biological aspects of burnout, a clear distinction between burnout and depression at somatic and biological levels remains inconclusive as well [10]. Even though numerous studies have tried to retrieve specific biomarkers for burnout [11], as well as biological parameters that could differentiate burnout from depression [12,13], so far the results are inconsistent.

Chronic psychosocial stress plays a central role in the etiology of both, burnout and depression [14]. Recently, there is a growing number of studies exploring mechanisms of stress adaptation and stress

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susceptibility. This led to the understanding that individual response to stress is mediated by the interplay between environmental factors and genetics [15]. A growing number of studies suggest that this interaction occurs through epigenetic mechanisms, whose main concept refers to variation in gene expression rather than gene sequence. Therefore, epigenetic mechanisms, including DNA methylation changes could play a crucial role in development of psychopathology [16]. In view of this, studies indicate epigenetic and functional changes of at least two types of stress-associated genes: those that directly govern the hypothalamic-pituitary-adrenal axis (HPA-axis) function, and those that cause long-term dysregulation of neuronal processes, and are significant for proper regulation of mood, emotions, and cognition [17].

A number of studies have shown that DNA methylation, as a reaction to external stress, plays an important role in pathogenesis of various stress-related psychiatric disorders, such as MDD and post-traumatic stress syndrome (PTSD) [18,19]. On the one hand, DNA methylation was thoroughly studied as a mediator in the effects of trauma and early life stress on mental health and the development of adult psychopathology [20,21]. On the other hand, studies observing epigenetic changes in correlations with chronic adult stress have expanded quite recently, even though the importance of chronic

psychosocial stress in psychopathology contribution has been well known [22]. In terms of work-related stress and DNA methylation, scientific evidence have just started emerging, resulting in only a few studies so far.

The aim of this review is to provide a comprehensive overview of changes in DNA methylation related to chronic psychosocial/work stress exposure, on the one hand and burnout/depression as the clinical outcome, on the other. In addition to state-of-the-art overview, the goal of this review is also to provide a scientific base for burnout biomarker research. Bearing in mind the difficult differential diagnosis between burnout and depression as well as possibly similar mechanisms in which work-related and chronic psychosocial stress in general affect DNA methylation, we are convinced that the results of this review could be useful starting point for research in this area.

## 2. Methodology

### 2.1. Study identification

We performed a literature search of studies associating chronic psychosocial/work stress or burnout/depression with DNA methylation. The literature reviewed here was identified via the MEDLINE (through

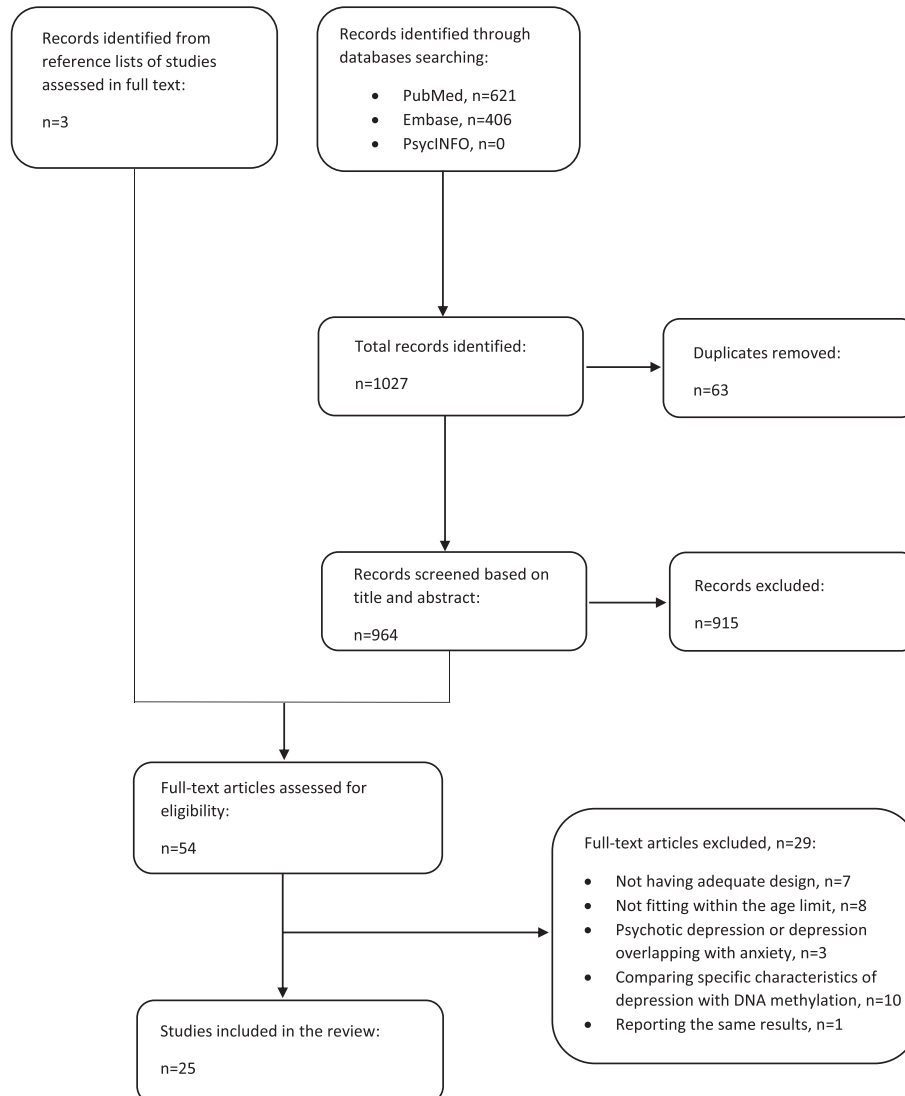


Fig. 1. PRISMA flow diagram of the study inclusion process.

PubMed), EMBASE and PSYCHINFO databases, using the keywords “work stress” OR “chronic psychosocial stress” OR “burnout” OR “depression”, crossed with “DNA methylation”. Additional articles were found scanning the list of references of original publications and review articles.

## 2.2. Study selection

Studies included in the review met the following criteria: 1) use of a case-control or cohort design; 2) performed on adult animal models or adult human population (18–65 years old); 3) use of quantitative methods to measure chronic psychosocial/work stress or clinical tools to assess burnout/depression; 4) use of at least one analysis investigating DNA methylation.

## 2.3. Data extraction

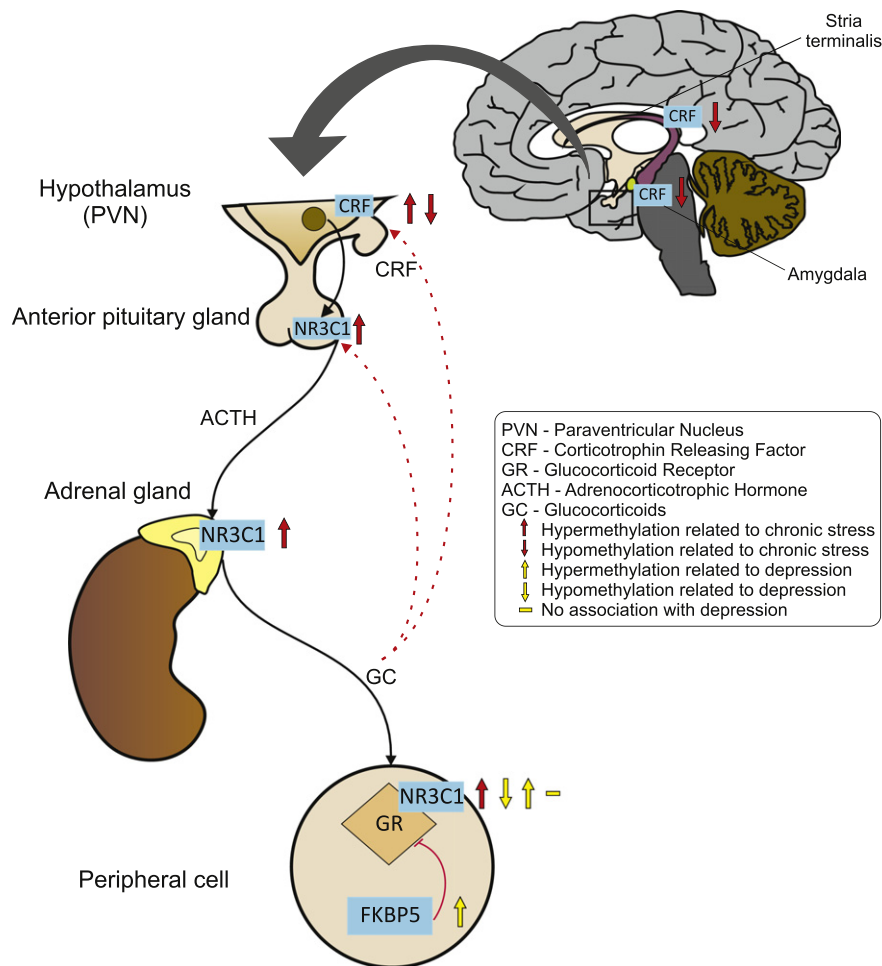
For each study, we extracted information on the following variables: 1) species, 2) experimental group characteristics, 3) sample size, 4) tissue used for DNA extraction, 5) gene and region examined, 6) DNA methylation assessment, 7) stress exposure, 8) effects of stress on

DNA methylation, 9) burnout/depression assessment, 10) effects of burnout/depression on DNA methylation.

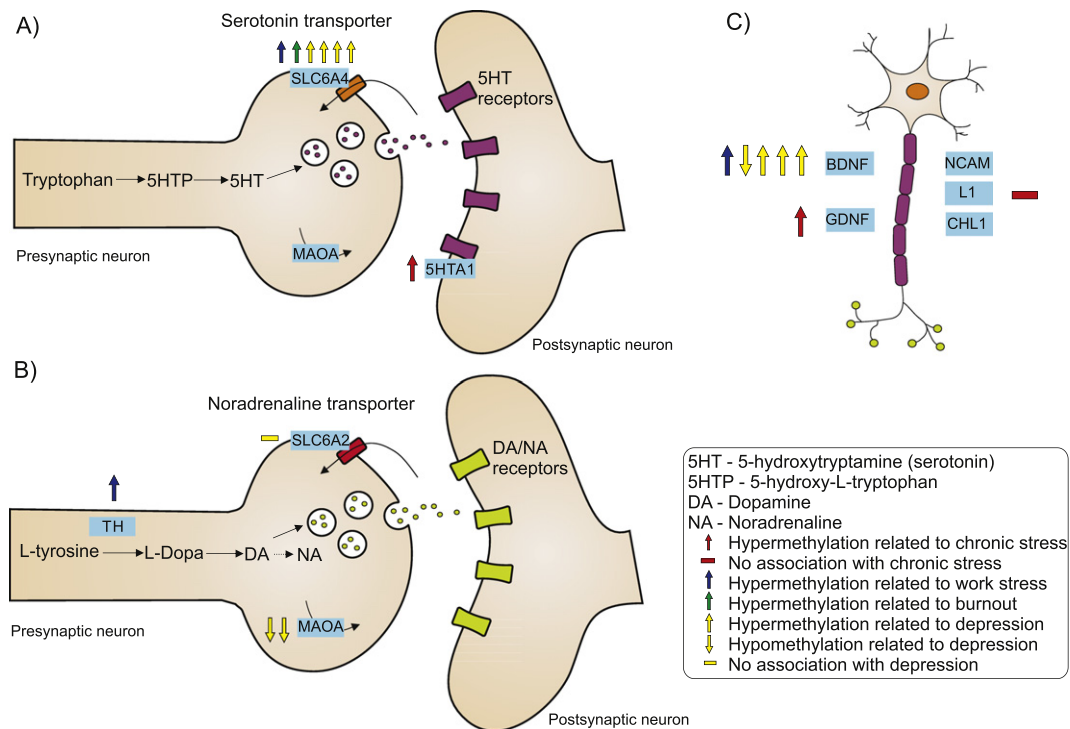
## 3. Results

Based on our search strategy, a total of 964 articles were identified. After an initial title and abstract scan, 51 studies were considered for further inspection. After full text inspection, 29 studies were excluded. Out of these, 7 studies did not meet the inclusion criteria, 9 did not fit within the age limit, 10 observed specific clinical characteristics of depression (processing of emotional stimuli, treatment response etc.), 3 assessed psychotic depression or depression overlapping with anxiety disorders and, finally, two article displayed results of the same study, so we included one of them in the review. 3 additional studies were included by scanning the references of relevant articles. An overview of the steps in the literature search is given in Fig. 1.

In total, 25 papers met all the criteria, including both, studies that used animal models (6) and human studies (19). Studies examining chronic stress and DNA methylation were predominantly performed on animal samples and generally focused on HPA-axis related genes (3 different genes in various tissue samples). On the other hand, the



**Fig. 2.** Summary of the results on DNA methylation of the HPA-axis genes. The figure presents the HPA-axis response to stress stimuli. Detection of environmental stressors by the limbic system induces neurons in the paraventricular nucleus (PVN) of the hypothalamus to produce corticotrophin releasing factor (CRF), which triggers secretion of adrenocorticotrophic hormone (ACTH) from the pituitary gland. This in turn leads to peripheral secretion of glucocorticoids (GC) from the adrenal glands into the blood stream and a negative feedback loop. The genes involved in this pathway are marked in blue: CRF—corticotrophin releasing factor gene; NR3C1—glucocorticoid receptor gene; FKBP5—FK506 binding protein 5 gene.



**Fig. 3.** Summary of the results on DNA methylation of the genes involved in neurotransmission and neural processes. A) Genes involved in serotonergic transmission; B) Genes involved in dopaminergic/noradrenergic transmission; C) Genes encoding for neural growth factors and neural adhesion molecules. The genes are marked in blue: SLC6A4—serotonin transporter gene; SLC6A2—noradrenaline transporter gene; 5HTA1—serotonin receptor gene; MAOA—monoamine oxidase A gene; TH—tyrosine hydroxylase gene; BDNF—brain derived neurotrophic factor gene; GDNF—glial cell line-derived neurotrophic factor gene; NCAM, L1, CHL1—genes encoding for neural adhesion molecules.

majority of studies on work stress, burnout, depression and DNA methylation were human and focused on genes involved in neurotransmission and neural processes (9 different genes). Results displaying DNA methylation of the HPA-axis genes are summarized in Fig. 2, whereas the ones showing DNA methylation patterns of the genes involved in neurotransmission and neural processes are given in Fig. 3.

### 3.1. Quality assessment and characteristics of included studies

We assessed the risk of bias and quality of interventional (animal) studies by adopting the SYRCL's risk of bias tool for animal studies [23]. We included items from selection bias and performance/detection bias groups and added two additional items relevant for the scope of this review (baseline DNA methylation assessment and assessment of stress-induced behavior). Two studies were of high quality (6–8 out of 8), three of intermediate quality (3–5 out of 8), and one study of low quality (2 out of 8). Quality of observational (human) studies was assessed with the Newcastle–Ottawa Scale (NOS) [24]. We modified the scale by adding an additional category for sample size, with a possible score of 0–3. An overall score of 0–4 was considered low quality, 5–8 intermediate, and 9 or more high quality. The majority of studies were of intermediate quality (5–8 out of 12). Quality assessment of included studies is presented in Appendix A.

Characteristics of included studies were described in four separate tables based on whether they focused on the effects of chronic psychosocial stress on DNA methylation (animal studies and a human study presented in a separate table), correlation between work stress/burnout and DNA methylation (human studies) or association between depression and DNA methylation (human studies).

### 3.2. Chronic psychosocial stress and DNA methylation in animal studies

Animal models were used in 6 studies [2,25–29] and the results are presented in Table 1. The majority of studies examined genes involved in HPA-axis regulation: i.e., corticotrophin-releasing factor gene (CRF) [26,27] and glucocorticoid receptor gene (GR) [25, 28]. Genes outside the HPA-axis investigated in the animal studies include the genes encoding for neural adhesion molecules of the immunoglobulin superfamily (NCAM, L1 and CHL1) [25], the serotonin receptor gene (5HT1A) [29] and the gene encoding for glial cell line-derived neurotrophic factor (GDNF) [2]. All the studies used candidate-gene approach, and observed DNA methylation in the CpG islands in the promoter region of determined genes. DNA methylation was assessed by pyrosequencing of bisulfate-converted DNA [26–28] or using bisulfite mapping and direct sequencing [2,25,29].

In the study of Desarnaud et al. [25], no association was found between social defeat stress and DNA methylation levels in the promoter region of GR gene or NCAM, L1 and CHL1 gene.

In the study of Uchida and colleagues [2], Chronic ultra-mild stress (CUMS) induced increased methylation levels in 2 CpG sites in the promoter region of the GDNF gene in the nucleus accumbens in the stress-vulnerable (BALB) mouse group as well as in 1 CpG site in the same region in the stress-resilient (B6) mouse group. Furthermore, increased DNA methylation was associated with the expression of depression-like behavior.

In the study of Witzman et al. [28], stress-induced changes in DNA methylation were observed in the CpG sites in exon 7 of GR gene in different tissues. Chronic psychosocial stress induced an increase in the overall methylation level of the gene in the adrenal and pituitary glands.

**Table 1**  
Animal studies and a human study on chronic psychosocial stress and DNA methylation.

Study (Quality score)	Experimental group, sex	Methylation analysis, N	Stress exposure	Tissue	Gene, region	Depression assesment	Effect of stress on DNA methylation	Effect of depression on DNA methylation
Desarnaud et al. [25] (2)	C57BL/6 J mice	Stressed and controls, 5 animals per group	Social defeat stress	Hippocampus	NCAM, L1, CHL1, NR3C1, promoters	/	No association	/
Elliot et al. [26] (7)	Stress-vulnerable and stress-resilient mice, 0% female	Stress-vulnerable and stress-resilient after exposure, controls	Chronic social stress (social defeat)	Hypothalamus	CRF, promoter	Anhedonia and social avoidance	Stress-vulnerable Average: ↓ (promoter)	/
Sterrenburg et al. [27] (4)	Wistar rat	Female/male stressed, female/male controls, 6 animals per group	Chronic variable mild stress	Hypothalamus (PVN), central amygdala (CeA), bed nucleus of the stria terminalis (BST)	CRF, promoter	Body weight loss	PVN Sum: ↑♀ 2 CpG ↑♀ BST Sum: ↓♂ 1 CpG ↓♀♂ 2 CpG ↓ 1 CpG ↑♂ 1 CpG ↓♀ CeA Sum: ↓♀ 2 CpG ↓♀ 1 CpG ↑♂ BALB: 2 CpG ↑ B6: 1 CpG ↑ (promoter) Pituitary gland Average: ↑ (promoter) Adrenal glands Average: ↑ (promoter) Blood Average: ↑ (promoter) PFC 1 CpG: ↑ Midbrain 1 CpG: ↑	/
Uchida et al. [2] (6)	Stress-vulnerable (BALB) and stress-resilient (B6) mice, 0% female	Before and after stress exposure	Chronic ultra-mild stress (CUMS)	Nucleus accumbens	GDNF, promoter	Anhedonia, increased immobility time in FST, social avoidance, novelty-suppressed feeding test	BALB: 2 CpG ↑ B6: 1 CpG ↑ (promoter)	Depressive-like behavior ↑
Witzmann et al. [28] (3)	Sprague Dawley rats, 0% female	Stressed and controls, 6 animals per group	Chronic psychosocial stress	Adrenal, pituitary, hypothalamus (PVN), hippocampus, blood	NR3C1, promoter	/	Pituitary gland Average: ↑ (promoter) Adrenal glands Average: ↑ (promoter) Blood Average: ↑ (promoter) PFC 1 CpG: ↑ Midbrain 1 CpG: ↑	/
Le Francois et al. [29] (3)	Stress-vulnerable (BALB) mice, 0% female	Stressed and controls, 15 animals per group	Unpredictable chronic mild stress	Prefrontal cortex (PFC) and midbrain	5HT1A, promoter	Deterioration of self-oriented behaviors (nest building and grooming)	PFC 1 CpG: ↑ Midbrain 1 CpG: ↑	/

↑ increased, ↓ decreased; ♀ females, ♂ males; PVN—periventricular nucleus; FST—Forced swim test.

The CRF gene was examined in two studies. In the one by Elliot et al. [26], chronic social stress induced a decrease in the average methylation level in the promoter region of the CRF gene in the paraventricular nucleus (hypothalamus), which was additionally associated with depressive-like behavior. In the other study [27], DNA methylation changes were sex-specific and brain center-specific (for different parts of limbic system). More specifically, female rats exposed to chronic variable mild stress had an increased overall methylation level in the paraventricular nucleus (hypothalamus) compared to female controls and methylation in 2 CpG sites increased after stress exposure. By contrast, in the BST, the overall methylation level was lower in stressed male rats compared to controls. In the central amygdala (CeA) tissue, the total DNA methylation level in stressed females was lower than in controls. Moreover, methylation levels were changed in individual CpG sites in both tissue samples and these changes were also sex-specific. All CpG sites observed were located in the promoter region of exon 1 and in the intronic sequence between exons 1 and 2 of the CRF gene.

In the recent study by le Francois et al. [29], DNA methylation changes were observed in 24 CpGs in the promoter region of 5HT1A gene. Unpredictable chronic mild stress exposure induced hypermethylation of

1CpG site in the promoter of the 5HT1A gene located in prefrontal cortex (PFC) and midbrain of stress-vulnerable (BALB) mice compared to controls.

### 3.3. Human studies

#### 3.3.1. Chronic psychosocial stress and DNA methylation

In the human study on effects of chronic stress on DNA methylation [30], chronic stress exposure was assessed using the Trier Inventory of Chronic Stress (TICS) [31] whereas depressive symptoms were assessed using the Beck Depression Index-II (BDI-II) [32]. Global DNA methylation was assessed using the PyroMark Q96 MD system. In addition, a gene-specific DNA methylation of CpG sites in amplicons 1 and 2 of the gene encoding for serotonin transporter (SLC6A4) was assessed using the Sequenom Epityper MassArray system. Chronic stress was correlated with increased global methylation, whereas depressive symptoms were associated with increased methylation levels of the SLC6A4 gene. However, these changes were 5-HTTLPR genotype dependent. (See Table 2.)



**Table 2**

A human study on chronic psychosocial stress and DNA methylation.

Study (Quality score)	Experimental group, sex	Methylation analysis, N	Stress exposure	Tissue	Gene, region	Depression assesment	Effect of stress on DNA methylation	Effect of depression on DNA methylation	Adjustments
Duman et al. [30] (6)	Healthy adults, 0% female	Before acute stress exposure, 71	Trier Inventory of Chronic Stress (TICS)	Blood	Global methylation, SLC6A4	BDI-II	Global: ↑  CpG island in amplicon 1: ↑ (SLC6A4)  (5-HTTLPR genotype dependent)	Average: ↑ (SLC6A4)  (5-HTTLPR genotype dependent)	5-HTTLPR genotype, no psychiatric diagnosis, no medication use

↑ increased, ↓ decreased; BDI-II—Beck Depression Inventory II.

**3.3.2. Work-stress/burnout and DNA methylation**

Three studies reported results on work-stress effects on DNA methylation. The results were summarized in Table 3. All studies used the candidate-gene approach and examined different genes. Two studies examined genes involved in the regulation of neurotransmission: SLC6A4 [33] and the gene encoding tyrosine hydroxylase (TH) [11], whereas one study examined the BDNF gene [34]. All studies used the same method for DNA methylation assessment—Human Methylation 450 BeadChip [11,34], whereas in one study, DNA methylation was assessed by direct sanger

bisulfite sequencing and afterwards verified with Methylation 450 BeadChip [33].

In the study of Alasaari et al. [33], DNA methylation was examined in 5CpG sites in the promoter region of the SLC6A4 gene. Work stress was associated with higher levels of DNA methylation in all 5CpG sites individually. In unadjusted (bivariate) analysis, burnout was not significantly associated to methylation levels. However, when mutually adjusted for both, burnout and work stress were significant contributors to higher methylation levels. In addition, this correlation was independent from the 5-HTTLPR genotype of the participants.

**Table 3**

Human studies on work stress, burnout and DNA methylation.

Study (Quality score)	Experimental group, sex	Methylation analysis, N	Stress exposure	Tissue	Gene, region	Burnout/depression assesment	Effect of work stress on DNA methylation	Effect of burnout/depression on DNA methylation	Adjustments
Alasaari et al. [33] (7)	Finish shift-working nurses, 100% female	High work stress group:24, low work stress group:25	JCQ	Peripheral blood leucocytes	SLC6A4, promoter	Burnout: MBI-GS Depressive symptoms: BDI	5 CpG: ↑	Burnout: ↑ (adjusted to WS)	Participants excluded: medication use, heavy smoking or high alcohol intake; Adjusted for 5-HTTLPR genotype No medical history of any psychiatric disorder
Miyaki et al. [11] (7)	Japanese manufacturing company workers, 9% female	Quartiles with lowest & highest job strain, 90 each; Q1 and Q4 compared	JCQ	Saliva	TH	Depressive symptoms: K6	4 CpG (promoter): ↑  12 CpG sites: ↑ 7 CpG sites: ↓ 2 CpG sites: ↑ (5' flanking region)  Average (promoter): ↑		
Song et al. [34] (7)	Japanese manufacturing company workers, 9% female	Quartiles with lowest & highest job strain, 90 each; quartiles with lowest & highest depression, 90 each; Q1 and Q4 compared	JCQ	Saliva	BDNF	Depressive symptoms: K6	Average: ↑ /  22 CpG sites: ↑ 28 CpG sites: ↓  Average: ↑	Depressive symptoms 3CpG (promoter): ↑ 2CpG (promoter): ↓  13 CpG sites: ↑ 43 CpG sites: ↓  Average (promoter): ↓  Average: ↓	Not implicated

↑ increased, ↓ decreased; JCQ—Job Content Questionnaire; MBI-GS—Maslach Burnout Index General Survey; BDI—Beck Depression Inventory; WS—work stress.

In the recent study conducted by Myaki and colleagues [11], DNA methylation was assessed in all CpG sites located throughout the TH gene and the 5' flanking region using DNA extracted from saliva samples. The average methylation level of all CpG sites was significantly increased in the highest job strain group (Q4) compared to the one of the lowest job strain group (Q1). In addition, hypermethylation associated with job strain was observed in the promoter region of the gene as well as in the majority of the individual CpG sites located throughout the whole gene sequence (64%).

Song and colleagues conducted a study comparing work stress and depressive symptoms among Japanese workers with DNA methylation of the brain derived neurotrophic factor (BDNF) gene [34]. DNA was extracted from saliva samples of the participants and all CpG sites (97) throughout the BDNF gene sequence were included in the methylation assessment. Work stress was associated with decreased average methylation of the gene whereas, in contrast, depressive symptoms contributed to hypermethylation of both- the whole gene and the promoter region.

### 3.3.3. Depression and DNA methylation

15 studies focused on the potential correlation between depression and DNA methylation [35–49]. The results were described in Table 4. The majority of studies used a candidate-gene approach, with only three epigenome-wide association studies (EWAS). Gene-specific DNA methylation was assessed by sodium bisulfite conversion and pyrosequencing [40,41,46,47], sodium bisulfite treatment and EpiTyper methylation analysis on MassArray platform (Sequenom) [38,39,42,45,48,49] or, in one study, using bisulfite conversion and methylation-specific quantitative PCR [44]. In EWAS, DNA methylation was assessed using Illumina Human Methylation27 BeadChip [35] or Illumina Human Methylation450 BeadChip [36,37].

In the EWAS study of Uddin et al. [35], individuals with a lifetime history of depression displayed a different methylation patterns compared to controls. More specifically, depressed cases had less genes with a defined methylation state (genes which are either methylated or unmethylated only in cases or controls, with methylated genes being defined as the ones with >80% methylation and unmethylated <20%) than unaffected individuals. Further functional pathway analyses indicated that clusters of these uniquely methylated and unmethylated genes were associated with distinct biological pathways associated with various mental disorders.

In an Australian study [36] of 24 monozygotic twin pairs discordant for depression, depression was associated with decreased global methylation levels in female discordant twin pairs, compared to control pairs. Furthermore, analysis showed a difference in intra-pair variance of methylation, with affected twins having a higher variance compared to their unaffected co-twin.

Numata and colleagues conducted a study investigating DNA methylation levels in two sets of samples, using an epigenome-wide approach [37]. In the discovery set, 393 CpG sites showed lower methylation levels in patients with MDD than in the controls. The replication study confirmed these results, displaying the same methylation changes in 84 CpG sites (out of 393). Among the CpG sites that demonstrated significant diagnostic differences in DNA methylation, three genes were considered to play an important role in MDD and were thus considered as potential biomarkers: DGHK, GSK3B and SGK1.

Among studies with a candidate-gene approach, the most extensively investigated was the promoter region of the serotonin transporter gene (SLC6A4), showing consistent findings. Results of the study by Philibert et al. [38] display a trend for higher methylation levels in individuals with a lifetime history of MDD, relative to controls. However, DNA methylation analyses were performed using

virally transformed lymphoblast in vitro, a procedure that the potential to affect DNA methylation. A study conducted on monozygotic twins [40], comparing 43 depression discordant pairs and 41 control pairs showed similar results. Accordingly, there was a significant intra-pair difference in DNA methylation levels of SLC6A4, with affected twins showing a higher methylation level compared to their unaffected co-twin. A recent study by de Booij et al. [41] compared a group of 33 individuals with MDD and 36 controls. There was a significant difference in methylation of 2 CpG sites in the promoter region of SLC6A4. However, this difference was displayed only in patients from the MDD group who were treated with SSRI compared to healthy controls.

DNA methylation of the BDNF gene was investigated in three studies. A Japanese study by Fuchikami et al. [42] compared DNA methylation in promoters of exons 1 and 4 in 20 individuals with MDD and 18 controls. There was a significant increase in the overall methylation levels in the MDD group but in the case of promoter 1 only. Moreover, the majority of individual CpG sites (71%) were hypermethylated in the MDD group, confirming a similar pattern. The same region (exon 1) of BDNF gene was assessed in a study of Carlberg et al. [44], performed on a larger sample (207 MDD patients and 278 controls), but with only 1 CpG site included. Similarly to the previous study, this CpG site was hypermethylated in patients with MDD. However, in adjusted analyses, the increase of methylation in MDD subjects was significantly associated with antidepressants therapy. In a study of D'Addario et al. [43], sample consisted of 41 patients with MDD and 44 controls. Sum methylation levels in the promoter region of BDNF gene were higher in the MDD group than in healthy controls.

Another gene observed in more than one study was NR3C1, coding for the glucocorticoid receptor (GR). The three studies investigated the same region—exon 7 and displayed different results. Results of the first [46] showed hypomethylation in 2 CpG sites of blood samples of 45 patients with MDD compared to 72 healthy controls. In the other study [45], observing 93 depression cases and 83 controls, no significant methylation difference was found. Finally, in a recent Thai study [47] comparing 29 medication-free MDD patients and 33 controls, there was a hypermethylation in 1 CpG site in female patients with MDD. In this study, global DNA methylation (LINE-1) was also assessed, however, no significant association was found with the presence of MDD.

Another gene related to GR is the FKBP5 binding protein 5 (FKBP5) gene that has been shown to influence GR sensitivity and thus plays a role in stress response regulation. Preliminary results of a recent study by Hohne et al. [48] suggest there is a higher average methylation level in intron 7 of the FKBP5 gene in individuals with a lifetime history of MDD ( $n = 61$ ) compared to controls ( $n = 55$ ). Both groups had been exposed to psychosocial stress (using Trier Social Stress Test), however, DNA methylation was assessed only after the exposure. Consequently, there is no data on the potential effect of psychosocial stress exposure on DNA methylation.

Melas et al. performed two studies, an original (82 depression cases and 92 controls) [45], and a replication study (17 cases with a lifetime history of depression and 27 controls) [49] on DNA methylation from saliva samples of participants. The observed gene region was exon 1 of monoamine oxidase A (MAOA), and displayed similar results, which is lower average methylation of this region as well as hypomethylation of a specific CpG site in female cases compared to controls.

Bayles and the colleagues [39] focused on DNA methylation of the norepinephrine transporter gene (SLC6A2), responsible for the reuptake of extracellular norepinephrine and dopamine. No significant difference was found between SLC6A2 DNA methylation levels in individuals with MDD and controls, but the study had a low power (4 MDD and 4 controls).

**Table 4**

Human studies on depression and DNA methylation.

Study (Quality score)	Experimental group, sex	Methylation analysis, N	Tissue	Gene, region	Depression assesment	Effect of depression on DNA methylation	Adjustments
Uddin et al. [35] (8)	Longitudinal Detroit Neighborhood Health Study, 60% female	33 lifetime depression history, 67 controls	Blood	Epigenome-wide association study	Depressive symptoms: PHQ-9. Cases: depressed mood/anhedonia, $\geq 1$ other symptom for $\geq 2$ weeks, and/or suicidal thoughts.	Different methylation patterns:  $\downarrow$ number of genes with a defined methylation state (cases) $\downarrow$ global $\downarrow$ discordant pairs (MDD)	No difference in demographic factors
Byrne et al. [36] (8)	MZ twins from Australian case-control study, 50% female	12 MDD discordant pairs; 12 control pairs	Peripheral blood leukocytes	EWAS global & individual genes	MDD: SSAGA	$\downarrow$ global $\downarrow$ discordant pairs (MDD)	Matched for age, smoking and alcohol consumption
Numata et al. [37] (9)	Case-control study over Japanese hospital patients, 84% female	20 medication-free MDD and 19 controls + 12 medication-free MDD and 12 controls (replication)	Peripheral blood leukocytes	Epigenome-wide association study	MDD: MINI	363 CpGs: $\downarrow$ (MDD) 84 CpGs: $\downarrow$ (MDD-replication)  <b>DGHH, GSK3B, SGK1</b> Individual CpGs: $\downarrow$ (MDD + MDD-replication)	No difference in demographic factors; no medication use
Bayles et al. [39] (3)	Participants from Australian case-control population study, 42% female	4 MDD, 4 controls	Blood	SLC6A2, promoter—2CpGs	MDD: MINI	No association	Participants with medical problems excluded
Philibert et al. [38] (6)	Iowa Adoption Studies participants, 50% female	68 MDD lifetime history, 124 controls	EBV-transformed lymphoblast	SLC6A4, promoter	Depressive symptoms: counts derived from SSAGA-II. MDD $\geq 5$ symptoms.	Sum: $\uparrow$ (MDD)	Not implicated
Zhao et al. [40] (9)	MZ twins from Vietnam Era Twin Registry, 0% female	43 depression discordant pairs, 41 control pairs	Peripheral blood leukocytes	SLC6A4, promoter	Depressive symptoms: BDI-II. MDD: SCID (DSM-III)	Average: $\uparrow$ intra-pair (depressive symptoms) 2 CpGs: $\uparrow$ (SSRI-treated patients with MDD)	Adjusted for physical activity, BMI, smoking, alcohol consumption, antidepressants use
Booij et al. [41] (9)	Ireland mental health services patients and controls, 64% female	33 MDD and 36 controls	Blood	SLC6A4, promoter	MDD: SCID BDI-II	2 CpGs: $\uparrow$ (SSRI-treated patients with MDD)	Matched for age, sex, BMI, smoking; participants excluded: using antipsychotics or mood stabilizers, with psychiatric/medical comorbidity, head injury; adjusted for type of AD used
Fuchikami et al. [42] (4)	Case-control study over Japanese hospital patients, 53% female	20 MDD, 18 controls	Blood	BDNF, promoter 1 and 4	MDD: MINI	Promoter 1 Promoter 4 Sum: $\uparrow$ No association 20 CpGs: $\uparrow$ 9 CpGs: $\downarrow$ (MDD)	Not implicated
D'Addario et al. [43] (8)	MDD patients and controls in Italy	41 MDD and 44 controls	Peripheral blood monocytes	BDNF, promoter	MDD: SCID	Sum: $\uparrow$ (MDD)	Matched for age
Carlberg et al. [44] (9)	Austria University Hospital, in- and outpatients with MDD, 61% female	207 MDD, 278 controls	Peripheral blood monocytes	BDNF, exon 1	MDD: SCID Depressive symptoms: BDI	1 CpG: $\uparrow$ (MDD on AD treatment)	Participants excluded: primary substance use disorder, organic/neurological cause of psychiatric symptoms; adjusted for genotype and AD use
Melas et al. [45] (7)	Swedish longitudinal population study, 100% female	82 depression, 92 controls	Saliva	NR3C1, 1F MAOA, exon 1	Depression: MDI	MAOANR3C1 Average: $\downarrow$ $\downarrow$ No association 1 CpG: $\downarrow$ (Depression)	Matched for childhood adversity; adjusted for age, smoking
Na et al. [46] (7)	Korea University Hospital, outpatients with MDD, 72% female	45 MDD and 72 controls	Blood	NR3C1, 1F	MDD: SCID	2 CpGs: $\downarrow$ (MDD)	Matched for age, gender; Participants with low IQ, neurological disease or brain lesions excluded

(continued on next page)



Table 4 (continued)

Study (Quality score)	Experimental group, sex	Methylation analysis, N	Tissue	Gene, region	Depression assesment	Effect of depression on DNA methylation	Adjustments
Nantharat et al. [47] (6)	Thailand hospital patients and controls, 74% female	29 medication-free MDD and 33 controls	Blood	Global DNA methylation, NR3C1, 1F	MDD: MINI	NR3C1, 1FGlobal 1 CpG: ↑ ♀ No association (MDD)	No medication use; Adjusted for gender
Hohne et al. [48] (10)	Participants from German longitudinal study, 54% female	61 lifetime history of MDD in remission and 55 controls, after psychosocial stress exposure (TSST)	Blood	FKBP5	BDI-II	Average: ↑ (MDD)	No difference in demographic factors; Participants using AD excluded; Adjusted for age, gender, genotype
Melas et al. [49] (4)	Swedish longitudinal population study, 64% female	17 lifetime history of depression and 27 controls	Saliva	MAOA, exon 1	Depression: MDI	Average: ↓ ♀ 1 CpG: ↓ ♀ (Depression)	Adjusted for age, gender, smoking, childhood adversity

↑ increased, ↓ decreased; ♀ females; MZ—monozygotic; EWAS—epigenome-wide association study; MDD—major depressive disorder; AD—antidepressants; PHQ-9—Patient Health Questionnaire; SSAGA—Semi-Structured Assessment for the Genetics of Alcoholism; MINI—Mini-International Neuropsychiatric Interview; SCID—Structured Clinical Interview for DSM Disorders; BDI/II—Beck Depression Inventory I/II; DSM—Diagnostic and Statistical Manual of Mental Disorders; MDI—Major Depression Inventory; TSST—Trier Social Stress Test; Diagnoses were based on DSM-IV, unless indicated.

#### 4. Discussion

In our review, we identified studies assessing potential correlations between chronic stress, work stress and burnout and depression with global DNA methylation and DNA methylation of 12 different individual genes. The majority of studies from our review were performed in the last few years, confirming the forthcoming expansion of epigenetic research, and offering potential tools for biomarker research.

Regarding the overlap between burnout and depression we need to distinguish between quantitative and qualitative symptoms of both disorders. Similar quality of symptoms is illustrated through a close link between (emotional) exhaustion as one of the main symptoms of burnout and the experience of depressed mood and anhedonia, the two core symptoms of depression [10]. In addition, many authors argue that burnout's clinical picture is often reminiscent of depression, with predominant feelings of helplessness, hopeless and powerless [50]. However, in clinical major depression (as diagnosed with DSM criteria) symptoms are required to have lasted for at least two weeks, and have to be severe as well as impairing the functional ability and quality of life of the person in question [51]. By contrast, duration of symptoms and distortion of functional ability are not explicitly defined in burnout self-report inventories, but are rather determined by the cut off scores that define burnout severity. Similarly, in several studies identified in this review, depression was operationalized by the presence of depressive symptoms as assessed by self-report inventories, such as Beck Depression Inventory (BDI) [32]. Evidently, the degree of symptom overlap between burnout and depression depends on the way burnout and depression are defined and assessed. Nevertheless, burnout or depressive symptoms on the one hand and clinical depression on the other might represent different phases in the stress process, i.e., as current states burnout or depressive symptoms could lead to depression as a clinical disorder in time if individual vulnerabilities and situational factors coincide [9,52]. Furthermore, it has been suggested that the pathophysiological changes underlying burnout may be less pronounced than those observed in major depression [53]. Therefore, in the following paragraphs, we focused on similarities and differences between burnout and depression on epigenetic level. More specifically, we described DNA methylation patterns of specific genes that could be considered as potential biomarkers of burnout.

Among the HPA-axis related genes, the NR3C1 gene, encoding for the GR was the most thoroughly examined. Whereas chronic psychosocial stress was associated with hypermethylation of this gene [28],

correlation with depression is confronting, suggesting association with both increased [47] and decreased [46] GR gene methylation or absence of methylation changes related to depression [40]. So far, there have already been many attempts to distinguish burnout and depression using different parameters related to cortisol activity. Recent studies on stressed workers suggest that hypercortisolism is linked with psychological distress and depression, whereas burnout is more likely followed by hypocortisolism [54]. These variations could be followed by different DNA methylation patterns of the GR gene. Even though we did not find any study observing methylation of this gene in relation to work stress and/or burnout, Menke et al. reported job-related exhaustion in association with increased GR expression and GR hypersensitivity [55]. The increased GR expression enhances negative feedback mediated by cortisol, leading to the HPA-axis hypo-reactivity and hypocortisolism [56]. In contrast, another study previously performed by the same author showed a reduction in GR-stimulated gene expression and the HPA-axis hyper-reactivity associated with major depressive disorder [57]. Different levels of the GR gene expression could potentially be explained by different methylation patterns of the GR gene (promoter), with hypomethylation leading to an increase and hypermethylation to a decrease in the expression of this gene. Additional, well-designed studies observing DNA methylation of this gene in relation to burnout are needed for further discussion and conclusions. Furthermore, studies comparing methylation patterns of this gene between individuals with burnout and depression are needed in order to explore the potential of this gene to discriminate burnout from depression on epigenetic level.

SLC6A4, the gene encoding the serotonin transporter was examined in the largest number of candidate-gene studies. This gene is generally most frequently associated with depression and a lot of research effort was invested so far in its biomarker potential concerning depression [19]. In addition, it was the only gene observed so far in relation to burnout [33]. Even though there was a correlation between burnout and work stress with SLC6A4 hypermethylation, these results were consistent with three studies showing the same methylation patterns in depression [38]. However, these are some interesting observation concerning the genotype and DNA methylation of this gene. Briefly, the SLC6A4 gene is characterized by a polymorphism in the promoter region (5-HTTLPR), defined by a short (S) and a long (L) allele [58,59]. Research suggest that carriers of the S allele exhibit more depressive symptoms, diagnosable depression, and suicidality in relation to stressful life events than individuals homozygous for the long allele [14].

Similar findings were shown in two studies included in our review. The study of Duman and colleagues showed that both chronic stress and depressive symptoms affected the SLC6A4 hypermethylation only in the homozygous carriers of the S allele. Moreover, in the study by Philibert et al., the short (S) 5HTTLPR allele was associated with a decreased SLC6A4 expression in patients with MDD. In contrast, the study of Alaasari et al. displays a genotype-independent hypermethylation of the SLC6A4 associated with work-related stress and burnout thus emphasizing predominant influence of environmental factors over genetic vulnerability. These results suggest a potential difference in gene-environment interaction that takes place in stress and depression vs. work stress and burnout pathways. Additional studies comparing SLC6A4 methylation in burnout and depression are necessary, taking into account the genetic polymorphism dependence of these methylation patterns.

Four studies examined DNA methylation of BDNF gene. In our review, three studies comparing individuals with MDD and healthy controls reported similar results suggesting association between MDD and BDNF hypermethylation. By contrast, in a study of Song et al. [34], work stress exposure was associated with increased average methylation of the whole BDNF gene, whereas depressive symptoms in individuals exposed to work stress were correlated with decreased methylation. However, this was the only study using saliva samples for DNA extraction. Additional studies are needed to further explore the role of BDNF methylation in this context, with attention to the specific regions of the gene and different DNA tissue sources. Diverse methylation patterns associated with work stress exposure and depressive symptoms in the same individuals could present an interesting starting point.

The gene for tyrosine hydroxylase (TH), an enzyme involved in catecholamines (dopamine, norepinephrine, and epinephrine) biosynthesis, did not gain much attention so far, as did the ones previously described. Even though catecholamines are neurotransmitters implicated in various physiological processes associated with mental health, there is still little evidence about epigenetic changes of the TH gene in response to stress. In the study from our review [11], work stress exposure was associated with increased average methylation of the promoter region and the whole gene sequence. However, we did not find any other studies examining TH methylation in the context of work stress/burnout, or any study comparing TH methylation and adult depression. Therefore, we cannot make any additional conclusions on works stress/burnout biomarker potential of this gene. Additional studies are needed to further explore TH methylation patterns in this context.

## 5. Conclusion

Given the fact that not much research has been done on this topic, making relevant conclusions at this point is quite challenging. Future studies examining DNA methylation patterns related to work stress/burnout and comparing them with the ones in depression could be a useful tool for a better understanding of epigenetic dimensions lying beyond these phenomena. Furthermore, thoroughly-designed longitudinal studies observing these changes are necessary for revealing the cause-effect relationship of epigenetics and psychopathology, unfolding the context in which these findings could be placed. When it comes to these studies, attention should be paid to: tissue relevance (brain vs. blood vs. saliva), clinical assessment variability, and confounding factors such as age, sex, smoking and alcohol consumption habits, use of antidepressants (or other drugs affecting DNA methylation) etc. which can all present a source of bias in statistical analyses. Nevertheless, DNA methylation research offers a whole new dimension of potential tools for a better understanding of the complex mechanisms that take place in the stress, human nature and psychopathology interplay and can thus present a new perspective of future work stress and burnout research.

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## Conflict of interest

The authors declare no conflict of interest.

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