Epigenomic Changes after Acupuncture Treatment in Patients Suffering from Burnout

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Abstract

Introduction: The effects of acupuncture treatment in patients suffering from burnout may imply an epigenetic control mediated by DNA methylation changes. In this observational study, a genome-wide characterization of epigenetic changes in blood DNA, before and after acupuncture treatment, was performed in a cohort of 11 patients suffering from burnout. Methods: Burnout was assessed using the Maslach Burnout Inventory (MBI) and DNA was extracted from blood samples and analyzed by Illumina EPIC BeadChip.

Results: Before acupuncture, all patients suffered of emotional exhaustion (EE) (MBI-EE score, 44 ± 6), 81\% suffered of depersonalization (DP) (MBI-DP score, 16 ± 6), and 72\% of low feelings of personal accomplishment (PA) (MBI-PA score, 29 ± 9). After acupuncture, all MBI dimensions improved significantly (EE, 16 ± 11 ($p = 1.5 \times 10^{-4}$); DP, 4 ± 5 ($p = 5.3 \times 10^{-4}$); and PA, 40 ± 6 ($p = 4.1 \times 10^{-3}$)). For each patient, both methylomes obtained before and after acupuncture co-clustered in the multidimensional scaling plot, indicating a high level of similarity. Genes corresponding to the 10 most differentially methylated CpGs showed enrichment in the brain dopaminergic signaling, steroid synthesis and in the insulin sensitivity pathways.

Conclusion: Acupuncture treatment was found to be highly effective on all burnout dimensions and the epigenetic targets identified were involved in some major disturbances of this syndrome.

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Introduction

Burnout is a maladaptive psychosocial response to chronic working stress clinically identified by high self-reported levels of emotional exhaustion (EE) and depersonalization (DP), as well as a low feeling of personal accomplishment (PA) [1, 2]. In the absence of medical care and work interruption, burnout may have harmful consequences, such as suicide [3]. In the 11th Revision of the International Classification of Diseases (ICD-11), burnout is coded as QD85 after the exclusion of other stress- and mood-related disorders [4]. The notion and term “burnout” historically originated in 1978 in the “Rose report” [5] as a result of an investigation commissioned by the USA Federal Aviation Administration, which aimed to understand the causes of series of plane collisions. Among the different methods used to assess burnout, the Maslach Burnout Inventory (MBI) remains the most recognized diagnostic tool [2, 6]. Burnout treatment usually involves stopping work for less than 6 months, sometimes associated with antidepressant treatment.

Derived from traditional Chinese medicine, acupuncture (“zhen jiu” in Chinese, meaning to burn and pierce) consists of the implantation and manipulation of thin stainless-steel needles at approximately 1–3 mm in the skin at distinct cutaneous locations (“acupoints”) for the purpose of rebalancing the path of “Qi” energy along “meridians” [7]. The World Health Organization proposed a nomenclature for 361 classical points located in the 14 principal meridians [8]. Neuroanatomy supports the choice of at least some acupoints [9].
The biological basis of acupuncture effects has been difficult to establish. Acupuncture stimulation may act via vagal modulation of the immune [10] and inflammatory systems [11]. It was also reported to act on neuronal regeneration, the neuroendocrine system, brain-gut peptide hormones, and oxidative stress [12]. It has also been shown to locally release adenosine that would exert an antinociceptive action via an adenosine A1 receptor [13] and to induce integrins and activate Erk1/2 [14]. In a recent study, the somatosensory autonomic reflexes and the vagal adrenal axis were shown to be implicated in the mode of action of electroacupuncture. Briefly, an inflammation was induced in a genetically modified mouse model with a conditional deletion of dorsal root ganglia derived neurons (PROKR2cre-DTR). The stimulation by electroacupuncture of the well-innervated acupoints (such as ST36) reduced inflammation, but only in the presence of PROKR2 neurons, and through the release of catecholamines. This experimental approach supports the implication of the vagal-adrenal axis in the mode of action of electroacupuncture [15].

In a three-arm study involving no acupuncture versus sham (i.e., the needle is implanted away from the acupoints) or true acupuncture for relieving stress-related symptoms in medical students, such as anxiety, depression, and burnout [16], only a placebo effect of acupuncture was observed. However, a specific effect of acupuncture has been observed in other studies. For example, Guan et al. [17] reported that the professional quality of life (ProQOL) scale established before and after acupuncture in 11 resident physicians experiencing a high level of job-related stress showed a significant score improvement posttreatment, with no negative impact of acupuncture. Another study in humans also reported a positive impact of acupuncture treatment on burnout-derived outcomes and concluded that acupuncture therapy could be a useful intervention to reduce burnout and was well accepted [18–20]. Of note, more than 100 different clinical trials registered in the United States National Institute of Health database (https://clinicaltrials.gov) are currently recruiting patients to assess acupuncture efficiency.

The epigenome is the interface between signals from the environment and genetic modifications that affect gene expression. Epigenetic factors modify the DNA structure through the (de)methylation of subsets of CpG islands, named differentially methylated regions (DMRs) [21]. Methylation in the promoter region induces a decrease in the expression of the corresponding gene [22]. Despite their potential relevance, epigenetic changes in patients suffering from burnout have been little studied. DNA methylation changes related to stress, burnout, and depressive conditions in humans have been reviewed by Bakusic et al. [23]. According to this review, the epigenetic effect of burnout was only reported in one study and consisted essentially in a decreased methylation of the serotonin transporter SLC6A4 [24].

Various studies have shown an impact of acupuncture on DNA methylation. Electroacupuncture has been reported to improve reproductive functions and decrease DNA methylation in the hippocampus of rats suffering from polycystic ovary syndrome (PCOS) [25]. Similarly, it has been shown to induce epigenetic and transcriptional changes in the adipose tissue of women suffering from PCOS [26]. Additionally, acupuncture decreased depressive-like behavior in rats in parallel with DNA methylation changes increasing brain-derived neurotrophic factor (BDNF) mRNA level in the hippocampus and protein levels in serum [27].

To our knowledge, no study has been performed on the possible effects of acupuncture treatment in burnout patients. In particular, no genome-wide trial has been conducted to investigate the blood DNA methylation pattern of humans during a burnout episode. The aim of this study was to assess the possible changes induced by acupuncture treatment on symptoms and on the genome-wide blood DNA methylation pattern in patients suffering from burnout.

Materials and Methods

Study Design and Participants

Participants were recruited at the time of their first medical visit to the private office of a medical doctor with a recognized qualification and traditional Chinese training in acupuncture. Inclusion criteria were adults >18 years suffering from burnout and willing to accept acupuncture treatment. Exclusion criteria were the presence of a preexisting psychiatric disorder, blood coagulation disorder, or an inability to consent to participate. Eleven (5 men; 6 women) patients were included and completed the entire study (mean age, 44 ± 10 [range, 26–59] years). Patients were heterogeneous in their occupations (mechanic, nursery director, compliance officer, trader, teacher, technician, asset manager, secretary, director, nurse, and human resources manager) and medical histories. Two patients regularly drank alcohol, one patient was treated by azathioprine, one patient presented congenital birth defects, one patient presented a poliniosis eczema, and one patient presented dorsal pain. Three women had previously been treated with acupuncture, but none of the men. All participants provided informed written consent. The study was approved by the Geneva cantonal ethics committee. We completed the STROBE checklist (see online supplementary material, available at www.karger.com/doi/10.1159/000521347).

Prior to acupuncture treatment, burnout was clinically measured using the MBI [6] and a blood sample was taken. During the final visit (average, 124 ± 52 days after inclusion), burnout was measured again using the MBI and another blood sample was taken. The use of alternative medicine by participants was reported by the Geneva cantonal ethics committee. We completed the STROBE checklist (see online supplementary material, available at www.karger.com/doi/10.1159/000521347).

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Epigenomic Changes after Acupuncture Treatment

A traditional Chinese medicine (TCM) diagnosis was systematically established by the general practitioner qualified in acupuncture and Chinese medicine, before each acupuncture session. The physician established the diagnosis taking into account the patient’s complaints, pulse and tongue analysis, as well as from the burnout syndrome assessments results. The physician conserved patient's complaints, pulse and tongue analysis, as well as from the puncture and Chinese medicine, before each acupuncture session.

The acupuncture treatment thus consisted of two acupuncture sessions, each lasting 20–30 min. A mean of 19 ± 5 acupuncture points were stimulated in some, but not all patients, depending on their medical history (online supplementary Table 1). In addition, 48 other acupuncture points were stimulated in some, but not all patients, depending on their medical history (online supplementary Table 1).

### Blood Methylation Analyses

DNA was extracted from 3 mL whole blood in freeze EDTA-coated tubes using the Puregene kit (Qiagen) in a P2 laboratory accredited by the Swiss Federal Coordinating Centre for Biotechnology. Blood was sampled twice in a similar time-related manner as patients treated with acupuncture (mean, 124 days ± 52), with a median of 1–4 mm per session. Eight acupuncture points were stimulated in all patients (HT7 [Shenmen], SP6 [Sanyinjiao], KI3 [Taixi], LR3 [Taichong], ST36 [Zusanli], SI3 [Houxi], PC6 [Neiguan], GB13 [Benshen]). These points had been previously reported or tested for improving insomnia, learning, memory, or reducing hypertension, nausea and pain in humans or rodents (Table 1). In addition, 48 other acupuncture points were stimulated in some, but not all patients, depending on their medical history (online supplementary Table 1).

### Methylome Data Analysis

The methylome data were analyzed at the genome level by comparing DNA methylations before and after acupuncture treatment from changes due to burnout improvement. Eight acupuncture points were stimulated in all patients (HT7 [Shenmen], SP6 [Sanyinjiao], KI3 [Taixi], LR3 [Taichong], ST36 [Zusanli], SI3 [Houxi], PC6 [Neiguan], GB13 [Benshen]). These points had been previously reported or tested for improving insomnia, learning, memory, or reducing hypertension, nausea and pain in humans or rodents (Table 1). In addition, 48 other acupuncture points were stimulated in some, but not all patients, depending on their medical history (online supplementary Table 1).

No.: numbering of the acupuncture points. Points: acupuncture points stimulated in all patients in the present study. Mean ± SD (standard deviation): mean of needle insertion per point per patient and the standard deviation. Physiological impact: physiological impact reported in other studies after stimulating the points individually or in dual combination, see references [66–73].

| No. | Points | Mean ± SD | Physiological Impact |
|-----|--------|-----------|----------------------|
| 1   | HT7    | 7±2       | Improved insomnia in a clinical trial [66], and insomnia in rats [67] |
| 2   | SP6    | 7±2       | Reduced hypertension, randomized controlled trial [68] |
| 3   | KI3    | 7±2       | Anti-inflammatory and antinociceptive effects in the arthritic rat [69] |
| 4   | LR3    | 4±3       | Anti-inflammatory and antinociceptive effects in the arthritic rat [69] |
| 5   | ST36   | 7±2       | Anti-inflammatory and antinociceptive effects in the arthritic rat [69] |
| 6   | SI3    | 6±3       | Feasibility study for acute neck pain in a randomized controlled trial [70] |
| 7   | PC6    | 3±1       | Reduced postoperative nausea (children) in a meta-analysis [71] |
| 8   | GB13   | 3±2       | Improved learning and memory in the rat [72, 73] |

Acupuncture Treatment

A traditional Chinese medicine (TCM) diagnosis was systematically established by the general practitioner qualified in acupuncture and Chinese medicine, before each acupuncture session. The physician conserved the diagnostics at his office. Patients underwent an average of 9 ± 2 acupuncture sessions, each lasting 20–30 min. A mean of 19 ± 5 DongBang® DB106 needles were implanted at a depth of 1–4 mm per session. Eight acupuncture points were stimulated in all patients (HT7 [Shenmen], SP6 [Sanyinjiao], KI3 [Taixi], LR3 [Taichong], ST36 [Zusanli], SI3 [Houxi], PC6 [Neiguan], GB13 [Benshen]). These points had been previously reported or tested for improving insomnia, learning, memory, or reducing hypertension, nausea and pain in humans or rodents (Table 1). In addition, 48 other acupuncture points were stimulated in some, but not all patients, depending on their medical history (online supplementary Table 1).

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Methylome Data Analysis

Epigenomic changes were analyzed at the genome level by comparing DNA methylations before and after acupuncture treatment in the “R” software environment for statistical computing using the package “missMethyl” [30], including functions from the packages “Minfi” [31] and “limma” [32]. Importantly, technical bias and batch effects were controlled. Technical bias due to both types of probes (Infinium I and II) present within each beadchip [33] was corrected by using the subset-quantile within array normalization (SWAN) method [34]. The “before” and “after” acupuncture DNA samples were always loaded on the same array to avoid batch effects in the analysis. The longitudinal design of the study did not allow to discriminate epigenomic variations due to the acupuncture treatment itself or to the spontaneous improvement of the burnout condition over time. The study design excluded epigenomic differences linked to genetic variations. Indeed, each pairwise-compared methylation was derived from the same genome of the same patient. Bayesian statistics were used to identify the most differentially methylated CpG producing log ratios and p values for the methylation changes using the “Limma” package [32]. The “lmFit” function fitted the M values using a linear regression model to recover methylation differences between both groups (before/after acupuncture) and the “eBayes” function was then applied on the fitted model to assess the statistical significance of the changes. We annotated the CpGs using the Bioconductor package named “IlluminaHumanMethylationEPICcano.ilm10b2.hg19”, which was specifically developed for the Illumina’s EPIC methylation arrays, and recovered gene names [35]. The entire analysis was iteratively performed 11 times to assess the consistency of the results.

Statistical Analyses

The statistical analyses were performed with the “R” statistical program (https://www.r-project.org/) version 4.0.0. For each patient, the “ggpaired” function graphically connects with lines the MBI sub-scores obtained before and after acupuncture. The “stat_compare_means” function executes pairwise t tests between the MBI sub-scores obtained before versus after acupuncture. The parameters were paired = TRUE and method = “t.test.” We used the same statistical pairwise t tests on the top 10 CpG sites identified as the most epigenetically affected after acupuncture, according to the method explained in the Methylome Data Analysis section. We reported the paired t tests derived p values in Figure 1 concerning the MBI sub-scores and in Figure 2 concerning the CpG methylation levels. In addition, we used retrospective powers to assess, at
the reached sample size, the probability to detect true improvements of MBI sub-scores after acupuncture. This is relevant with regards to the relatively small size of the cohort. The threshold for the powers is 80%. We calculated the powers in R using the “power.t.test” function and reported their values in Figure 1. Power calculation was based on the differences between both corresponding means in each MBI sub-scores, the standard deviation of the measures, the number of patients (n = 11), and the significance level setup at 0.05.

Pathways
Enrichment analysis of pathways was performed with the genes annotated in order to identify enrichments and networks based on “a database of known and predicted protein-protein interactions,” including “direct (physical) and indirect (functional) associations” identified by the STRING functional annotation protein network database [36]. The analysis was performed online (http://string-db.org/).

Results
Clinical Data
All patients suffered from EE (MBI-EE, 44 ± 6 [range, 31-54]; threshold ≥27) before acupuncture (Fig. 1a). For the two other dimensions, 81% (9/11) suffered from DP (MBI-DP, 16 ± 6 [4–28]; threshold ≥10) and 72% (8/11) presented low feelings of PA (MBI-PA, 29 ± 9 [11–41]; threshold <34) (Fig. 1b, c). All MBI dimensions improved significantly after the acupuncture sessions according to paired t tests (EE, 16 ± 11 [p = 1.5 × 10−4]; DP, 4 ± 5 [p = 5.3 × 10−4]; and PA, 40 ± 6 [p = 4.1 × 10−3]) (Fig. 1a–c).

These results were in line with the previously reported positive impact of acupuncture on burnout-derived measures (Table 2).

Epigenomic Changes and Biological Pathways
In the multidimensional scaling plot, both methylomes obtained from each individual (before and after acupuncture) correctly segregated together as expected when considering the same genetic origin of samples (Fig. 2a).

The top 10 CpGs were those whose methylation values measured before and after acupuncture presented the most significant differences with uncorrected p values ranging from 10−5 to 10−6 (Table 3). CpGs with higher uncorrected p values were not relevant considering the relatively small sample size. CpGs were annotated with regard to the genes in which (or in the vicinity of which) they were found. Their methylation levels were either up- or down-methylated after acupuncture treatment.

Increased methylation levels were observed in four CpG sites annotated with four genes in all patients as follows: adenomatous polyposis coli (APC) gene (cg22035501); dopamine receptor D5 (DRD5) gene (cg26296488); hormone-sensitive lipase (LIPE) gene
Epigenomic Changes after Acupuncture Treatment

Discussion

Our study findings show that acupuncture has a significant positive impact on MBI scores (Fig. 1) and induces methylation changes, which are particularly marked in the selection of the top 10 CpGs. These suggest poten-
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The Insulin Signaling Pathway: LIPE (cg01537765) and PRKAR1B (cg19157140)

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(Table 4). The PRKAR1B gene, whose methylation was decreased by acupuncture (Fig. 2b), encodes a regulatory subunit of the cyclic AMP-dependent protein kinase A (PKA) involved as LIPE in the insulin signaling pathway (Table 4). In addition, the two genes are interconnected as it has been shown that an overexpression of the catalytic unit of PKA increases LIPE transcription [37]. The possible involvement of the PRKAR1B gene in burnout has not been studied, but a genome-wide mRNA expression analysis in rats showed that electroacupuncture increased PRKAR1B expression in the periaqueductal gray region, a critical target of acupuncture-mediated analgesia [38].

The LIPE gene, whose methylation was increased by acupuncture (Fig. 2b), encodes the hormone-sensitive lipase. The specific functions of the latter are to mobilize stored fat during catabolic periods by hydrolyzing cell triglycerides in adipose tissue and to provide free cholesterol, a key substrate for steroid hormone synthesis, by hydrolysing cholesteryl esters in steroidogenic tissues [37]. Burnout has been shown to increase salivary cortisol [39]. In particular, the acupuncture-induced hypermethylation of LIPE that provides the key substrate for cortisol synthesis [37] may exert its beneficial effect by participating in reducing cortisol levels.

A decrease of insulin resistance by acupuncture has recently been shown in a meta-analysis comprising 10 randomized controlled trials including 737 patients in the context of PCOS. Results showed a significant decrease in the body mass index and waist-to-hip ratio in the acupuncture-treated group, as well as improvements in fasting plasma glucose [40]. Benrick et al. [41] also reported that electroacupuncture increased whole-body glucose uptake in women suffering from PCOS. LIPE expression has been shown recently to decrease insulin sensitivity [42]. The acupuncture-induced hypermethylation of LIPE (Fig. 2b) would therefore increase insulin sensitivity. This effect would be beneficial in the case of a burnout syndrome that was reported to be associated with insulin resistance [43]. Acupuncture would therefore decrease the risk of this side effect of the burnout.

cAMP Signaling Pathway: LIPE (cg01537765) and DRD5 (cg26296488)

The second pathway detected by STRING relates to cAMP signaling and involves the LIPE and DRD5 genes (Table 4). The DRD5 gene, whose methylation was increased by acupuncture (Fig. 2d), encodes a dopamine receptor. Dopamine, via DRD5 in particular, is a modulator of synaptic plasticity in the central nervous system. It acts by stimulation of long-term potentiation (LTP) of field potential, a process mediated by the cAMP signaling pathway [44, 45]. DRD5 was shown to be associated with psychiatric disorders, such as attention-deficit/hyperactiv-

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Table 4. Pathways detected by the STRING functional annotation protein network for the top 10 CpG site genes

| ID          | Term description                  | FDR  | Genes                                    |
|-------------|-----------------------------------|------|------------------------------------------|
| hsa04910    | Insulin signaling pathway         | 0.0434 | LIPE, PRKAR1B                           |
| hsa04024    | cAMP signaling pathway            | 0.045  | LIPE, DRD5                              |
| SM00248     | Ankyrin repeats                   | 0.0465 | ANKR1D11, ANK1S1B                       |

Ankyrin Repeat Pathway: ANKR1D11 (cg11673833) and ANK1S1B (cg15097794) Genes

The third pathway detected by STRING concerns two sites (cg11673833 and cg15097794) associated with ankyrin repeats-containing genes, i.e., the ANKR1D11 and ANK1S1B genes (Table 4). The ANKR1D11 gene, whose methylation was decreased by acupuncture (Fig. 2e), has been reported to regulate pyramidal neuron migration and dendritic differentiation in the developing mouse cerebral cortex. It also controls the BDNF and the tropomyosin related kinase B (TrkB) and is involved in histone acetylation-mediated epigenetic modifications [49]. The ANK1S1B gene, whose methylation was decreased by acupuncture (Fig. 2e), codes for a synaptic scaffold protein that has been associated with cognitive ability, antipsychotic drug and corticosteroid responses, and body mass index in genome-wide association studies [50]. Thus, the ankyrin repeat containing proteins being involved in synaptic plasticity are highly relevant as targets of the acupuncture treatment in the burnout.

PCMI (cg25454546), ASPSCR1 (cg23632839), APC (cg22035501), LARP4 (cg05043645), and cg22113197

These four genes are also highly relevant, independent of the STRING pathways. The PCMI gene, whose methylation was decreased by acupuncture (Fig. 2e), regulated the proliferation of neural progenitor cells in mice [51, 52]. In humans, PMC1 was found to increase the availability of the dopamine receptor D2 (DRD2) in the brain and associations between PMC1 genetic variants and schizophrenia have been described [53]. The intravenous administration of a synthetic corticotropin-releasing hormone in humans increased both the cortisol stress hormone in blood and dopamine in the dorsal striatum according to positron emission tomography scans per-
formed with a dopamine receptor radio-ligand [54]. The acupuncture induced-hypermethylation of DRD5 and the expected PMC1-induced DRD2 upregulation, together with the role possibly played by increased brain dopamine levels in the burnout, strongly suggests that a dysregulation of the dopaminergic system is involved in the burnout syndrome.

Of note, Parkinson’s disease, considered as a dopamine dysregulation syndrome [55], has also been proposed as a good candidate for acupuncture treatment. Indeed, the latter was found to induce an increase in the striatum synaptic dopamine level and to improve motor functions in a Parkinson’s disease mouse model [56]. According to a meta-analysis including 42 different studies, acupuncture induced a four-fold increase in dopamine levels ($p < 0.00001$) measured in dopaminergic neurons in rodent models of Parkinson’s disease [57].

The main function of ASPSCR1 (synonym “TUG”), whose methylation was increased by acupuncture (Fig. 2c), is to sequester the glucose transporter GLUT4 in intracellular vesicles in muscle and fat cells [58]. Insulin induces the translocation of GLUT4 to the cell surface and increases glucose uptake by cleavage of ASPSCR1 [59], although it is difficult to speculate how the decreased ASPSCR1 expression induced by acupuncture might affect insulin sensitivity.

The APC gene, whose methylation was increased by acupuncture (Fig. 2d), encodes a tumor suppressor regulating the WNT signaling pathway [60]. No evidence for an association of APC with burnout, acupuncture, or insulin was found. However, it has been reported that APC is a key organizer of nicotinic synapses. It binds to both
neuroligin and neurexin and is required for a precise alignment between the pre- and postsynaptic membranes and for the coordination in their maturation [61, 62]. For this reason, it was therefore considered as associated with neuronal plasticity in the model shown in Figure 3.

The LARP4 gene, whose methylation was decreased by acupuncture (Fig. 2e), binds the poly-A tail of mRNA molecules and activates PKA [63]. The cAMP/PKA cascade has been reported to play a role in the neuroprotective effect of acupuncture [64, 65]. These previous findings are confirmed by the identification of PRKAR1B and LARP4 as targets for acupuncture treatment in this study. A summary of the known associations between the top 10 genes of this study and relevant STRING pathways or biological entities is shown in Table 5.

A model summarizing the epigenomic impact of acupuncture in the burnout syndrome is shown in Figure 3. It is noteworthy that PCM1, ASPSCR1, and LARP4 are all intimately connected with two of the enriched pathways identified in this study, i.e., dopamine and cAMP/PKA and the insulin signaling pathway.

Limitations

Our study has some limitations, notably the small sample size. The major limitation of the acupuncture treatment concerns the stimulation of defined points applied systematically to each patient, irrespective of their condition. Although the points chosen should not vary in the same patients from one session to another and between patients for methodological reasons, we consider it to be incompatible with good acupuncture practice. Indeed, each patient presenting for the same diagnosis of burnout, a psychic and physiological state, is influenced by all kinds of individual factors that are carefully considered in the acupuncture care and will lead to defining the points to stimulate based on a global medical history. Moreover, the choice of stimulation points may vary from one session to another, depending on the condition of the patient at the time of the consultation. Another difficulty in drawing conclusions from this study concerns the fact that treatment by a general practitioner is never limited to needles. Medical care is always associated with empathic listening, psychological support and, above all, assistance to make personal or environmental changes that will call on the patient’s own resources.

Conclusions

This longitudinal study revealed the high efficiency of acupuncture on all burnout dimensions and allowed to detect important acupuncture-induced epigenetic changes in specific genes. Analysis of the genes and pathways involved should help to better understand the molecular dysfunctions underlying the burnout syndrome.

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Statement of Ethics

This monocentric observational study involving human subjects complies with the Declaration of Helsinki, the Human Research Act, and with the ordinance on Human Research with the Exception of Clinical trials. All the 11 patients included were free to participate and signed an informed consent. This study does not involve vulnerable participants. Study approval statement: The ethics committee of the canton of Geneva (CCER) approved this human research project under the name of the Epitac study (authorization No. 2019-00290, registry https://raps.swissethics.ch/). Consent to participate statement: All patients included in the study signed an informed consent form (ICF) and agreed with the publication of their epigenome data and their MBI assessments.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.P. recruited the patients, completed the case report forms, collected the blood, and performed acupuncture treatment. L.S. was in charge of DNA extraction and methylome data analysis. A.P.-G. was in charge of the study design, funding, and supervision of the whole project. All authors participated in writing the manuscript.

Data Availability Statement

The epigenomic datasets generated and analyzed during the current study are available in the Gene Expression Omnibus (GEO) repository under accession number GSE184202 (https://www.ncbi.nlm.nih.gov/geo).
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