

Epigenomic Changes after Acupuncture Treatment in Patients Suffering from Burnout

Marc Petitpierre^a Ludwig Stenz^b Ariane Paoloni-Giacobino^b^aGeneral Medicine, Acupuncture and Chinese Pharmacotherapy Office, Rolle, Switzerland; ^bDepartment of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland

Keywords

Acupuncture · Burnout · Clinical research · Cohort study · Dopamine · Epigenetic

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 Bead-Chip. **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.

© 2021 The Author(s).
Published by S. Karger AG, Basel

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 pollinosis 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 using the International Complementary and Alternative Medicine Questionnaire (French version) [28]. Patients were treated with acupuncture according to the Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) [29] (see online supplementary Table 1).

Table 1. Acupuncture points systematically stimulated in the patient cohort ($n = 11$) and references to their previously reported impact

No.	Points	Mean \pm SD	Physiological Impact
1	HT7 (Shenmen)	7 \pm 2	Improved insomnia in a clinical trial [66], and insomnia in rats [67]
2	SP6 (Sanyinjiao)	7 \pm 2	
3	KI3 (Taixi)	7 \pm 2	
4	LR3 (Taichong)	4 \pm 3	Reduced hypertension, randomized controlled trial [68]
5	ST36 (Zusanli)	7 \pm 2	
6	SI3 (Houxi)	6 \pm 3	Anti-inflammatory and antinociceptive effects in the arthritic rat [69]
7	PC6 (Neiguan)	3 \pm 1	Feasibility study for acute neck pain in a randomized controlled trial [70]
8	GB13 (Benshen)	3 \pm 2	Reduced postoperative nausea (children) in a meta-analysis [71]
9	GV24 (Shenting)	3 \pm 2	Improved learning and memory in the rat [72, 73]

No.: numbering of the acupuncture points. Points: acupuncture points stimulated in all patients in the present study. Mean \pm 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].

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 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 acupoints 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 acupoints 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 Coordination Centre for Biotechnology. Blood was sampled twice in a similar time-related manner as patients treated with acupuncture (mean, 124 days \pm 52), with the aim to help discriminate CpG methylation changes subsequent to acupuncture treatment from changes due to burnout improvement. Once extracted, DNA was quantified using both the Nanodrop and the Qubit. The extraction produced an average of 72 ± 35 μ g of DNA per sample; DNAs were of excellent quality (260/280 ratio, 1.8 ± 0.02). Bisulfite conversion was performed on 1 μ g DNA eluted into 14 μ L of EB buffer using the EZ DNA methylation kit (Zymo). Bisulfite-converted DNA was processed on the EPIC array (Illumina) at the genomic platform of the University of Geneva (Geneva, Switzerland). All 24 methylome profiles obtained passed the internal quality controls for the EPIC arrays (staining, extension, hybridization, target removal, bisulfite conversion, specificity, and non-polymorphic).

Methylome Data Analysis

Epigenomic changes were analyzed at the genome level by comparing DNA methylations before and after acupuncture treat-

ment 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 methylome 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 “IlluminaHumanMethylationEPICanno.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

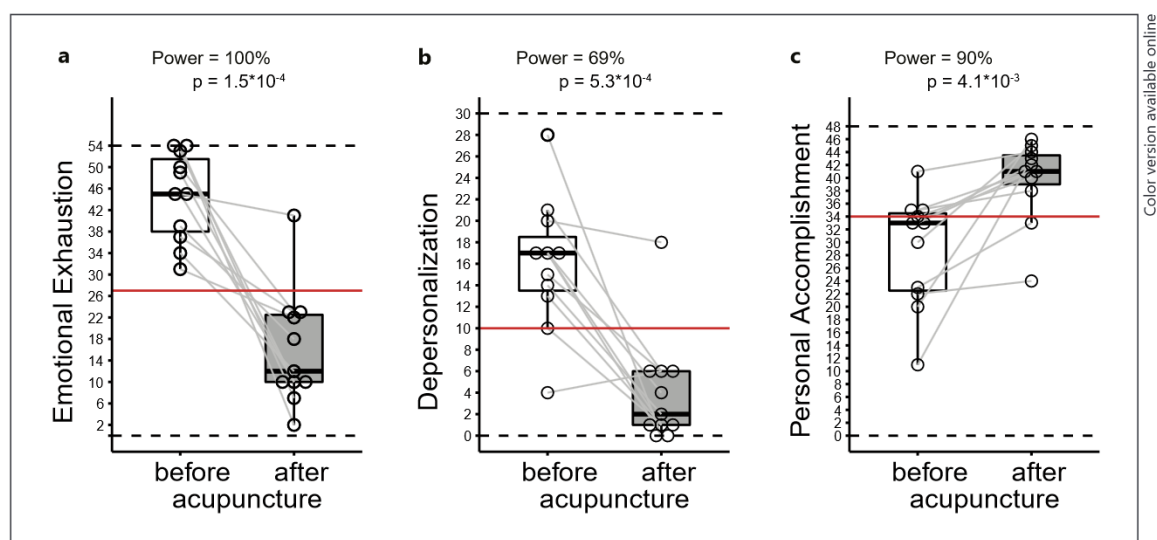


Fig. 1. MBI-derived burnout measures before and after acupuncture for all included patients. Each dot represents an MBI sub-score obtained from a patient. Burnout was measured before and after acupuncture using the MBI [6]. Red horizontal lines indicate the clinically established burnout thresholds: emotional exhaustion ≥ 27 , depersonalization ≥ 10 , and personal accomplishment < 34 [2]. Black dashed lines represent the up and down limit values in each MBI dimension. Indicated p values are derived from t tests of paired groups comparing the data obtained before versus after

acupuncture. The retrospective powers indicated on the top of each graphic (**a**, **b**, and **c**) correspond to the probabilities to detect true differences in the MBI sub-scores after acupuncture. The powers are sufficient concerning the improvements observed in both the emotional exhaustion and the personal accomplishment dimensions, but insufficient for the change observed in the depersonalization dimension of the burnout. **a** MBI dimension: emotional exhaustion. **b** MBI dimension: depersonalization. **c** MBI dimension: sense of personal accomplishment.

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 [$p = 1.5 \times 10^{-4}$]; DP, 4 ± 5 [$p = 5.3 \times 10^{-4}$]; and PA, 40 ± 6 [$p = 4.1 \times 10^{-3}$]) (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

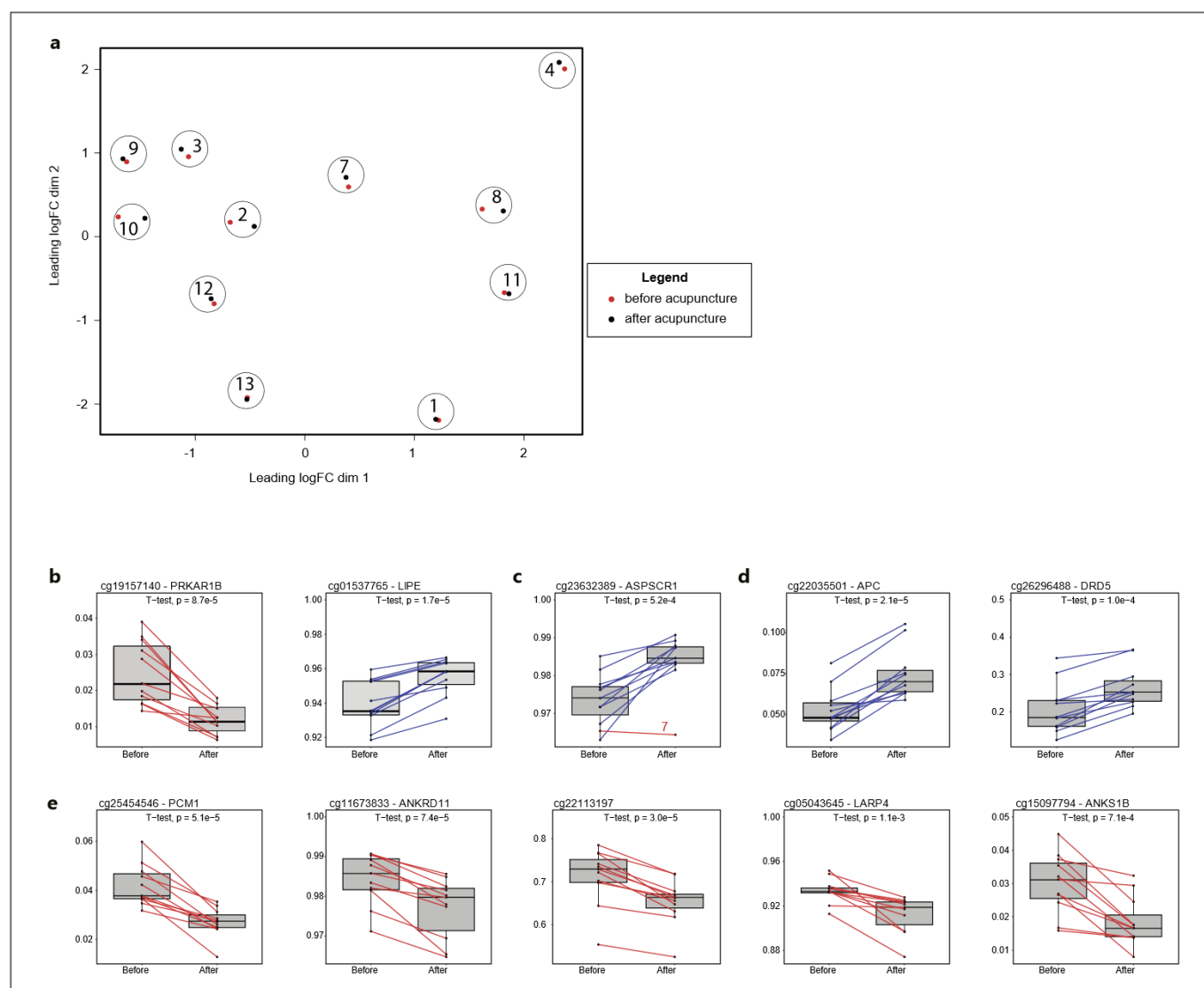


Fig. 2. Epigenomic changes after acupuncture treatment in burnout patients. **a** Multidimensional scaling plot of the 22 patient blood methylomes obtained before and after acupuncture. **b–e** Individual CpG sites with decreased methylation in the second samples (red lines) and increased methylation (blue lines). Indicated *p* values are derived from *t* tests of paired groups. **b** CpG sites showing changes of methylation in all patients. **c** CpG site showing changes of methylation across all patients, except for patient "7." **d** CpG sites with increased methylation after acupuncture in all patients. **e** CpG sites with decreased methylation after acupuncture in all patients.

(cg01537765); and alveolar soft part sarcoma chromosomal region candidate 1 (ASPSCR1) gene (cg23632389) (except for patient "7") (Fig. 2b–d). Decreased methylation levels were observed in six CpG sites annotated with five genes in all patients as follows: pericentriolar material 1 (PCM1) gene (cg25454546); ankyrin repeat domain 11 (ANKRD11) gene (cg11673833); no gene annotated (cg22113197); La ribonucleoprotein 4 (LARP4) gene (cg05043645); ankyrin repeat and sterile alpha motif domain containing 1B (ANKS1B) gene (cg15097794); and protein kinase CAMP-dependent type I regulatory subunit beta (PRKAR1B) gene (cg19157140) (Fig. 2b, e). The STRING analysis revealed that PRKAR1B and LIPE genes

belong to the insulin-signaling pathway (hsa04910, FDR = 0.043), DRD5 and LIPE to the cAMP signaling pathway (hsa04024, FDR = 0.045), and that ANKRD11 and ANKS1B were enriched for ankyrin repeats (SM00248, FDR = 0.0465) (Table 4).

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-

Table 2. Impact of acupuncture on burnout-related outcomes

Studies	Cohort	Assessment	Intervention(s)	Effect(s) after intervention
Present study	11 burnout patients	MBI-EE, MBI-DP, MBI-PA	8–9 sessions of acupuncture; the most used points were: HT7 (Shenmen) , SP6 (Sanyinjiao) , KI3 (Taixi), LR3 (Taichong) , ST36 (Zusanli) , SI3 (Houxi), *PC6 (Neiguan) , GB13 (Benshen), and GV24 (Shenting)	Decreased MBI-EE (44.6 to 16.2, $p = 1.5 \times 10^{-4}$) and MBI-DP (16.0 to 24.1, $p = 5.3 \times 10^{-4}$), increased MBI-PA (28.8 to 39.7, $p = 4.1 \times 10^{-3}$)
[17]	11 resident physicians	Mean ProQOL-burnout sub-score	3 auricular acupuncture sessions in one month; points not reported	Decreased mean ProQOL-burnout sub-score from 26.55 to 24.05 ($p = 2 \times 10^{-3}$)
[18]	19 caregivers	Lipp's burnout sub-score	10 sessions of acupuncture; the most used points were: ST36 (Zusanli) , EX-HN3 (Yintang), LR3 (Taichong) , BL62 (Shenmai), *PC6 (Neiguan) , SP6 (Sanyinjiao) , LI4 (Hegu), CV4 (Guanyuan), KI3 (Taixi), GB34 (Yanglingquan)	Decreased Lipp's burnout sub-score from 20.6 to 13.6 ($p = 1.1 \times 10^{-3}$)
[19]	37 caregivers	Mean ProQOL-burnout sub-score	16 sessions of auricular acupuncture on five points	Decreased mean ProQOL-burnout sub-score from 22.9 to 21.3 ($p = 6 \times 10^{-3}$)
[16]	82 students	MBI-SS-EE, MBI-SS-C, MBI-SS-AE	8 sessions of true electroacupuncture vs. sham electroacupuncture vs. control (no intervention); ST36 (Zusanli) , *PC6 (Neiguan) , GB14 (Yangbai), GV20 (Baihui) , and "four Shishencong points"	Electroacupuncture specific decrease in MBI-SS-EE ($p = 4 \times 10^{-3}$), MBI-SS-C ($p = 2 \times 10^{-2}$) without specific changes in MBI-SS-AE ($p = 7 \times 10^{-2}$)
[20]	20 patients	HR, HRV	6 sessions of either needle or laser-based acupuncture on four points: GV20 (Baihui) , *PC6 (Neiguan) , HT7 (Shenmen) , and LR3 (Taichong)	Decreased HR after needle acupuncture and decreased HRV after both needle and laser-based acupuncture

Studies: studies found to assess acupuncture effects on burnout referenced [16–20]. Cohort: total number of individuals and brief description of the cohort. Assessment: measures of the outcome (burnout) before and after the intervention (acupuncture). MBI: Maslach Burnout Inventory, including measures of emotional exhaustion (MBI-EE), depersonalization (MBI-DP), and personal accomplishment (MBI-PA). Professional Quality of Life (ProQOL) scale including a mean burnout sub-score [74]. The MBI student survey (MBI-SS) includes measures of emotional exhaustion (MBI-SS-EE), cynicism (MBI-SS-C), and academic efficacy (MBI-SS-AE). HR: heart rate. HRV: heart rate variability. Intervention(s): description of the acupuncture treatment with number of sessions and stimulated points. Points used in two different studies appear in bold and in three different studies in bold and highlighted. *PC6 (Neiguan) was common to all studies reporting the targeted points. Effect(s) after intervention: impact of the intervention (acupuncture) on the outcome (burnout) with mean scores, sub-scores, and p values.

Table 3. The top 10 CpG sites differentially methylated after acupuncture

No.	ID	CpG type	Chr	Position	Strand	Type	Gene	logFC	p value	Region	Specific gene region
1	cg25454546	Shore	8	17781589	+	I	PCM1	−0.54	1.5E−6	promoter	5'UTR
2	cg23632389	Shore	17	79953908	−	I	ASPSCR1	0.55	4.9E−6	body	exon6
3	cg19157140	Island	7	766323	−	I	PRKAR1B	−0.67	7.1E−6	promoter	5'UTR
4	cg11673833	Island	16	89345548	−	I	ANKRD11	−0.35	7.1E−6	body	exon10
5	cg22035501	Open sea	5	112073398	−	I	APC	0.41	1.4E−5	promoter	TSS
6	cg15097794	Island	12	99288663	+	I	ANKS1B	−0.56	2.5E−5	body	5'UTR
7	cg22113197	Open sea	10	35902589	+	II	N/A	−0.38	2.7E−5	N/A	N/A
8	cg26296488	Island	4	9783192	−	I	DRD5	0.45	2.9E−5	promoter	TSS
9	cg01537765	Island	19	42914828	+	I	LIPE	0.37	3.5E−5	body	exon2
10	cg05043645	Open sea	12	50836089	−	II	LARP4	−0.39	3.5E−5	body	intron8

No.: numbering of the top 10 sites. ID: identity of the CpG site. CpG type: type of CpG site depending on its sequence context. Chr (chromosome), position, and strand: exact location of the CpG sites in the genome. Type: probe type, either I or II. Gene: name of the gene associated with the site. logFC: change in methylation with decreased methylation after acupuncture in the case of negative logFC and increased methylation after acupuncture in the case of positive logFC. p value: statistical significance of the change without correction for multiple testing. Region: location of the CpG site in the promoter or in the body of the gene. N/A: not available. Specific gene region: precise location of the CpG in the 5'-untranslated region (5'UTR), in an exon or intron, or in the transcriptional start site (TSS).

tial links of these genes with the symptoms specifically alleviated by the acupuncture treatment or with more general possible effects of acupuncture. Three pathways were detected by the STRING analysis.

Insulin Signaling Pathway: LIPE (cg01537765) and PRKAR1B (cg19157140)

The first pathway detected by STRING concerns insulin signaling and involves the LIPE and PRKAR1B genes

(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 hydrolysing 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/hyperac-

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	ANKRD11, ANKS1B

tivity disorder in humans [46]. In rats, DRD5 was reported to mediate the acquisition of fear [47] or, inversely, the positive effect of physical training on chronic stress depression [48]. The acupuncture-induced hypermethylation of DRD5 observed in this study might help to decrease the harmful consequences of the burnout on mental health by decreasing dopamine action in some areas of the brain.

Ankyrin Repeat Pathway: ANKRD11 (cg11673833) and ANKS1B (cg15097794) Genes

The third pathway detected by STRING concerns two sites (cg11673833 and cg15097794) associated with ankyrin repeats-containing genes, i.e., the ANKRD11 and ANKS1B genes (Table 4). The ANKRD11 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 ANKS1B 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.

PCMC1 (cg25454546), ASPSCR1 (cg23632389), APC (cg22035501), LARP4 (cg05043645), and cg22113197

These four genes are also highly relevant, independent of the STRING pathways. The PCMC1 gene, whose methylation was decreased by acupuncture (Fig. 2e), regulated the proliferation of neural progenitor cells in mice [51, 52]. In humans, PCMC1 was found to increase the availability of the dopamine receptor D2 (DRD2) in the brain and associations between PCMC1 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-

Fig. 3. Model of the epigenomic impact of acupuncture in burnout. The ID code of CpGs indicated below their corresponding genes appear either with a black dot when more methylated or with a white dot when less methylated after acupuncture treatment. Genes associated with these CpGs are shown in a box. Physiologic links appear as arrows for an expected positive impact, as lines for connections, and as inverted-T lines for repression. Pathways detected by STRING are underlined.

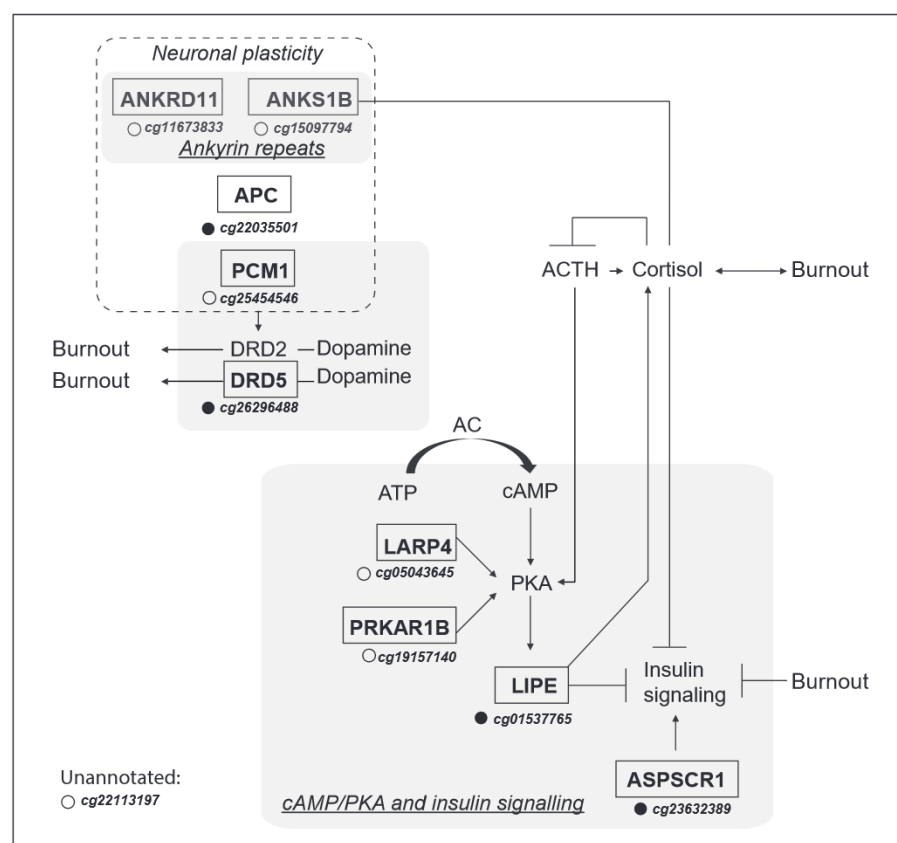


Table 5. Reported associations between genes and insulin, cAMP, neuronal plasticity and dopamine pathways

Gene	Insulin	cAMP	Neuronal plasticity	Dopamine
PCM1				
ASPSCR1				
PRKAR1B				
ANKRD11				
APC				
ANKS1B				
N/A				
DRD5				
LIPE				
LARP4				

Gene names annotated from the top 10 CpG sites mostly affected in their methylation status after acupuncture treatment in patients suffering from burnout and associated with insulin, cAMP, neuronal plasticity, and dopamine according to the literature. Associations appear in grey rectangles.

formed with a dopamine receptor radio-ligand [54]. The acupuncture induced-hypermethylation of DRD5 and the expected PCM1-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.

Acknowledgement

We thank Mylene Docquier and Isabelle Durussel-Gerber at the Genomic Platform of the University of Geneva for their help in the processing of the samples into the Epic array.

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.

Funding Sources

This study was funded by the Foundation “Lebherz – Cornelius Celsus (LCC)” (<https://www.fondation-lcc.ch/>).

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>).

References

- Maslach C, Schaufeli WB, Leiter MP. Job burnout. *Annu Rev Psychol*. 2001;52:397–422.
- Rotenstein LS, Torre M, Ramos MA, Rosales RC, Guille C, Sen S, et al. Prevalence of burnout among physicians: a systematic review. *JAMA*. 2018;320(11):1131–50.
- Pompili M, Rinaldi G, Lester D, Girardi P, Ruberto A, Tatarelli R. Hopelessness and suicide risk emerge in psychiatric nurses suffering from burnout and using specific defense mechanisms. *Arch Psychiatr Nurs*. 2006;20(3):135–43.
- Khoury B, Kogan C, Daouk S. International classification of diseases 11th edition (ICD-11). In: Zeigler-Hill V, Shackelford TK, editors. *Encyclopedia of personality and individual differences*. Cham: Springer International Publishing; 2017. p. 1–6.
- Samra R. Brief history of burnout. *BMJ*. 2018;363:k5268.
- Maslach C, Jackson SE. The measurement of experienced burnout. *J Organiz Behav*. 1981;2(2):99–113.
- Acupuncture. *NIH Consens Statement*. 1997;15(5):1–34.
- WHO Scientific Group on International Acupuncture Nomenclature. *A Proposed standard international acupuncture nomenclature: report of a WHO Scientific Group*. Geneva: World Health Organization; 1991. p. 30.
- Briggs JP, Shurtleff D. Acupuncture and the complex connections between the mind and the body. *JAMA*. 2017;317(24):2489–90.
- Torres-Rosas R, Yehia G, Peña G, Mishra P, del Rocio Thompson-Bonilla M, Moreno-Eutimio MA, et al. Dopamine mediates vagal modulation of the immune system by electroacupuncture. *Nat Med*. 2014;20(3):291–5.
- Lim HD, Kim MH, Lee CY, Namgung U. Anti-inflammatory effects of acupuncture stimulation via the vagus nerve. *PLoS One*. 2016;11(3):e0151882.
- Kou RZ, Chen H, Yu ML, Xu TC, Fu SP, Lu SF. Acupuncture for behavioral changes of experimental depressive disorder: a systematic review and meta-analysis. *Sci Rep*. 2017;7(1):9669.
- Goldman N, Chen M, Fujita T, Xu Q, Peng W, Liu W, et al. Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. *Nature Neuroscience*. 2010;13:883.
- Shin HC, Park JY, Lim HD, Namgung U. Induction of $\alpha 6$ and $\beta 1$ integrins by acupuncture stimulation in rats. *Biochem Biophys Res Commun*. 2017;491(3):629–35.
- Liu S, Wang Z, Su Y, Qi L, Yang W, Fu M, et al. A neuroanatomical basis for electroacupuncture to drive the vagal-adrenal axis. *Nature*. 2021;598(7882):641–5.
- Dias M, Vellarde GC, Olej B, Teófilo Salgado AE, de Barros Rezende I. Effects of electroacupuncture on stress-related symptoms in medical students: a randomised placebo-controlled study. *Acupunct Med*. 2014;32(1):4–11.
- Guan X, Citkovitz C, Kraus T, Garcia A, Kesavan Nasir M. Assessing the impact of acupuncture therapy on medical resident well-being: a feasibility and acceptance study. *Obstet Gynecol*. 2018;132:36S.
- de Oliveira CCC, Scivoletto S. Impact of acupuncture on stress levels of professionals working with maltreated children. *Acupunct Med*. 2017;35(4):303–4.
- Reilly PM, Buchanan TM, Vafides C, Breakey S, Dykes P. Auricular acupuncture to relieve health care workers' stress and anxiety: impact on caring. *Dimens Crit Care Nurs*. 2014;33(3):151–9.
- Litscher G, Liu CZ, Wang L, Wang LP, Li QQ, Shi GX, et al. Improvement of the dynamic responses of heart rate variability patterns after needle and laser acupuncture treatment in patients with burnout syndrome: a transcontinental comparative study. *Evid Based Complement Alternat Med*. 2013;2013:128721.
- Perez MF, Lehner B. Intergenerational and transgenerational epigenetic inheritance in animals. *Nat Cell Biol*. 2019;21:143–51.
- Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev*. 2002;16(1):6–21.
- Bakusic J, Schaufeli W, Claes S, Godderis L. Stress, burnout and depression: a systematic review on DNA methylation mechanisms. *J Psychosom Res*. 2017;92:34–44.
- Alasaari JS, Lagus M, Ollila HM, Toivola A, Kivimäki M, Vahtera J, et al. Environmental stress affects DNA methylation of a CpG rich promoter region of serotonin transporter gene in a nurse cohort. *PLoS One*. 2012;7(9):e45813.
- Cui P, Ma T, Tamadon A, Han S, Li B, Chen Z, et al. Hypothalamic DNA methylation in rats with dihydrotestosterone-induced polycystic ovary syndrome: effects of low-frequency electroacupuncture. *Exp Physiol*. 2018;103(12):1618–32.
- Kokosar M, Benrick A, Perfilov A, Nilsson E, Kallman T, Ohlsson C, et al. A single bout of electroacupuncture remodels epigenetic and transcriptional changes in adipose tissue in polycystic ovary syndrome. *Sci Rep*. 2018;8(1):1878.
- Jiang H, Zhang X, Lu J, Meng H, Sun Y, Yang X, et al. Antidepressant-like effects of acupuncture-insights from DNA methylation and histone modifications of brain-derived neurotrophic factor. *Front Psychiatry*. 2018;9:102.
- Druart L, Pinsault N. The I-CAM-FR: a French translation and cross-cultural adaptation of the I-CAM-Q. *Medicine*. 2018;5(3):72.
- MacPherson H, Altman DG, Hammerslag R, Youping L, Taixiang W, White A, et al. Revised standards for reporting interventions in clinical trials of acupuncture (STRICTA): extending the CONSORT statement. *J Evid Based Med*. 2010;3(3):140–55.
- Phipson B, Maksimovic J, Oshlack A. MissMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. *Bioinformatics*. 2016;32(2):286–8.
- Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics*. 2014;30(10):1363–9.
- Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47.
- Dedeurwaerder S, Defrance M, Calonne E, Denis H, Sotiriou C, Fuks F. Evaluation of the Infinium methylation 450K technology. *Epigenomics*. 2011;3(6):771–84.
- Maksimovic J, Gordon L, Oshlack A. SWAN: subset-quantile within array normalization for Illumina Infinium Human Methylation450 Bead Chips. *Genome Biol*. 2012;13(6):R44.
- Hansen KD. IlluminaHumanMethylation-EPICanno.ilm10b2.hg19: Annotation for Illumina's EPIC methylation arrays. 2016. R package version 0.6.0.
- Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*. 2019;47(D1):D607–13.
- Holysz M, Derebecka-Holysz N, Trzeciak WH. Transcription of LIPE gene encoding hormone-sensitive lipase/cholesterol esterase is regulated by SF-1 in human adrenocortical cells: involvement of protein kinase A signal transduction pathway. *J Mol Endocrinol*. 2011;46(1):29–36.
- Wang K, Xiang X-H, Qiao N, Qi J-Y, Lin L-B, Zhang R, et al. Genomewide analysis of rat periaqueductal gray-dorsal horn reveals time-, region- and frequency-specific mRNA expression changes in response to electroacupuncture stimulation. *Sci Rep*. 2014;4(1):6713.
- Pilger A, Haslacher H, Meyer BM, Lackner A, Nassan-Agha S, Nistler S, et al. Midday and nadir salivary cortisol appear superior to cortisol awakening response in burnout assessment and monitoring. *Sci Rep*. 2018;8(1):9151.
- Zheng R, Qing P, Han M, Song J, Hu M, Ma H, et al. The effect of acupuncture on glucose metabolism and lipid profiles in patients with PCOS: a systematic review and meta-analysis of randomized controlled trials. *Evid Based Complement Alternat Med*. 2021;2021:5555028.
- Benrick A, Kokosar M, Hu M, Larsson M, Maliqueo M, Marcondes RR, et al. Autonomic nervous system activation mediates the increase in whole-body glucose uptake in response to electroacupuncture. *FASEB J*. 2017;31(8):3288–97.
- Morigny P, Houssier M, Mairal A, Ghilain C, Mouiel E, Benhamed F, et al. Interaction between hormone-sensitive lipase and ChREBP in fat cells controls insulin sensitivity. *Nat Metab*. 2019;1(1):133–46.
- Fernandez-Montero A, García-Ros D, Sánchez-Tainta A, Rodríguez-Mourille A, Vela A, Kales SN. Burnout syndrome and increased insulin resistance. *J Occup Environ Med*. 2019;61(9):729.

- 44 Papaleonidopoulos V, Kouvaros S, Papatheodoropoulos C. Effects of endogenous and exogenous D1/D5 dopamine receptor activation on LTP in ventral and dorsal CA1 hippocampal synapses. *Synapse*. 2018;72(8):e22033.
- 45 Yang HW, Zhou LJ, Hu NW, Xin WJ, Liu XG. Activation of spinal d1/d5 receptors induces late-phase LTP of C-fiber-evoked field potentials in rat spinal dorsal horn. *J Neurophysiol*. 2005;94(2):961–7.
- 46 Johansson S, Hallelund H, Halmøy A, Jacobsen KK, Landaas ET, Dramsdahl M, et al. Genetic analyses of dopamine related genes in adult ADHD patients suggest an association with the DRD5-microsatellite repeat, but not with DRD4 or SLC6A3 VNTRs. *Am J Med Genet B Neuropsychiatr Genet*. 2008;147b(8):1470–5.
- 47 Inoue T, Izumi T, Maki Y, Muraki I, Koyama T. Effect of the dopamine D(1/5) antagonist SCH 23390 on the acquisition of conditioned fear. *Pharmacol Biochem Behav*. 2000;66(3):573–8.
- 48 Kang J, Wang Y, Wang D. Endurance and resistance training mitigate the negative consequences of depression on synaptic plasticity through different molecular mechanisms. *Int J Neurosci*. 2020;130(6):541–50.
- 49 Ka M, Kim WY. ANKRD11 associated with intellectual disability and autism regulates dendrite differentiation via the BDNF/TrkB signaling pathway. *Neurobiol Dis*. 2018;111:138–52.
- 50 Younis RM, Taylor RM, Beardsley PM, McClay JL. The ANKS1B gene and its associated phenotypes: focus on CNS drug response. *Pharmacogenomics*. 2019;20(9):669–84.
- 51 Ge X, Frank CL, Calderon de Anda F, Tsai LH. Hook3 interacts with PCM1 to regulate pericentriolar material assembly and the timing of neurogenesis. *Neuron*. 2010;65(2):191–203.
- 52 Zhang W, Kim PJ, Chen Z, Lokman H, Qiu L, Zhang K, et al. MiRNA-128 regulates the proliferation and neurogenesis of neural precursors by targeting PCM1 in the developing cortex. *Elife*. 2016;5.
- 53 Monroe TO, Garrett ME, Kousi M, Rodriguiz RM, Moon S, Bai Y, et al. PCM1 is necessary for focal ciliary integrity and is a candidate for severe schizophrenia. *Nat Commun*. 2020;11(1):5903.
- 54 Payer D, Williams B, Mansouri E, Stevanovski S, Nakajima S, Le Foll B, et al. Corticotropin-releasing hormone and dopamine release in healthy individuals. *Psychoneuroendocrinology*. 2017;76:192–6.
- 55 Warren N, O’Gorman C, Lehn A, Siskind D. Dopamine dysregulation syndrome in Parkinson’s disease: a systematic review of published cases. *J Neurol Neurosurg Psychiatry*. 2017;88(12):1060–4.
- 56 Kim SN, Doo AR, Park JY, Bae H, Chae Y, Shim I, et al. Acupuncture enhances the synaptic dopamine availability to improve motor function in a mouse model of Parkinson’s disease. *PLoS One*. 2011;6(11):e27566.
- 57 Ko JH, Lee H, Kim SN, Park HJ. Does acupuncture protect dopamine neurons in Parkinson’s disease rodent model? A systematic review and meta-analysis. *Front Aging Neurosci*. 2019;11(102):102.
- 58 Bogan JS, Hendon N, McKee AE, Tsao TS, Lodish HF. Functional cloning of TUG as a regulator of GLUT4 glucose transporter trafficking. *Nature*. 2003;425(6959):727–33.
- 59 Habtemichael EN, Li DT, Camporez JP, Westergaard XO, Sales CL, Liu X, et al. Insulin-stimulated endoproteolytic TUG cleavage links energy expenditure with glucose uptake. *Nat Metab*. 2021;3(3):378–93.
- 60 Serre L, Stoppin-Mellet V, Arnal I. Adenomatous polyposis coli as a scaffold for microtubule end-binding proteins. *J Mol Biol*. 2019;431(10):1993–2005.
- 61 Rosenberg MM, Yang F, Mohn JL, Storer EK, Jacob MH. The postsynaptic adenomatous polyposis coli (APC) multiprotein complex is required for localizing neuroligin and neurexin to neuronal nicotinic synapses in vivo. *J Neurosci*. 2010;30(33):11073–85.
- 62 Temburni MK, Rosenberg MM, Pathak N, McConnell R, Jacob MH. Neuronal nicotinic synapse assembly requires the adenomatous polyposis coli tumor suppressor protein. *J Neurosci*. 2004;24(30):6776–84.
- 63 O’Toole KT, Deming PB. Novel molecular interaction between cyclic-AMP dependent protein kinase a holoenzymes and La-related protein 4. *FASEB J*. 2017;31(S1):770–1212.
- 64 Li QQ, Shi GX, Yang JW, Li ZX, Zhang ZH, He T, et al. Hippocampal cAMP/PKA/CREB is required for neuroprotective effect of acupuncture. *Physiol Behav*. 2015;139:482–90.
- 65 Liu JH, Wu ZF, Sun J, Jiang L, Jiang S, Fu WB. Role of AC-cAMP-PKA cascade in antidepressant action of electroacupuncture treatment in rats. *Evid Based Complement Alternat Med*. 2012;2012:932414.
- 66 Wu P, Cheng C, Song X, Yang L, Deng D, Du Z, et al. Acupoint combination effect of Shenmen (HT 7) and Sanyinjiao (SP 6) in treating insomnia: study protocol for a randomized controlled trial. *Trials*. 2020;21(1):261.
- 67 Zhu YH, Yang CH, He LX, Qiao L, Cheng CS. [Effect of electroacupuncture at “Shenmen” (HT7) and “Sanyinjiao” (SP6) on energy metabolism in paraventricular nucleus of hypothalamus of insomnia rats]. *Zhen Ci Yan Jiu*. 2019;44(3):170–5.
- 68 Zhang J, Lyu T, Yang Y, Wang Y, Zheng Y, Qu S, et al. Acupuncture at LR3 and KI3 shows a control effect on essential hypertension and targeted action on cerebral regions related to blood pressure regulation: a resting state functional magnetic resonance imaging study. *Acupuncture in medicine*. *Acupunct Med*. 2021;39(1):53–63.
- 69 Yang F, Gong Y, Yu N, Yao L, Zhao X, Hong S, et al. ST36 acupuncture alleviates the inflammation of adjuvant-induced arthritic rats by targeting monocyte/macrophage modulation. *Evid Based Complement Alternat Med*. 2021;2021:9430501.
- 70 Sun ZR, Yue JH, Tian HZ, Zhang QH. Acupuncture at Houxi (SI 3) acupoint for acute neck pain caused by stiff neck: study protocol for a pilot randomised controlled trial. *BMJ Open*. 2014;4(12):e006236.
- 71 Zhang Y, Zhang C, Yan M, Wang N, Liu J, Wu A. The effectiveness of PC6 acupuncture in the prevention of postoperative nausea and vomiting in children: a systematic review and meta-analysis. *Paediatr Anaesth*. 2020;30(5):552–63.
- 72 Cui S, Xu M, Huang J, Wang QM, Lai X, Nie B, et al. Cerebral responses to acupuncture at gv24 and bilateral gb13 in rat models of Alzheimer’s disease. *Behav Neurol*. 2018;2018:8740284.
- 73 Yang Y, Hu S, Lin H, He J, Tang C. Electroacupuncture at GV24 and bilateral GB13 improves cognitive ability via influences the levels of Aβ, p-tau (s396) and p-tau (s404) in the hippocampus of Alzheimer’s disease model rats. *Neuroreport*. 2020;31(15):1072–83.
- 74 Heritage B, Rees CS, Hegney DG. The ProQOL-21: a revised version of the professional quality of life (proqol) scale based on rasch analysis. *PLoS One*. 2018;13(2):e0193478.