Saudi Journal of Biological Sciences 29 (2022) 2299–2305

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Preksha Dhyāna meditation induces alterations at the transcriptome level in novice and healthy college students

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ARTICLE INFO

Article history:
Received 19 October 2021
Revised 10 November 2021
Accepted 28 November 2021
Available online 3 December 2021

Keywords:
Preksha Dhyana
Transcriptome
Up-and down-regulated genes
Cellular pathways

ABSTRACT

Background: The human transcriptome across a variety of cell types and tissues are affected by stress and other psychological factors. *Preksha Dhyana* meditation (PM) is effective at improving cognitive skills in novice healthy college student meditators after 8 weeks of intervention, but the molecular and cellular mechanisms involved in these improvements are still largely unknown.
Methods: In order to decipher potential mechanisms at the cellular level, transcriptomic profiling analyses, from peripheral blood, were performed at baseline and 8 weeks post-intervention in 18-paired participants (RNASeq).
Results: At the transcriptomic level, 494 genes were nominally differentially expressed (p-*value* ≤ 0.05) between baseline and 8 weeks post-intervention. Our data showed that 136 genes were upregulated, while 358 genes were downregulated. These genes were enriched in several cellular pathways including innate and adaptive immunity, cell signaling, and other metabolic processes.
Conclusions: Overall, our findings indicate that *PM* meditation affects gene expression patterns from whole blood in novice healthy college students. Improvements at the cognitive skills were also mirrored with changes at RNA expression profiling.

1. Introduction

Meditation practices are increasingly involved in daily activities to reduce anxiety, life stress conditions, pain, and depression (Felder et al., 2012; Jarrett et al., 2012). Meditation techniques are considered clinical intervention tools in combatting depression, ADHD, pain management, drug abuse, and addiction (Speca et al., 2000; Zylowska et al., 2008; Kuntz et al., 2018). Although some practices may differ in detail, but their main focus remains the stress relief through purification of emotions and self-recognition. Meditation practices among college students shows measurable benefits including increased focus and attention (Black et al., 2009; Travis et al., 2009). Meditation improved examination scores for college students and reduced mind wandering (Mrazek et al., 2013). Randomized controlled trials have also demonstrated benefits of meditation for students with autism and other intellectual disabilities (Serwacki and Cook-Cottone, 2012). Recently, the effect of mindfulness on depression, stress, and academic performance was assessed among medical students in Saudia Arabia (Alzahrani et al., 2020). Recent data showed that mindfulness is inversely associated with depression and stress,

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.11.060
1319-562X/

but not with the academic performance, and this study showed that mindfulness can predict depression and stress among college students (Alzahrani et al., 2020).

Despite these benefits, the underlying cellular mechanisms involved in relief outcomes remain largely unknown, though preliminary studies suggest gene expression and epigenetic changes play a role in post meditation relief feelings. Yoga and relaxation practices significantly and rapidly affect global gene expression in peripheral blood mononuclear cells (PBMCs) (Qu et al., 2013). Relaxation also induces temporal transcriptome alterations in inflammatory pathways, and energy metabolism (Bhasin et al., 2013). Recently, a link between meditation and the immune system, human microbiota, and epigenetics was proposed (Househam et al., 2017). Although the relationship between meditation practices and healthy gut microbiota remains to be identified, data showed the positive impact of meditation on functional gastrointestinal disorders (Schoultz et al., 2013; Kuo et al., 2015; Schoultz et al., 2015). At the epigenetic levels, the blood methylome of experienced meditators demonstrated 61 differentially methylated sites within only 8 h of mindfulness-based practice (Chaix et al., 2020). The genes associated with these sites were involved in cellular functions such as immune response and inflammation. Furthermore, rapid changes in histone deacetylases and inflammatory gene expression were reported in expert meditators (Kaliman et al., 2014).

Although several meditation studies were conducted, the impact of meditation on the molecular and cellular targets specifically in novice meditators remain unclear. Recently, we confirmed the beneficial effects of *PM* on the cognitive skills of healthy college students after 8 weeks of intervention (Pragya et al., 2021). The current study follows this initial observation and sets out to investigate the molecular and cellular effects of *PM* meditation.

2. Materials and methods

2.1. Ethics committee and institutional review board (IRB) approval process

The proposal was approved by the Florida International University (FIU) ethics and IRB committees (IRB-17-0108-CR02). All participants signed informed consent forms to be enrolled in the study. All participants were given ID numbers according to the IRB rules. The study was registered in Clinicaltrials.gov with an Identifier: ***NCT03779269***.

2.2. Study participants and experimental design

A total of 142 participants were enrolled in the study. The study design and the different types of PM meditation were previously described (Pragya et al., 2021). Blood samples were drawn and stored in the Biorepository at the John P. Hussman Institute for Human Genomics at the University of Miami. The meditators (n = 51) blood samples were drawn, and the RNA was extracted from all the subjects at baseline and by the end of the 8 weeks meditation sessions. Due to quality control issues and pairing of the samples, only 18 samples (for RNASeq) were finally included in the downstream analyses.

2.3. RNA extraction from different blood samples

Purple-top EDTA tubes were used for blood collection per manufacturers recommendations on the Autogen FlexStar instrument (Catalog # AGKT-WB-640). fPAX RNA Blood tubes (ThermoFisher Scientific, USA) were collected and stored at −20 °C for at least 24 h before being permanently stored at −80 °C. RNA extraction was performed using GLOBINclear human RNA extraction kit (Catalog # AM1980, ThermoFisher Scientific, USA). RNA was treated with DNAse and the blood samples from all groups were kept at −80 °C until further use. All samples were tracked within the facility implemented Nautilus® Laboratory Information Management System.

2.4. RNA Sequencing

Blood cell composition was determined using the *minfi* function [estimateCellCounts] (Houseman et al., 2012). Due to our paired-design, Wilcoxon Signed Rank Test was used to measure cell type composition differences between pre- and post-intervention samples. Only NK (0.032, p = 0.03) and monocytes (0.0787, p = 0.04) showed a small trend, while Granulocytes, B cells, and CD4T did not show significant differences (Supplementary data S1).

RNA from 18 total individuals in pairs (pre- and post-intervention) with RNA integrity numbers >6 (Agilent Bioanalyzer) were subjected to downstream RNAseq analysis in the John P. Hussman Institute for Human Genomics, Center for Genome Technology Sequencing Core. Briefly, total RNA was quantified and qualified using the Agilent Bioanalyzer. Then 400 ng of total RNA was used as input for the NuGEN Universal Plus mRNA-Seq kit with AnyDeplete Globin (Tecan Genomics, Redwood City, CA) per the manufacturer's instructions to create polyA selected RNA and globin depleted sequencing libraries. Each sample was sequenced to more than 30 million raw single end 100 bp reads on the Illumina NovaSeq 6000.

2.5. Differential gene expression analysis and statistical analyses

Sequencing data were processed with a bioinformatics pipeline including quality control, alignment to the GRCh37 (hg19) human reference genome with STAR aligner v2.5.0a (Dobin et al., 2013) and gene quantification performed with the Gene Counts STAR function against the GENCODE v19 annotation gene set. Count data was input into edgeR software (Robinson et al., 2010) for differential expression analysis. Counts were normalized using the trimmed mean of M−values (TMM) method to account for compositional difference between the libraries (Robinson and Oshlack, 2010). Differential expression analysis between groups was performed for paired samples adjusting for differences between individuals using an additive linear model with individual as the blocking factor. For this we used the generalized linear model likelihood ratio test (glmLRT) implemented in edgeR. Protein coding genes with a nominal p-value $\leq$ 0.05 and the average log counts per million across the samples of at least 0 were considered differentially expressed.

2.6. Pathway analysis

The set of up-and down-regulated genes was used to identify Pathways and interaction networks affected between the 8-weeks post-intervention and the baseline. Differentially expressed genes were assessed for enrichment in functional pathways using Ingenuity Pathway Analysis (Qiagen, Germantown, MD). Up-regulated genes and down-regulated genes were analyzed in separate analyses. A p-value $\leq$ 0.01 of a pathway was considered significant.

3. Results

3.1. Transcriptional profiling comparison between pre- and post-intervention in whole blood cells

We first set out to determine transcriptomic changes, in whole blood, induced by PM meditation. The analysis of 18 pairs of participants revealed 494 nominally statistically significant differentially expressed genes ($p \leq 0.05$) as a result of the meditation intervention (Fig. 1, Supplementary file S2). Among those genes, 136 genes were upregulated, and 358 genes were downregulated. Table 1 depicts the top twenty upregulated genes, while Table 2 shows the top twenty downregulated genes. The list of all identified genes in the experiment is illustrated in the supplementary file S3 & S4.

4. PM is capable of inducing alterations in different cellular and metabolic pathways

The up-and down-regulated genes were separately used to identify the potential cellular pathways modulated by PM. The identified pathways are illustrated in Table 3(A & B). The identified enriched pathways were interferon signaling pathway (overlap of

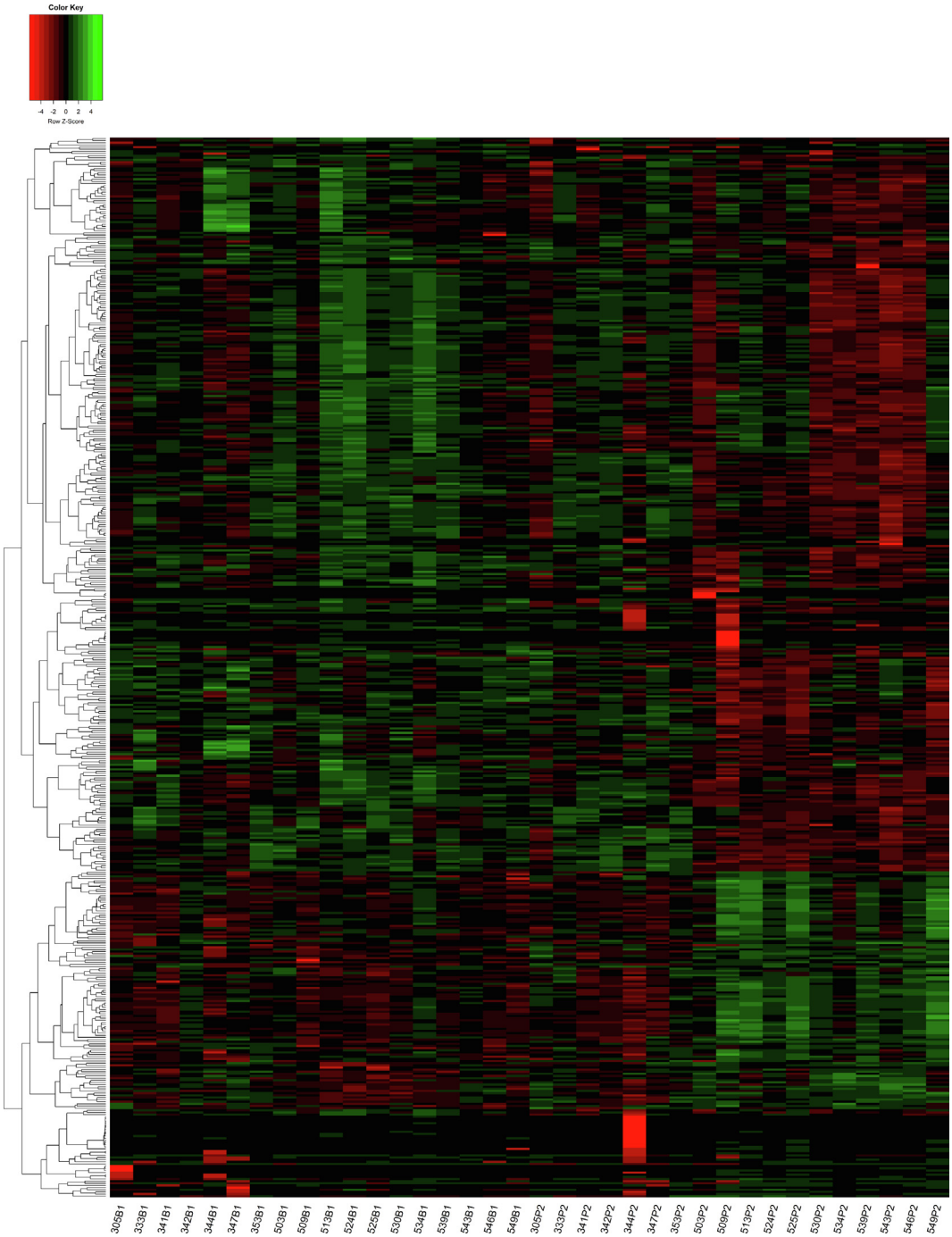

Fig. 1. Supervised heatmap of the significantly and differentially expressed genes between the baseline and 8 weeks post-intervention. The figure depicts the 494 affected genes (Y-axis) in the 36 samples (X-axis). The color key shows the scale of regulation (-4 to 4), and green color denotes the upregulation while the red color denotes the downregulation.

Table 1
The list of the 20 most upregulated genes in the study.

Ensembl_ID	Gene_name	logFC	linearFC	logCPM	P-Value
ENSG00000163492.9	CCDC141	1.74E+00	3.33E+00	1.13E+00	1.26E−02
ENSG00000178404.5	DDC8	1.65E+00	3.14E+00	1.83E+00	2.22E−03
ENSG00000155657.19	TTN	1.30E+00	2.46E+00	4.86E+00	2.58E−02
ENSG00000078114.14	NEBL	1.28E+00	2.44E+00	5.36E−01	1.59E−02
ENSG00000124575.5	HIST1H1D	1.22E+00	2.33E+00	7.72E−01	9.62E−03
ENSG00000042832.7	TG	1.21E+00	2.31E+00	1.38E+00	5.36E−05
ENSG00000115239.17	GPR75-ASB3	1.20E+00	2.30E+00	2.71E−02	1.04E−02
ENSG00000213132.2	AC022498.1	1.19E+00	2.28E+00	8.68E−01	1.80E−03
ENSG00000008086.6	CDKL5	1.18E+00	2.27E+00	6.10E−01	2.01E−02
ENSG00000197580.7	BCO2	1.14E+00	2.20E+00	5.12E−02	1.41E−02
ENSG00000164082.10	GRM2	1.13E+00	2.18E+00	3.27E−01	4.77E−03
ENSG00000169031.14	COL4A3	1.10E+00	2.14E+00	5.21E−01	5.04E−03
ENSG00000198518.5	HIST1H4E	1.05E+00	2.07E+00	1.49E+00	3.73E−02
ENSG00000160229.7	ZNF66	9.36E−01	1.91E+00	9.87E−01	8.18E−03
ENSG00000181722.11	ZBTB20	9.36E−01	1.91E+00	2.49E+00	4.31E−02
ENSG00000130997.12	POLN	9.25E−01	1.90E+00	1.91E−01	2.90E−02
ENSG00000176438.8	SYNE3	9.12E−01	1.88E+00	3.33E+00	1.13E−03
ENSG00000081052.10	COL4A4	8.87E−01	1.85E+00	5.02E−01	8.81E−03
ENSG00000188167.4	TMPPE	8.82E−01	1.84E+00	2.41E+00	2.18E−02
ENSG00000212743.1	DKFZP667F0711	8.77E−01	1.84E+00	1.60E+00	1.00E−02

Table 2
The list of the 20 most downregulated genes in the study.

Ensembl_ID	Gene_name	logFC	linearFC	logCPM	p-value
ENSG00000125618.12	PAX8	−1.33E+00	−2.52E+00	7.26E−01	7.64E−03
ENSG00000089685.10	BIRC5	−1.09E+00	−2.13E+00	1.18E+00	1.07E−02
ENSG00000182272.7	B4GALNT4	−1.09E+00	−2.12E+00	5.01E−01	4.40E−02
ENSG00000134321.7	RSAD2	−1.08E+00	−2.11E+00	5.37E+00	1.17E−02
ENSG00000112290.8	WASF1	−1.07E+00	−2.10E+00	2.82E−02	7.83E−03
ENSG00000154165.3	GPR15	−1.06E+00	−2.08E+00	6.63E−02	8.67E−03
ENSG00000165949.8	IFI27	−1.05E+00	−2.07E+00	3.45E+00	2.96E−02
ENSG00000137807.9	KIF23	−1.04E+00	−2.05E+00	4.71E−01	1.97E−02
ENSG00000241043.1	GVQW1	−1.04E+00	−2.05E+00	2.34E−01	4.19E−02
ENSG00000075218.14	GTSE1	−1.02E+00	−2.03E+00	1.43E−01	1.53E−02
ENSG00000177469.12	PTRF	−9.91E−01	−1.99E+00	4.51E−02	2.92E−02
ENSG00000124019.9	FAM124B	−9.39E−01	−1.92E+00	1.23E−01	2.72E−02
ENSG00000165244.6	ZNF367	−9.39E−01	−1.92E+00	7.26E−01	7.99E−03
ENSG00000029993.10	HMGB3	−9.11E−01	−1.88E+00	5.15E−01	8.49E−03
ENSG00000105991.7	HOXA1	−9.11E−01	−1.88E+00	2.63E−01	1.45E−02
ENSG00000135047.10	CTSL	−8.91E−01	−1.85E+00	2.66E+00	1.51E−04
ENSG00000171766.11	GATM	−8.86E−01	−1.85E+00	4.58E−01	1.59E−02
ENSG00000172426.11	RSPH9	−8.86E−01	−1.85E+00	8.23E−01	4.67E−03
ENSG00000141682.11	PMAIP1	−8.83E−01	−1.84E+00	1.82E+00	5.79E−03
ENSG00000134548.5	C12orf39	−8.82E−01	−1.84E+00	1.02E+00	1.48E−02

Table 3
The affected canonical pathways when upregulated (A) and downregulated (B) genes were used in Ingenuity pathway analysis.

Name of pathway	p-value	Overlap
(A) Top Canonical Pathways		
Anandamide Degradation	1.75E−02	33.3% (1/3
Transcriptional Regulatory Network in Embryonic Stem Cells	4.00E−02	3.7% (2/54
Phosphatidylcholine Biosynthesis I	4.03E−02	14.3% (1/7)
Phosphatidylethanolamine Biosynthesis II	5.15E−02	11.1% (1/9)
NAD Phosphorylation and Dephosphorylation	7.36E−02	7.7% (1/13)
(B) Top Canonical Pathways		
Interferon Signaling	1.82E−05	16.7% (6/36)
Th1 and Th2 Activation Pathway	8.64E−05	6.4% (11/172)
Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I	3.42E−04	18.2% (4/22)
Prolactin Signaling	1.68E−03	7.4% (6/81)
Unfolded protein response	1.79E−03	8.9% (5/56)

16.7% of the total pathway genes), the Th1 and Th2 activation pathway (overlap of 6.4%), and the pyrimidine Deoxyribonucleotides De Novo Biosynthesis I pathway (overlap 18.2%). Prolactin signaling pathway was also among the affected pathways with an overlap of 7.4% (6 genes among the total 81 pathway genes). Finally, the unfolded protein response pathway revealed that 8.9% were among the downregulated genes (5 genes of 56 total genes).

In addition, other pathways were also enriched such as anandamide degradation pathway (33.3% overlap), transcriptional regulatory network in embryonic stem cells pathway (3.7% overlap),

phosphatidylcholine Biosynthesis I pathway (14.3% overlap), Phosphatidylethanolamine Biosynthesis II pathway (11.1% overlap), and NAD phosphorylation and dephosphorylation pathway (7.7% overlap).

5. Discussion

Recently, we reported the efficacy of *PM* in improving cognitive skills after 8 weeks of intervention (Pragya et al., 2021). While RNA profiling and DNA methylation have been shown to be altered by meditation and mindfulness (Kaliman et al., 2014), the different designs made the results difficult to compare across studies. In some studies, participants were expert or experienced in meditation (Chaix et al., 2020), while in our study the age group was between 18 and 24 and all of the participants were healthy college students and novice meditators. The PM protocol studied here is unique in that while it included relaxation periods similar to other Yoga session, most of the intervention is based on attention and brain function. Thus, while previous Yoga and meditation studies clearly showed the benefits on the emotional, psychological, and health outcome of practicing individuals (Astin et al., 2003; Sharma et al., 2008; Kuntsevich et al., 2010; Buric et al., 2017; Lim et al., 2018), the cellular and molecular mechanisms involved in improvements remain poorly understood.

Several groups have attempted to address this question. For example, Su Qu et al. reported a rapid and a robust change in gene expression after short time of Yoga practice in peripheral blood lymphocytes using microarrays analysis platform (Qu et al., 2013). In addition, Bhasin et al. showed that relaxation response (RR) induces changes at the transcriptome levels with special emphasis on energy metabolism, insulin secretion, and inflammatory pathways (Bhasin et al., 2013). Similarly, in our study several genes of the total 494 significantly differentially expressed genes were involved in brain functions, muscle, and cardiac relaxation and cellular signaling, several pathways involved in the immune system were most enriched as well. Both studies showed cognitive skills improvements correlated with most of the affected pathways which included important known pathways in signaling pathways, cytokine release, and innate and adaptive immunity. Interestingly, *NF-κβ* expression level decreased although the trend toward downregulation was not statistically significant (p = 0.059) (Supplementary file S5). The gene was previously shown to be downregulated in RR study (Bhasin et al., 2013). This modest decrease can be related to the extended intervention (8 weeks post intervention). Clearly, the possibility of a more significant decrease of *NF-κβ* at earlier stages cannot be ruled out. Noteworthy, although *NF-κβ* is considered a major player in proinflammatory signaling pathways, the inflammation process is complex and multifactorial (Lawrence, 2009). *CCDC14* was the most differentially expressed gene as a result of PM intervention. This gene is involved in radial migration and centrosomal function in neurons (Hutchins et al., 2016). *DDC8* was also found to be upregulated, and the gene is suggested to play a regulatory role in brain trauma injuries (Jaworski et al., 2007). *TTN* showed upregulation and has its role in skeletal and cardiac muscles synthesis (Hinson et al., 2015). *NEBL* was also among the upregulated genes, and it is believed to be involved in the actin binding and the assembly of Z-disk (Li et al., 2004). *TG* was found to be upregulated, and it is a gene involved in thyroid hormone metabolism (Watanabe et al., 2018). Other genes involved in structural activities were also found to be upregulated at 8 weeks post intervention compared to baseline. *CDKL5* a cyclin dependent kinase like 5 is a structural protein expressed in different body tissues, especially the brain and it is involved in brain development and function (Bartnik et al., 2011). Studies suggested that the CDKL5 protein is involved in the formation, growth, and movement (migration) of nerve cells (neurons), as well as cell division. It also plays a role in the transmission of chemical signals at the connections (synapses) between neurons. Interestingly, three putative transcriptional factors were among the top 20 upregulated genes (*ZNF66*, *ZBTB20*, and *ZNF573*). These transcriptional factors belong to the zinc finger protein family. *PAX8* was the most upregulated gene in the list of the 348 downregulated genes. *PAX8* is a transcriptional factor, and it plays critical role in epithelial cell survival and proliferation (Di Palma et al., 2013). *BIRC5* interacts with the PI3k/Akt signaling pathway and is involved in the esophageal squamous cell cancer (ESCC). Data revealed that *BIRC5* can inhibit the migration and invasion of tumor cells and regulate the expression of angiogenesis-associated factors. In addition, the PI3K/Akt signaling pathway is able to regulate the expression of *BIRC5*, which affects the development of ESCC (Shang et al., 2018). *RSAD2* was also among the most downregulated genes, and it is involved in interferon gamma (INFγ) signaling and innate immunity (Jang et al., 2018). *WASF1* is involved in signaling and it plays critical role in actin cytoskeleton required for membrane ruffling (Ito et al., 2018). *GPR15* encodes for a G protein-coupled receptor and involved in cell signaling as well. *IFI27* is an interferon alpha inducible protein 27, and it is involved in INFγ and innate immunity. Knockdown of *IFI27* inhibited cell proliferation and invasion in oral squamous cell carcinoma (Wang et al., 2018). Interestingly, two transcriptional factors were also among the most downregulated genes, *ZNF367* and *HOXA1*. *ZNF367* belongs to the Zinc finger protein family, while *HOXA1* belongs to Homeobox A1 group of genes involved in several developmental processes in the brain and the cardiovascular system (Tischfield et al., 2005; Naef et al., 2018).

Interestingly, some genes involved in epigenetic mechanisms such as HDMS demethylate histones, chromatin modifying enzymes, and chromatin organization were found to be affected (Onder et al., 2012; Shi and Tsukada 2013). In experienced meditators, a reduced expression of histone deacetylase genes such as *HDAC2*, 3, and 9 was detected along with a decreased expression of proinflammatory genes (*RIPK2* and *COX2*) (Kaliman et al., 2014). Our data showed that HDAC2 expression was decreased after 8 weeks (logFC −0.26, p = 0.08), while other *HDACs* showed unchanged patterns with the intervention. *COX2* expression pattern was unchanged in our study, although *COX20* was slightly decreased (logFC −0.16, and p = 0.26). *RIPK2* was also found to be downregulated after 8 weeks (logFC −0.26, p = 0.35). Our findings showed that prolactin pathways signaling was among the enriched pathways. Prolactin (PRL) is a hormone with pleiotropic effects on the growth, development, immunoregulation, and metabolism (Ben-Jonathan et al., 2006). In this study, two major pathways were also enriched as a result of intervention. The interferon signaling pathway and Th1 and Th2 activation pathway and both pathways play central roles in innate and acquired immunity (Nallar and Kalvakolanu, 2014).

Our study had its own limitations which included that we did not specifically have a control group without PM intervention with paired blood samples at two timepoints, though it has been reported that control groups showed no significant pattern after 8 h of leisure activities (Chaix et al., 2020). Furthermore, the sampling time interval between baseline and 8 weeks is a snapshot of time post-intervention and possibly misses changes occurring early or even after the 8-week time point. We believe that the analysis of *PM* effects at RNA expression might reveal different mechanisms of regulation if performed shortly after the intervention. In Chaix et al. study, 61 Differentially methylated sites (DMS) were identified in the intervention group after only one day (8 h) of intensive mindfulness practices (Chaix et al., 2020). Critically, our sample size in this study is limited and thus is underpowered to detect all but the strongest effects. Thus, we are only reporting

nominally significant *p*-values for expression. While we acknowledge the possibility that this may include some false positives, as a hypothesis generating pilot study these results warrant further validation and replication in subsequent work.

To the best of our knowledge, our study is the first to analyze meditation effects at the gene expression profiling level in novice meditators for a time span of 8 weeks. These findings will assist in designing shorter and targeted intervention approaches of *PM* to compare the affected molecular and cellular targets at short- and long-term intervention. Taken together, in addition to its beneficial effect on the cognitive skills, several genes and cellular pathways were affected. Our data revealed the differential expression patterns of several genes and cellular pathways involved in immune system, and other essential cellular pathways. These results should encourage in-depth investigation of stepwise meditation protocols not only in healthy subjects, but also in disease states.

6. Conclusion and future directions

Our group is conducting CpG methylation analysis of the same study group (baseline vs 8 weeks postintervention). The future study will reveal alterations at the methylation level which will strengthen the concept of beneficial effects of PM at the cognitive skills, RNA profiling, and DNA methylation.

Declaration of Competing Interest

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

Acknowledgements

We are thankful for Jain Education and Research Foundation for funding the study. We appreciate the assistance offered by Mr. Rajeev Jain and Jain studies Program at FIU. We are also deeply grateful to the research team involved in guiding the practicing students through the meditation process and blood sample collection. The authors declare no conflict of interest. BA, SUP, and AG assessed and analyzed the transcriptomics data and helped in manuscript writing. NDM assisted in data analysis. Additionally, PU, PV, NM, HE, and SCP were involved in data collection, data management, and writing the manuscript. DM was involved in guiding and mentoring the whole study and was involved in writing the manuscript. We appreciate Dr. Ray Prather for critically reading the manuscript and Allan Salinas for assistance in editing.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.11.060.

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