

## Effects of Traditional Japanese Massage Therapy on Gene Expression: Preliminary Study

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### Abstract

**Objectives:** Changes in gene expression after traditional Japanese massage therapy were investigated to clarify the mechanisms of the clinical effects of traditional Japanese massage therapy.

**Design:** This was a pilot experimental study.

**Settings/location:** The study was conducted in a laboratory at Tsukuba University of Technology.

**Subjects:** The subjects were 2 healthy female volunteers (58-year-old Participant A, 55-year-old Participant B).

**Interventions:** The intervention consisted of a 40-minute full-body massage using standard traditional Japanese massage techniques through the clothing and a 40-minute rest as a control, in which participants lie on the massage table without being massaged.

**Outcome measures:** Before and after an intervention, blood was taken and analyzed by microarray: (1) The number of genes whose expression was more than double after the intervention than before was examined; (2) For those genes, gene ontology analysis identified statistically significant gene ontology terms.

**Results:** The gene expression count in the total of 41,000 genes was 1256 genes for Participant A and 1778 for Participant B after traditional Japanese massage, and was 157 and 82 after the control, respectively. The significant gene ontology terms selected by both Participants A and B after massage were “immune response” and “immune system,” whereas no gene ontology terms were selected by them in the control.

**Conclusions:** It is implied that traditional Japanese massage therapy may affect the immune function. Further studies with more samples are necessary.

### Introduction

MASSAGE THERAPY IS ONE OF THE MOST popular complementary and alternative medicine therapies in the world, and many scientific studies on it have been published in the last 2 decades in Western countries.<sup>1,2</sup> In some studies,<sup>3,4</sup> the number and activity of natural killer cells increased following massage therapy. Some studies revealed an elevation in secretory immunoglobulin A concentration after massage therapy,<sup>5</sup> after traditional Japanese massage therapy,<sup>6</sup> and after a 10-minute back rub.<sup>7</sup> These findings suggest that various kinds of massage therapies improve the immune function. However, the mechanism of the clinical effects of massage therapies remains unknown, so the study focused on changes in gene expression after massaging. This is the first report to demonstrate that traditional Japanese massage therapy alters the expression of many genes in the human body.

### Materials and Methods

Two (2) healthy women in their 50s who liked receiving massage were recruited. They gave their informed consent to participate in the study. They were asked to refrain from strenuous exercise for 12 hours and from eating and drinking for 2 hours before the test.

The participants presented themselves to the laboratory at 2:30 PM. After resting on the massage table for 30 minutes, blood (2.5 mL) was drawn by venous puncture for the pre-intervention sample, and then a 40-minute intervention (traditional Japanese massage or rest) was performed. After the intervention, blood (2.5 mL) was drawn again for the postintervention sample. The blood samples were collected in a PAXgene Blood RNA Kit (QIAGEN, Valencia, CA) and quickly taken to Mitsubishi Chemical Medience Corporation (Tsukuba branch, Japan) where total RNA was extracted,<sup>8</sup> and then the extracted RNA was taken to DNA Chip

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Research Inc. (Yokohama, Japan) to be analyzed by the microarray technique.

RNA from the unfractionated white blood cells was analyzed using an Agilent human 44K microarray. First, the number of genes whose expression was more than double after the intervention than before was examined. Next, in those genes, the gene expression profiles were identified by gene ontology analysis.

### Interventions

The participants received a 40-minute massage intervention. It was performed on the full body, except the face, head, and abdomen. Standard traditional Japanese massage techniques,<sup>6</sup> consisting mainly of kneading and lesser amounts of stroking and pressing through the clothing were used, with intensity of stimulation being within the range of comfort. The traditional Japanese massage therapy was performed by a female therapist holding Japan's national massage practitioner license with 20 years of treatment experience. The participants also received a 40-minute rest intervention as a control, in which they lay on the massage table without massaging. The two interventions were performed at a 1-week interval.

### Microarray hybridization and data acquisition

Equal amounts of total RNA preparations from the two participants' blood samples were mixed in each group and analyzed by the microarray technique. Two hundred and fifty (250) ng of total RNA was reverse transcribed to cDNA using T7 Promoter Primer and MMLV-RT. Then, the cDNA was converted to cRNA using T7 RNA polymerase, which simultaneously amplifies the target material and incorporates cyanine (Cy)3- or Cy5-labeled cytidine triphosphate (CTP). Equal amounts of Cy3-labeled cRNA derived from the controlled sample and Cy5-labeled cRNA derived from the massaged sample were mixed. The mixture was applied to a Whole Human Genome Oligo Microarray (G4112F, Agilent Technologies, CA) on which 41,000 genes (including transcripts) were spotted, and hybridization was allowed to proceed for 17 hours at 65°C as described by the manufacturer. After hybridization, the arrays were washed and scanned using a DNA microarray scanner (Agilent G2565BA). The image files were extracted using Agilent Feature Extraction software version 10.7.3.1 applying LOWESS background subtraction and dye normalization.

### Gene ontology analysis

Gene ontology analysis was performed utilizing GeneSpring GX10.0 software. The biologic meaning of the dis-

criminated genes was examined based on gene ontology. Gene ontology analysis was performed ( $p$ -value < 0.1 was considered significant) using the False Discovery Rate correction test. Gene ontology terms judged significant by this examination were regarded as specific terms for discriminated genes.

This study was approved by the Medical Ethics Committee of Tsukuba University of Technology and performed according to the ethical standards set forth in the Helsinki Declaration of 1964 and its amendment of 2000.

### Results

The demographics of the participants and the study results are shown in Table 1. The number of genes whose expression was more than double after the massage intervention than before was 1256 of the total 41,000 genes for Participant A and 1778 genes for Participant B, whereas those after the rest intervention as the control were 157 and 82 for Participants A and B, respectively. Next, genes whose expression was more than double after each intervention than before were annotated by functional domain databases,<sup>9</sup> assigned with gene ontology terms, and the significant gene ontology terms that were selected for both Participants A and B in each intervention were determined. In the results, the significant gene ontology hit for the gene ontology terms "immune response" and "immune system" in the massage intervention, whereas the significant gene ontology hit for no gene ontology term in the control.

### Discussion

For participants, the genes count that expressed more twice after massage than before was larger than that in the control. This suggests that stimulation by traditional Japanese massage therapy activates the human body positively, compared with mere rest. Significant gene ontology terms common to both participants after massage were "immune response" and "immune system." These imply that traditional Japanese massage therapy may affect the immune function and that traditional Japanese massage therapy may help prevent and treat immune disorders.

### Conclusions

These findings support the results of previous studies, which suggested that massage therapy including traditional Japanese massage may improve immune function.<sup>1-7</sup> In the present study, many genes expressed in traditional Japanese massage and gene ontology categories related to immune function were

TABLE 1. RESULTS OF MICROARRAY ANALYSIS

| Participants |   | Count of gene expression |                      | Results of gene ontology (GO) analysis  |                                  |  |
|--------------|---|--------------------------|----------------------|---|----------------------------------|--|
| ID           | Demographics                            | Control                  | Massage <sup>a</sup> | GO accession (from amiGO <sup>9</sup> ) | GO TERM                          | Corrected p Value                          |
| A            | Female<br>58 years old<br>155 cm, 54 kg | 157                      | 1256                 | GO:0006955                              | Immune response                  | 1.453 <sup>-8</sup>                        |
| B            | Female<br>55 years old<br>161 cm, 58 kg | 82                       | 1778                 | GO:0002376<br>GO:0006955                | Immune system<br>Immune response | 4.003 <sup>-6</sup><br>1.379 <sup>-9</sup> |
|              |   |                          |                      | GO:0002376                              | Immune system                    | 8.811 <sup>-7</sup>                        |

<sup>a</sup>Massage, traditional Japanese massage therapy.

selected significantly only in the case of massage, not the rest intervention. These results may be due to the specific effects of traditional Japanese massage therapy that induces a comfortable feeling in the person receiving the massage.

Further studies should be conducted with more samples to clarify the mechanisms of the clinical effects of traditional Japanese massage therapy.

### Acknowledgments

The present study was supported by a competitive research project program grant from Tsukuba University of Technology, 2009. The principal investigator was Nozomi Donoyama.

### Disclosure Statement

No competing financial interests exist.

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