Correlation Between DNA Methylation of TRPA1 and Chronic Pain States in Human Whole Blood Cells

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Abstract

Objectives. Neuro-immune interactions with functional changes in the peripheral blood cells including changes in the transient receptor potential ankyrin 1 (TRPA1) appear to play a pivotal role in the development of chronic pain in humans. The aim of this study was to examine the association between TRPA1 DNA methylation in whole blood cells and the pain states in chronic pain patients.

Methods. After collecting blood samples from 12 chronic pain patients, the authors measured DNA methylation levels in whole blood cells. Significant associations between the patient’s demographic data and the chronic pain states were determined by a multiple linear regression analysis that used age, body mass index, pain duration, depression, anxiety, cognitive impairment, activities of daily living, neuropathic pain, and pain states as the dependent variables, and the TRPA1 DNA methylation levels as the independent variables.

Results. Multiple regression analysis revealed a significant correlation between increases of the methylation levels of the CpG island in the TRPA1 gene and increases in the number of neuropathic pain symptoms, which were evaluated using the Douleur Neuropathique 4 (DN4) questionnaire. Decreases in the TRPA1 mRNA expression were also significantly related to increases in the DN4 score. The presence of a burning sensation, which is one of pain symptoms in the DN4 questionnaire, was significantly correlated with the increase in DNA methylation level of TRPA1.

Conclusions. TRPA1 DNA methylation levels in whole blood cells appear to be associated with pain symptoms in chronic pain patients.

Key Words. DN4; Epigenetics; Neuropathic pain; TRPA1

Introduction

Recent progress in pain research has revealed the importance of the neuro-immune interaction in the development of chronic pain [1,2]. It has also been suggested that endogenous signals, such as danger-associated molecular patterns originating from the degeneration af-
ter peripheral nerve injury, might initiate innate immune responses through Toll-like receptors (TLR) in the neuronal cells, and leads to the development of chronic pain [2]. A recent animal study reported that interactions between TLR-4 and transient receptor potential ankyrin 1 (TRPA1) in the neurons were crucial for the development of neuropathic pain [3]. In the peripheral blood mononuclear cells, increases in the TLR-4 responsiveness have also been reported in both the rat model of neuropathic pain and in chronic pain patients [4].

The increase in methylation near gene promoters is thought to be correlated with the suppression of transcription. A recent human study in monozygotic twins reported that decreases in the heat pain threshold were due to either an increase in the DNA methylation levels at the TRPA1 promoter sites or a decrease in the TRPA1 mRNA expression in whole blood cells [5]. Based on these previous findings, we decided to further investigate the relationship between the chronic pain states and DNA methylation of the TRPA1 genes in the whole blood cells of chronic pain patients.

Methods

This study was approved by the Ethics Committee of the Hyogo College of Medicine. A total of 12 patients suffering from chronic back pain or postherpetic neuralgia without malignant diseases were enrolled in this prospective cohort study that examined the epigenetics of chronic pain. Written informed consent was obtained from all subjects.

Patients ranged in age from 44 to 81 years and all of the enrolled participants completed the Self-Rating Questionnaire for Depression (SRQ-D), State-Trait Anxiety Inventory 1 (STAI-1), Barthel Index, and Mini-Mental State Examination (MMSE). SRQ-D was used to evaluate the depression status [6], while anxiety levels were assessed using STAI-1 [7]. The Barthel Index was used to evaluate activities of daily living [8], and MMSE was used to evaluate the cognitive impairment [9].

Chronic pain status was evaluated using either the Douleur Neuropathique 4 (DN4) questionnaire or the short-form McGill Pain questionnaire (SF-MPQ). We used the DN4 to discriminate neuropathic pain from other pain states [10]. The SF-MPQ, which was created in order to specifically assess pain states [11], consists of the sensory pain rating index (S-PRI), the affective PRI (A-PRI), the total PRI (T-PRI), the visual analog scale (VAS), and the present pain intensity (PPI).

Whole blood was collected from each patient, and stored at -30°C until analyzed. Genome-wide assays of DNA methylation were performed by Q&G Science Co., Ltd. (Fukushima, Japan) in 12 patients using the Illumina HumanMethylation450 BeadChip (Illumina Inc., San Diego, CA, USA). From the collected data, we selected 13 β-values from either the CpG islands or CpG island shores of the TRPA1 gene (chr8: 72984967, 72987499, 72987744, 72987762, 72987797, 72987836, 72987838, 72987870, 72987872, 72987916, 72987924, 72988280, 72988608 bp), which represented the methylation ratio for each of the analyzed CpG sites (0–1). The genome-wide mRNA expression essay was performed by Oncomics Co., Ltd. (Nagoya, Japan) in 10 patients using the SurePrint G3 Human Gene Expression 8x60K v2 Microarray Kit (Agilent Technologies, Santa Clara, CA, USA).

Statistics

All statistical analyses were performed using SPSS Statistics 21 software (IBM, Chicago, IL). To investigate the association between the patient status and the DNA methylation levels at the CpG sites of the TRPA1 gene, we performed multiple linear regression analysis using a stepwise variable selection. The dependent variables included the age, body mass index (BMI), pain duration, or each of the scores for SRQ-D, STAI-1, MMSE, Barthel Index, DN4, and SF-MPQ, while the 13 β-values of the DNA methylation levels of the TRPA1 gene were used as the independent variables. As we evaluated 13 test parameters, the statistical significance was set at \( p < 0.0038 \) after a Bonferroni adjustment—that is, \( (0.05/13 = 0.0038) \).

After confirming that the DN4 score was significantly correlated with the TRPA1 methylation level, we used Spearman’s rank-order correlation to determine the association between the DN4 score and the TRPA1 mRNA expression level, with the statistical significance set at \( p < 0.05 \). We also performed multiple linear regression analysis to examine the correlations between each pain symptom in the DN4 questionnaire and the DNA methylation level.

Results

The DNA methylation level at the CpG island of the TRPA1 gene (cg01610488, chr8: 72987870) was selected for predicting which of the dependent variables were significant (Table 1). Results indicated there was a significant correlation between the increase in the DNA methylation level at the CpG island of the TRPA1 gene and the increase in the DN4 score, which represents the diversity of the neuropathic pain symptoms (Figure 1). There was also a significant relationship between the decrease in the TRPA1 expression and the increase in the DN4 score (Figure 1).

Within the DN4 questionnaire, the prevalence of pain symptoms was as follows: burning (50.0%), painful cold (16.7%), electric shocks (41.7%), tingling (25.0%), prickling pain (8.3%), numbness (58.3%), itching (8.3%), hypesthesis to touch (25.0%), hypesthesis to prick (25.0%), and allodynia (8.3%). The presence of burning sensation showed a significant correlation to the increase in the level of TRPA1 DNA methylation (\( p = 0.001 \)).

Although the increase in the DNA methylation level at the CpG island of the TRPA1 gene (cg12668482, chr8: 72987762) was not statistically significant, it tended to
be associated with the decrease in the Barthel Index score, which is used to assess daily routine physical functions (Table 1). The increase in the DNA methylation level (cg01610488, chr8: 72987870) was also tended to be associated with the increases in SRQ-D and STAI-1 scores (Table 1).

Discussions

The present study demonstrated that the increased methylation level of the CpG island in the TRPA1 gene in whole blood cells was associated with the increase in the DN4 score. The DN4 questionnaire consists of 10 items that rate neuropathic pain symptoms, with higher scores indicating neuropathic pain [10]. The presence of burning sensation was significantly correlated with the DNA methylation level of TRPA1. Given that there are decreases in the heat pain threshold in chronic pain patients [12–14] and additionally the decrease in the heat pain threshold is associated with an increase in the DNA methylation level of the TRPA1 gene in whole blood cells of monozygotic twins [5], it is likely that the DNA methylation of TRPA1 is correlated with chronic pain states.

Table 1  Demographic data of patients, pain states, and the results of the multiple linear regression analysis

<table>
<thead>
<tr>
<th>Patients' characteristics</th>
<th>Multiple linear regression analysis</th>
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<tbody>
<tr>
<td></td>
<td>r</td>
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<tr>
<td>Age (years)</td>
<td>69.3 ± 11.3</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>7/5</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>24.4 ± 3.4</td>
</tr>
<tr>
<td>Pain duration (years)</td>
<td>8.5 [2.5–15.0]</td>
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<tr>
<td>SRQ-D (0–36)</td>
<td>8.0 [4.0–13.5]</td>
</tr>
<tr>
<td>STAI-1 (20–80)</td>
<td>30.0 [26.8–42.0]</td>
</tr>
<tr>
<td>Barthel Index (0–100)</td>
<td>100.0 [98.8–100.0]</td>
</tr>
<tr>
<td>MMSE (0–30)</td>
<td>28.5 [26.5–30.0]</td>
</tr>
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</table>

Pain states

| DN4 (0 – 10) | 3.0 [1.0–4.0] | 0.82 | 0.001* |
| SF-MPQ       | S-PRI (0–33)  | 5.0 [3.5–8.3] | NS |
| A-PRI (0–12) | 1.0 [0.0–2.3] | NS |
| T-PRI (0–45) | 8.0 [4.3–10.3] | NS |
| VAS (0–100 mm)| 55.5 [28.5–72.3] | NS |
| PPI (0–5)    | 2.0 [1.8–2.0] | NS |

Each data point represents the mean ± SD or the median [25th–75th percentile]. *P < 0.0038 indicates significance. A-PRI, affective pain rating index; BMI, body mass index; DN4, Douleur Neuropathique 4; MMSE, Mini-Mental State Examination; NS, not selected; PPI, present pain intensity; SF-MPQ, short-form McGill Pain Questionnaire; S-PRI, sensory pain rating index; SRQ-D, Self-Rating Questionnaire for Depression; STAI, State-Trait Anxiety Index; T-PRI, total pain rating index; VAS, visual analog scale.

Figure 1  DNA methylation at the CpG island of the TRPA1 gene is associated with the Douleur Neuropathique 4 (DN4) score (A). Similarly, TRPA1 mRNA expression is also associated with the DN4 score (B).
It is well-known that chronic pain can limit the daily activities of patients [15,16]. Our current study found that DNA methylation of TRPA1 tended to suppress the routine daily activities of the enrolled participants, and that there was a diversity of chronic pain symptoms that were associated with the DNA methylation of TRPA1. Thus, DNA methylation might be responsible for suppressing routine daily activities of chronic pain patients. Moreover DNA methylation of TRPA1 tended to relate with depression and anxiety in the present study. A recent animal study revealed that TRPA1 function might be involved in depression and anxiety [17]. As chronic pain reportedly augments depression and/or anxiety with the suppression of activities of daily living [18,19], DNA methylation of TRPA1 might be a mediator among chronic pain, daily activities, depression, and anxiety, respectively.

Persistent pain animal models that use nerve injury techniques have reported finding several changes in the DNA methylation at the peripheral nerve, dorsal root ganglions, spinal dorsal horn, and the brain [20–22]. In humans, however, there are only a few studies at the present time that have examined the epigenetic changes in chronic pain patients [23,24]. Further investigations will need to be undertaken in order to determine the specific association between the epigenetics and the chronic pain states in various human tissues.

In neuronal cells, TRPA1 expression is regulated by neurotrophins such as artemin and nerve growth factor [25]. However, the regulation of TRPA1 expression in blood cells has yet to be definitively investigated. In human monocytes, temperature has been reported to affect the TRPA1 mRNA expressions that regulate cytokine secretion, such as tumor necrosis factor (TNF) -α or interleukin (IL) -10 [26]. Chronic pain patients also exhibit increased plasma concentrations of TNF-α and decreased levels of IL-10 [27–29]. Although the mechanisms responsible for the epigenetic changes in the TRPA1 gene of circulating cells linked to chronic pain remain unknown, in chronic pain states, it is possible that changes in the TRPA1 DNA methylation that regulate the TRPA1 expression may affect cytokine secretion from the immune cells.

**Conclusions**

DNA methylation level of TRPA1 in whole blood cells appears to be correlated with pain symptoms in chronic pain patients.

**References**


