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# Identification of transcription factors activated by methylmercury in mouse brain

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**ABSTRACT** — Methylmercury is a harmful heavy metal that selectively disrupts the central nervous system. To clarify the transcription induction mechanism that operates in the brain in response to methylmercury exposure, we used a protein/DNA binding assay to identify transcription factors activated in the cerebella of mice administered methylmercury. We identified PAX4, PAX6, TCF3 and HMGA1 as transcription factors activated by methylmercury.

**Key words:** Methylmercury, Transcription factor, Brain

## INTRODUCTION

Methylmercury is an environmental pollutant that causes serious central nervous system disorders such as sensory paralysis, speech disorders, motor ataxia, and narrowing of the visual field (Bakir *et al*., 1973). However, the mechanisms underlying methylmercury-induced toxicity and associated biological defensive responses are not well understood, and determination of their molecular mechanism is necessary.

We previously performed expression variation analysis of gene clusters in the brain tissue of mice that were administered methylmercury, and identified chemokines (CCL3 and CCL4) (Kim *et al*., 2013), secretoglobin (SCGB3A1) (Takahashi *et al*., 2013) and tumor necrosis factor α (TNFα) (Iwai-Shimada *et al*., 2016) as genes whose expression were increased specifically in the brain in response to methylmercury. This suggests that there is a transcription mechanism that is activated by methylmercury specifically in the brain. NF-E2-related factor 2 (Nrf2) (Kumagai *et al*., 2013) and nuclear factor-kB (NF-kB) (Kim *et al*., 2012) are transcription factors known to be activated by methylmercury, but the transcription mechanisms underlying methylmercury-induced toxicity remain unexplained. Therefore, in this study, we comprehensively searched for transcription factors activated by methylmercury treatment using a protein/DNA array assay (a membrane array method that can simultaneously investigate the activity of 345 transcription factors) (Tokumoto *et al*., 2014).

## MATERIALS AND METHODS

### Animal experiments

All experiments were performed in accordance with the Regulations for Animal Experiments and Related Activities at Tohoku University. C57BL/6 male mice (8 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Mice were housed in a controlled

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environment (temperature 22 ± 2°C, relative humidity 55 ± 20%, and 12-hr light/12-hr dark cycle). The mice were allowed free access to chow (CE2; CLEA, Tokyo, Japan) and water *ad libitum*. After an adaptation period, mice were randomly divided into control (n = 5) and methylmercury-treated (n = 5) groups. Methylmercuric chloride (Sigma-Aldrich, St. Louis, MO, USA), dissolved in physiological saline, was administered via a single subcutaneous injection at dose of 20 mg/kg. Cerebella were dissected 24 hr after the injection.

### Preparation of nuclear fractions

Cerebella were homogenized in a hypotonic buffer (10 mM HEPES–KOH [pH 7.9], 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol [DTT]) containing protease inhibitor (Roche, Indianapolis, IN, USA) for 30 min on ice. Nonidet P-40 (1%) was added, and homogenates were centrifuged at 2,300 × g at 4°C. The supernatant was removed, and the pellet was resuspended in nuclear lysis buffer (10 mM HEPES–KOH [pH 7.9], 400 mM NaCl, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 1 mM DTT, 5% glycerol) containing protease inhibitor (Roche). The resulting suspension was sonicated and centrifuged at 15,000 × g at 4°C to obtain the nuclear fraction. The protein concentration of each fraction was determined using a DC protein assay kit (Bio-Rad, Hercules, CA, USA).

### Protein/DNA array assay

Protein/DNA array assays were conducted using a Combo Protein/DNA Array (Affimetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. In brief, 20 µg of nuclear proteins were incubated with biotin-labeled DNA-binding probes. The protein–DNA complexes were separated from free probes using spin columns. The protein-bound probes were eluted and hybridized to a membrane dotted with 345 transcription factor consensus sequences. The membrane was washed and blocked with blocking buffer, and then incubated with horseradish peroxidase (HRP)-coupled streptavidin. Immunoreactive proteins were detected by enhanced chemiluminescence (Immobilon Western Chemiluminescent HRP Substrate, Millipore, Billerica, MA, USA). Dot density was measured using ImageJ 1.50i software (National Institutes of Health, Bethesda, MD, USA).

## RESULTS AND DISCUSSION

Male C57BL/6 mice were subcutaneously administered methylmercuric chloride (20 mg/kg). Their cerebella were extracted 24 hr later and a nuclear extract was prepared. Pathological and behavioral abnormalities were not observed in the mice at this dosage, and an increase in the expression of SCGB3A1, which is induced in a brain-specific manner by methylmercury, was confirmed (data not shown). We then subjected the nuclear extract to a protein/DNA array assay to comprehensively investigate the ability of 345 transcription factors to bind to consensus sequences. This identified four transcription factors (PAX4, PAX6, TCF3 and HMGA1) whose consensus sequence-binding activity levels were increased by more than a factor of five by methylmercury in comparison with

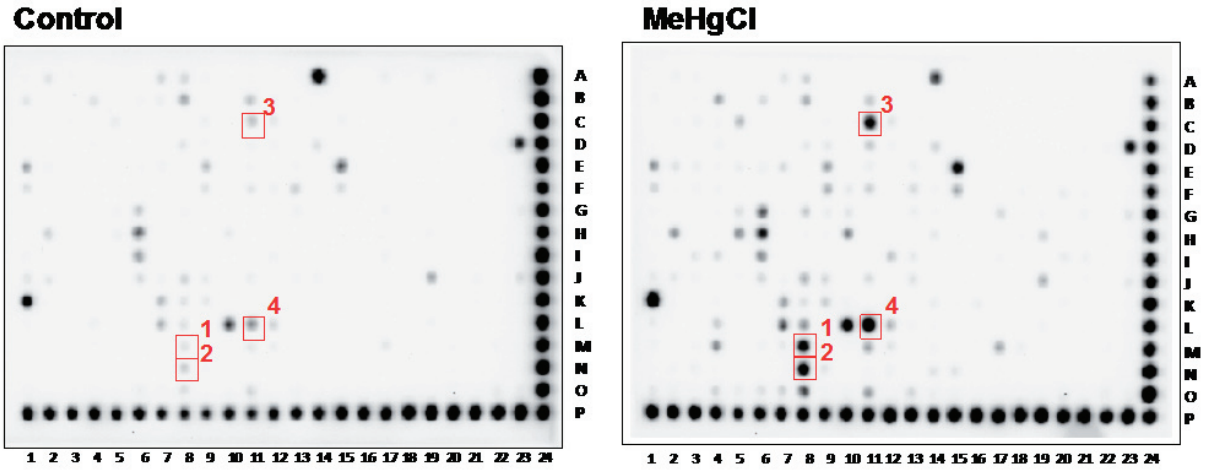


**Fig. 1.** Change in transcription factor binding activity to consensus sequence in cerebella of methylmercury-treated mice. C57BL/6 mice were injected subcutaneously with MeHgCl (20 mg/kg). At 24 hr after injection, cerebella were dissected and the DNA binding activity of transcription factors was evaluated using protein/DNA array assay. The density of dots within the red boxes increased more than 5-fold.

**Table 1.** Transcription factors activated by methylmercury in mouse cerebellum.

| No. | Gene name | NM number | Description | Fold change |
|---|---|---|---|---|
| 1 | PAX4 | NM_011038 | paired box 4 | 17.0 |
| 2 | PAX6 | NM_013627 | paired box 6 | 13.3 |
| 3 | TCF3 | NM_011548 | transcription factor 3 | 6.5 |
| 4 | HMGA1 | NM_001025427 | high mobility group AT-hook 1 | 5.2 |

control conditions (Fig. 1 and Table 1). PAX4 and PAX6 are members of the paired box (PAX) family that participate in the development of various organs mainly in the prenatal period (Feiner *et al*., 2014). PAX6 particularly is known to play an important role in the differentiation of various types of neuron (Maekawa *et al*., 2005). It is possible that PAX family members are important transcription factors for the regulation of gene groups that respond to methylmercury; this suggestion is supported by a recent report by Takemoto *et al*., which showed that PAX family members, including PAX4 and PAX6, are activated in a primary culture of rat astrocytes treated with methylmercury (Takemoto *et al*., 2016). TCF3 is a helix-loop-helix-type transcription factor belonging to the E protein family, and participates in lymphocyte production (Ikawa *et al*., 2006). HMGA1 has been shown to be associated with breast cancer onset, but its function is almost unknown (Sepe *et al*., 2016). Further investigations of the regulation of gene expression in brain tissue by the transcription factors identified in this study may provide clues regarding the mechanism underlying the central nervous system-specific toxicity of methylmercury.

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**Conflict of interest----** The authors declare that there is no conflict of interest.

## REFERENCES

Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalidi, A., al-Rawi, N.Y., Tikriti, S., Dahahir, H.I., Clarkson, T.W., Smith, J.C. and Doherty, R.A. (1973): Methylmercury poisoning in Iraq. Science, **181**, 230-241.

Feiner, N., Meyer, A. and Kuraku, S. (2014): Evolution of the vertebrate Pax4/6 class of genes with focus on its novel member, the Pax10 gene. Genome Biol. Evol., **6**, 1635-1651.

Ikawa, T., Kawamoto, H., Goldrath, A.W. and Murre, C. (2006): E proteins and Notch signaling cooperate to promote T cell lineage specification and commitment. J. Exp. Med., **203**, 1329-1342.

Iwai-Shimada, M., Takahashi, T., Kim, M.S., Fujimura, M., Ito, H., Toyama, T., Naganuma, A. and Hwang, G.W. (2016): Methylmercury induces the expression of TNF-alpha selectively in the brain of mice. Sci. Rep., **6**, 38294.

Kim, M.S., Takahashi, T., Lee, J.Y., Hwang, G.W. and Naganuma, A. (2012): Methylmercury induces CCL2 expression through activation of NF-kappaB in human 1321N1 astrocytes. J. Toxicol. Sci., **37**, 1275-1278.

Kim, M.S., Takahashi, T., Lee, J.Y., Hwang, G.W. and Naganuma, A. (2013): Global chemokine expression in methylmercury-treated mice: methylmercury induces brain-specific expression of CCL3 and CCL4. J. Toxicol. Sci., **38**, 925-929.

Kumagai, Y., Kanda, H., Shinkai, Y. and Toyama, T. (2013): The role of the Keap1/Nrf2 pathway in the cellular response to methylmercury. Oxid. Med. Cell Longev., **2013**, 848279.

Maekawa, M., Takashima, N., Arai, Y., Nomura, T., Inokuchi, K., Yuasa, S. and Osumi, N. (2005): Pax6 is required for production and maintenance of progenitor cells in postnatal hippocampal neurogenesis. Genes Cells, **10**, 1001-1014.

Sepe, R., Piscuoglio, S., Quintavalle, C., Perrina, V., Quagliata, L., Formisano, U., Terracciano, L.M., Fusco, A. and Pallante, P. (2016): HMGA1 overexpression is associated with a particular subset of human breast carcinomas. J. Clin. Pathol., **69**, 117-121.

Takahashi, T., Kim, M.S., Saito, T., Lee, J.Y., Hwang, G.W. and Naganuma, A. (2013): Brain-specific induction of secretoglobin 3A1 expression in mice treated with methylmercury. J. Toxicol. Sci., **38**, 963-965.

Takemoto, T., Ishihara, Y., Tsuji, M., Kawamoto, T. and Yamazaki, T. (2016): Transcription factor activation in rat primary astrocytes exposed to methylmercury. Fundam. Toxicol. Sci., **3**, 63-65.

Tokumoto, M., Lee, J.Y., Fujiwara, Y. and Satoh, M. (2014): Alteration of DNA binding activity of transcription factors in NRK-52E rat proximal tubular cells treated with cadmium. J. Toxicol. Sci., **39**, 735-738.